Department of Chemistry

Chemistry of Bicyclic Sesquiterpenes Isolated from *Dittrichia graveolens* L. Greuter in Western Australia

Aboaleid Ali M. Rtimi

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of
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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university.

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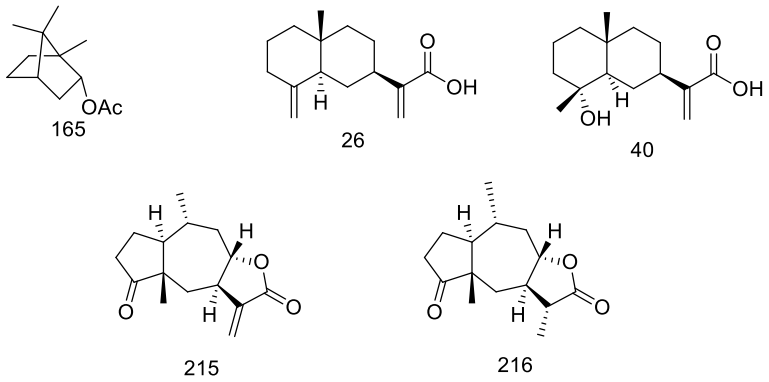
Date: 22/03/2016

Abstract

The species *Dittrichia graveolens* is a shrub native to Mediterranean basin. Over the past century, it has been introduced into many countries including Australia. The chemical composition of *Dittrichia graveolens* growing in Australia is yet to be thoroughly investigated. The compounds in the resin of the aerial parts of the *D. graveolens* species grown in the south west of Western Australia (Balingup) was studied by using a rapid extraction technique using ethanol. This solvent was effective to isolate and characterise the main components within the resin of the plant. Five compounds were isolated from the plant **26**, **40 165**, **215** and **216** in a considerable amount (> 0.5% w/w dry plant). The absolute configuration of compound **216** was not reported at the outset of this study.

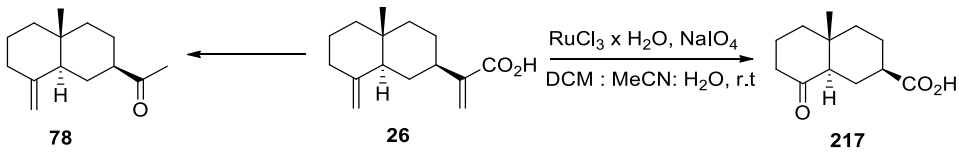


For a large scale extraction, the plant material was extracted with 2% sodium hydrogen carbonate solution which allowed extraction of the plant on > 10 kg scale. The extraction using aqueous solution of 2% sodium bicarbonate showed the presence of the five compounds illustrated above.

The bioactivity of the crude extract, costic **26** and ilicic acid **40** were investigated against alfalfa seed germination. Crude extract and ilicic acid at (21 mM) showed high inhibitory activity against seed germination whereas costic acid showed moderate inhibition activity at the same concentration.

The chemistry of the bicyclic sesquiterpenes costic and ilicic acid were made the focus of this work. The sesquiterpene acids were selectively functionalised to introduce

complexity to the molecule. For instance, the oxidation of the alkene groups in the costic acid gave interesting ketoacid **217** which is a useful intermediate in synthesis.



78 **26** **217**

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Lastly, I am extremely grateful to my father, Ali, and my mother, Aisha who raised me and taught me to study hard and to give priority in my life to the quest for knowledge. I am grateful to my brothers and sisters who encouraged me.

Contents

List of Abbreviations

AIBN	2,2'-Azobis(2-methylpropionitrile)
BHT	2,6-Di-tert-butyl-4-methylphenol
br	Broad
$CDCl_3$	Deuterated chloroform
COSY	Correlation spectroscopy
Cat	Catalytic
CSA	Camphorsulfonic acid
m-CPBA	*m*-Chloroperoxybenzoic acid
d	Doublet
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	Dichloroethane
DCM	Dichloromethane
DIBALH	Diisobutylaluminium hydride
DMA	Dimethylamine
DMF	*N*,*N*-Dimethylformamide
DMS	Dimethylsulfide
DMSO	Dimethyl sulfoxide
eq	Equivalent(s)
HMPA	Hexamethylphosphoramide
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single-quantum correlation
Hz	Hertz
h	Hour(s)
IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
L	Litres
LDA	Lithium diisopropylamide
M	Molar
m/z	Mass to charge ratio
m.p.	Melting point

m	Multiplet
mg	Milligrams
MHz	Megahertz
mL	Millilitres
mmol	Millimoles
mol	Moles
n-BuLi	*n*-Butyllithium
NBS	*N*-bromosuccinimide
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser enhancement spectroscopy
ppm	parts per million
q	Quartet
rt	Room temperature
s	Singlet
SEM	Standard error of the mean
sp	Septet
t	Triplet
TBS	*t*-Butyldimethylsilyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
w/v	Weight per volume
w/w	Weight per weight

Chapter 1

Introduction

Plants of Asteraceae (Asteraceae) family have been a rich source of sesquiterpenes and have been studied by chemists around the world.[1,2] *Dittrichia graveolens* is a species within the Asteraceae family. *D. graveolens* is not native to Australia, however it was introduced from the Mediterranean basin in 1860s. It is an annual and grows to 20 – 50 cm tall with a pleasant aromatic smell. It flowers in late summer and produces fluffy seeds which allows it to spread quickly (Figure 1.1).[3] The first chemical investigation to this plant growing in Turkey and Egypt showed it to be an excellent source for sesquiterpenes.[4, 5] *D. graveolens* is now a common sight along roads, railway lines and in clearings in the south west of Western Australia (Figure 1.1). The initial interest in this plant in Western Australia was a comment from a local sheep farmer who claimed that about 200 of his sheep died in a paddock that contain *D. graveolens* and studies have shown that the mortality was due to head seeds which were penetrating to jejunal mucosa.[3] In addition, a historical story from the north of Italy mentioned that fishermen steeped this plant in water then used the extract to facilitate catching of the fish.[6]

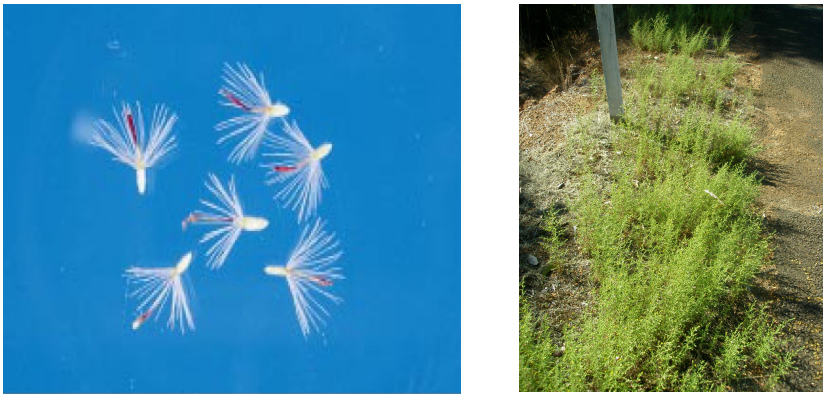


Figure 1.1: *Dittrichia graveolens* and fluffy seeds

1. 1 **Bicyclic Sesquiterpenes biosynthesis**

Sesquiterpenes are a diverse class of natural product with promising biological activity. Sesquiterpenes contain 15 carbon atoms and comprise 3 isoprene units. Most sesquiterpenes are found in plants. These compounds are synthesised from mevalonate in the cytosol or deoxyxylulose pathway in the plant plastids.[7] Sesquiterpenes have been isolated in acyclic, monocyclic, bicyclic, tricyclic forms. This thesis will concentrate on the bicyclic sesquiterpenes. The general families of bicyclic sesquiterpenes incorporating 5, 6 and 7 membered rings are eudesmane **1**, eremophilane **2**, cadinane **3**, guaiane **4** and drimane **5** (Figure 1.2). The four decalin structures only differ by the location of the substituents. The eudesmane **1** contains two axial methyl groups (groups 14 and 15) at C4 and C10 and an equatorial isopropyl group attached to C7. However, eremophilane **2** differ from **1** only by the position of the bridgehead methyl group that is connected in **2** at C5. Cadinane **3** does not contain bridgehead methyl groups. The methyl groups are attached to C3 and C9 and the isopropyl group attached to C6. Unlike the first three sesquiterpenes, guaiane **4** contains an azulene framework as opposed to a decalin ring. Guaiane **4** contains a substituent methyl groups attached to C4 and C10, and isopropyl group attached to C7. Drimane **5** contains a decalin ring with a bridgehead methyl group attached to C10. Drimane **5** does not have an isopropyl substituent instead two methyl groups are attached to C4.

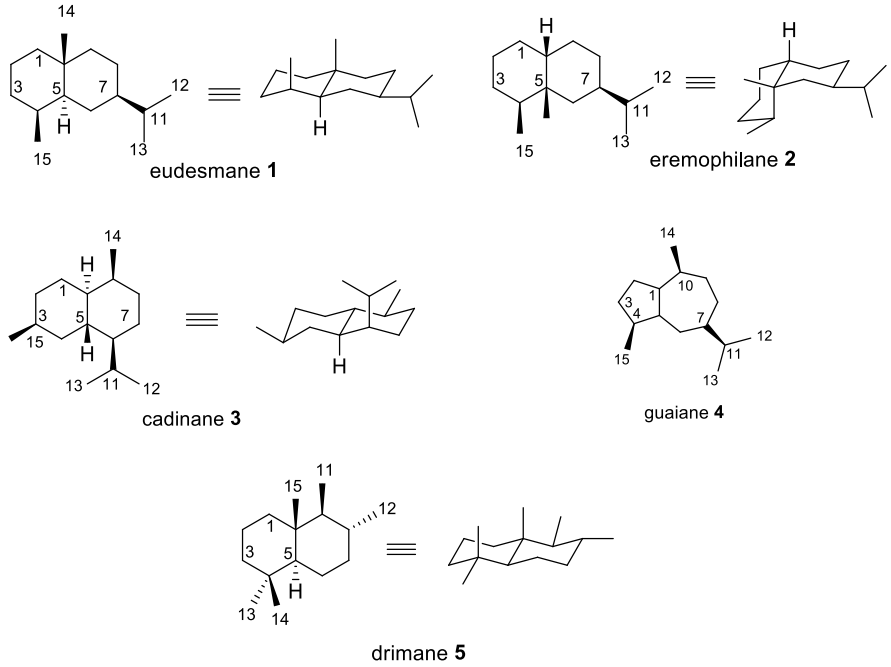
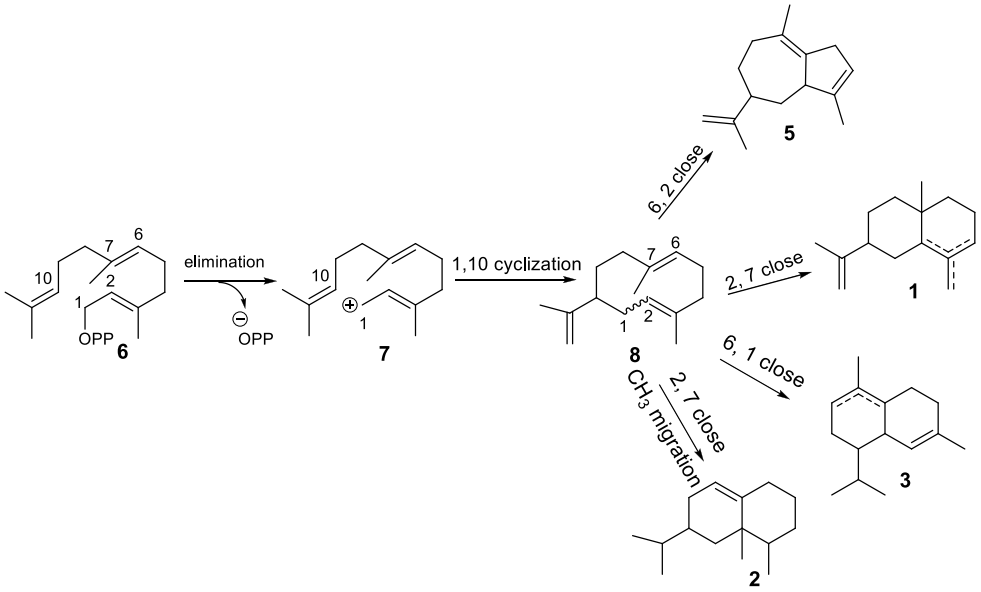


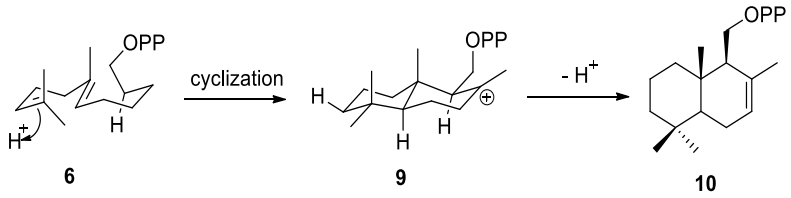
Figure 1.2: Structure of general bicyclic sesquiterpenes

The 15 carbon atoms of all sesquiterpenes are derived from farnesyl pyrophosphate. Cyclisation of farnesyl pyrophosphate produces the extensive variety of sesquiterpenes structures and stereochemistries. Elimination of pyrophosphate from farnesyl pyrophosphate **6** gives the *trans*-farnesyl cation **7** (Scheme 1.1),[8, 9] which undergoes a 1,10-cyclization to form (*E,E*)-germacradienyl **8** which contains a cyclodecane ring. This cyclodecane ring can cyclise in two different ways to produce different bicyclic sesquiterpenes. A 6,2-cyclization forms the guaiane skeleton **5** whereas a 2,7–cyclization gives the bicyclic eudesmane skeleton **1**. In addition, sesquiterpene skeletons **3** and **2** were synthesised from related cyclisation pathways (Scheme 1.1). A 1,6-cyclization forms the cadinane **3** whereas a 2,7-cyclization gives eremophilane **2** (Scheme 1.1).



Scheme 1.1: Biosynthesis of the guaiane **5,** eudesmane skeleton **1**, cadinane **3** and eremophilane **2**

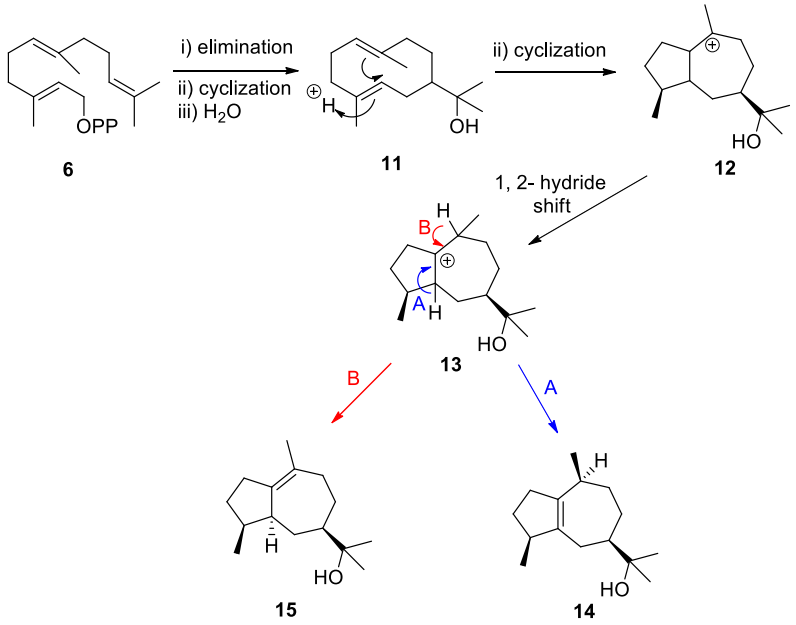
Drimane derivative **10** is synthesised from a different pathway (Scheme 1.2). Drimane **10** is synthesised from one step cyclisation of farnesyl pyrophosphate **6** to give the drimenyl phosphate **10**.



Scheme 1.2: Biosynthesis of drimane skeleton **10**

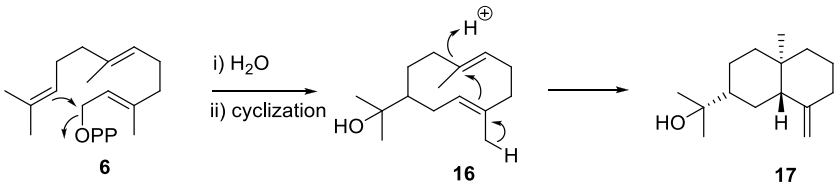
1. 2 **Biosynthesis of bicyclic guaiane**

Guaiane derivatives **14** and **15** are synthesised via two cyclization reactions.[10] The cyclization of farnesyl pyrophosphate **6** gives the alcohol **11**. An acid promoted transanular cyclisation between the two alkenes of the cyclodecane ring of allylic alcohol **11** gives the guaianyl cation **12**. A 1,2-hydride shift forms the intermediate guaiyl bridgehead cation **13**. Elimination of H^+ through pathway A gives the guaiol **14**, whereas the elimination of H^+ through pathway B leads to bulnesol **15**. (Scheme 1.3).



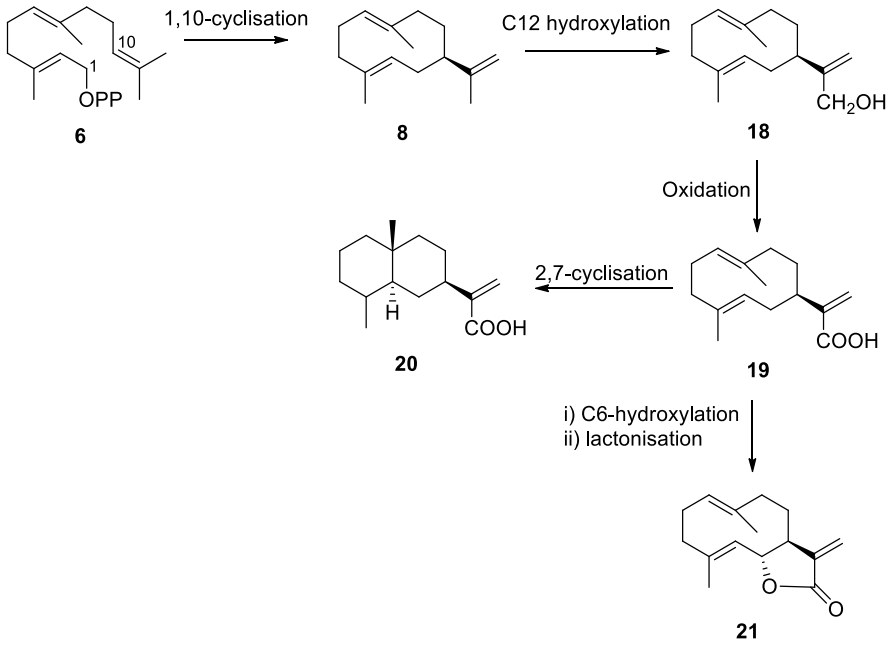
Scheme 1.3: biosynthesis of guaiol **14** and bulnesol **15**

Eudesmol is synthesised through hydration at C11 and 1,10-cyclization of farnesyl pyrophosphate **6** to give the germacradienol **16**. This is followed by 2,6-cyclization to form *β*-eudesmol **17** (Scheme 1.4).



Scheme 1.4: Biosynthesis of *β*-eudesmol **17**

A 1,10-cyclization of farnesyl pyrophosphate **6** by germacrane A synthase gave the germacrane A **8** in *Asteraceae* (Scheme 1.5). Hydroxylation of germacrane **8** at C12 forms the allylic alcohol **18** followed by oxidation at C12 to form germacrane acid **19.** A 2,7-cyclisation of germacrane acid **19** afforded the sesquiterpene acid **20**. However, hydroxylation of acid **19** at C6 followed by lactonization to form (+)-costunolide **21**.[11,12]



Scheme 1.5: Biosynthesis of costunolide and bicycilic acid

1. 3 **Biological activity of bicyclic-sesquiterpenes**

The biological activities of sesquiterpenes have been extensively reported.[13-16] Many sesquiterpenes have diverse biological potential such as insect repellence and anti-inflammatory, anti-bacterial, anti-cancer properties. Bicyclic sesquiterpenes are among the sesquiterpenes that exhibit curative effects against many plant and human disorders. The high biological activity of bicyclic sesquiterpenes is linked to presence of a range of *α, β*-unsaturated carbonyl groups (eg. Figure 1.3) which can react with biological nucleophiles to form stable adducts by Michael addition reaction.[17,18] An example of a biological nucleophile is the thiol present in the cysteine residue of proteins.[17]

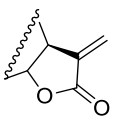
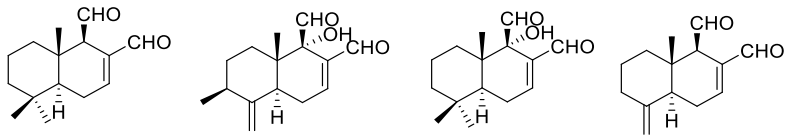


Figure 1.3: *α,β*-unsaturated carbonyl

1.3.1 Insect antifeedant

Many sesquiterpenes have antifeedant activity, which means they are unpalatable to insects and other herbivores. It is probably the main purpose of these secondary metabolites. The most studied insect antifeedant are polygodial **22**, muzigadial **23** and warburganal **24**. They were isolated from folk medicinal East African *Warburgia* tree (Canellaceae). Compounds **22** and **24** showed high antifeedant actions against African armyworms, *Spodoptera littoralis* and *S. exempta* larvaes at 0.1 ppm.[19,20] The polygodial **22** was also isolated from bark of *Drimys winteri* and their antifeedant activity was investigated against *Spodoptera littoralis* (Lep., Noctuidae) in leaf discs with choice and no choice assay. The assay was conducted on the sweet pepper leaves. In the choice test, the treated leaf discs and non-treated leaf discs (control) were put together in the same container, whereas the no choice test only the treated discs were put in container. Polygodial **22** was repellent by 94.7% in choice test and 53.8% in no choice test at 1000 ppm with DC_{50} = 708 ppm.[13] Gerard *et al,* have isolated the sesquiterpenes polygodial **22** and 9-deoxymuzingadial **25** from *Pseudowintera colorata* (Raoul), which is a tree native to New Zealand. Both compounds **22** and **25** showed high antifeedant and insecticidal activity in cloth consumed by *Anthrenocerus australis* and *Tineloa bisselliella* larvae ($p < 0.001$ compared to control $p < 0.05$) with minimum concentration of 0.8 mg/g (Table 1.1).[21]



22 **23** **24** **25**

Figure 1.4: Structures of polygodial **22**, muzigadial **23,** warburganal **24** and 9-deoxymuzigadial **25**

Table 1.1: polygodial **22** and 9-deoxymuzigadial activity **25**.[21]

Treatment (mg/g wool)	Mean cloth consumption (mg)		Survival (%, SE in brackets)	
	A. australis	*T. bisselliella*	*B. australis*	*T. bisselliella*
Polygodial				
3.0	39	-0.6	95.1 (2.7)	0.0
1.5	9.4	2.1	98.3 (1.7)	8.5 (3.6)
0.8	14.8	2.4	98.3 (1.7)	13.6 (4.5)
0.4	19.0	10.3	98.2 (1.7)	55.3 (7.3)
0.2	41.1	15.7	100	97.4 (2.5)
9-deoxymuzigadial				
3.0	2.5	-0.6	98.3 (1.7)	2.0 (2.0)
1.5	4.0	0.9	96.6 (2.3)	1.7 (1.7)
0.8	9.1	0.8	95.1 (2.8)	0.0
0.4	24.1	15.6	100	84.0 (5.2)
0.2	30.1	22.5	100	95.8 (2.9)
Untreated	39.2	22.2	100	95.7 (2.9)
Ethyl acetate	41.6	20.0	100	98.0 (2.0)
SED	4.3	1.9		

Watanabe *et al*., reported that the costic acid **26** and ilicic acid methyl ester **27** were isolated from *Callitris glaucophylla* heartwood.[14] Costic acid **26** and ilicic acid methyl ester **27** were investigated for their repellent activity against the termite *Coptotermes formosanus* using filter paper test in concentration of 0.5% (w/w) (Figure 1.5). Costic acid **26** and ilicic acid methyl ester **27** showed high repellent percentage activity (68.9% ± 10.1 SEM) and (66.7% ± 7.5 SEM) respectively against the *Coptotermes formosanus*, compared with the commercial antitermitic agent alkyl ammonium chloride (46% ± 10.0 SEM).

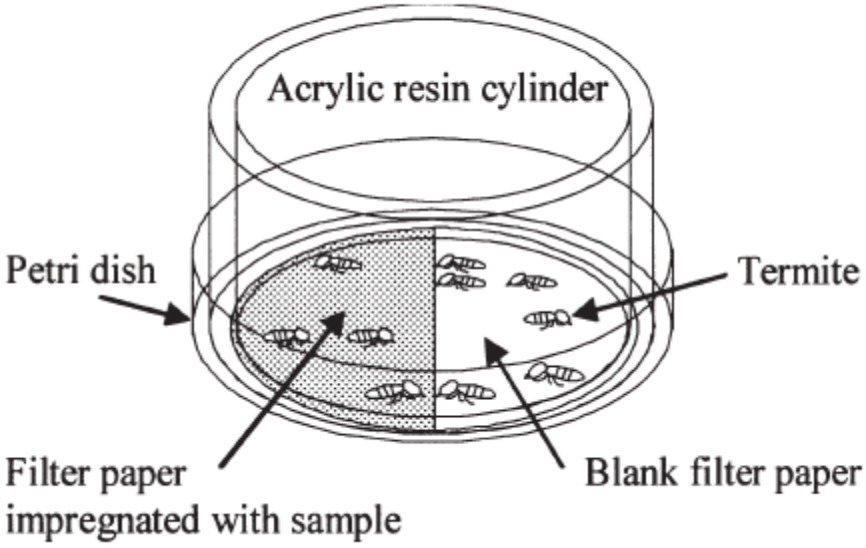


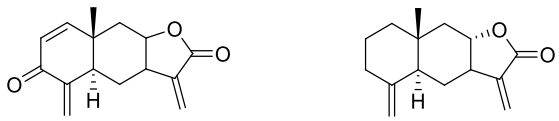
Figure 1.5: filter paper repellent test by Watanable., *et al*.[14]

26 **27**

Figure 1.6: Structures of costic acid **26** and ilicic acid methyl ester **27**

Encelin **28** has been isolated from *Encelia actoni* and *Enelia asperifolia* and exhibited a high toxicity and antifeedant activity against the polyphagous *Spodopetra littoralis* (Noctuidae).[22] Encelin **28** was incorporated into an artificial diet at concentration 0.5-5.0 µmol g^{-1} fresh weight and presented to neonate larvae *S. littoralis* (n = 30) for six days. Encelin **28** significantly reduced the growth of surviving larvae at 0.5 µmol g^{-1} (20% compared to control 100%).[22] LD_{50} of encelin **28** was measured after 48 h (LD_{50} = 60 µg per larvae). A structurally similar compound isoalantolactone **29** was isolated from *Inula racemosa*.[23] It was mixed with wheat seeds 50, 75, 100 and 125 µg g^{-1} (w/w). Deterrence of isoalantolactone **29** was measured against rice weevils (*Sitophilus oryzae* L.). The treated seeds with isoalantolactone showed a strong repellence against the *S. oryzae* .The Compound **29** exhibited high insecticidal activity

against *S. oryzae* when the wheat seeds treated with 200 to 3000 µg g^{-1}. The LC_{50} of compound **29** was 123.57, 707.21, 655.31 and 610.50 µg g^{-1} at 5, 10, 15 and 20 days respectively. In addition, isoalantolactone **29** caused 100% mortality in *S. oryzae* within 5 days when seeds were treated with 3000 µg g^{-1}(Table 1.2).[23]



28 **29**

Figure 1.7: Structures of encelin **28** and isoalantolactone **29**

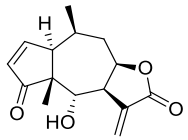
Table 1.2: Mortality (%) of adults *S. oryzae* held on wheat seeds treated with **29**.[23]

Dosages of **29**	Days after treatment			
(µg/g)	5	10	15	20
200	11.9±2.38**	16.67±4.77**	17.95±5.13**	17.95±5.13**
400	19.04±4.77**	23.81±6.31**	30.77±8.89**	35.91±6.79**
600	33.33±4.77**	55.38±8.60**	56.41±9.25**	58.97±6.79**
1000	42.86±4.13**	64.28±4.13**	69.23±4.45**	74.36±2.57**
2000	76.19±2.38	100	100	100
3000	100**	100	100	100
NC	0	0	0	0
PC	86.54±3.29	100	100	100
LC_{50}	1234.57	707.21	655.31	610.51

: Significant difference between compound **29 and aluminium phosphide treated control at 5% & 1% levels by *T*-test. PC: Positive Control. NC: Negative Control.

1.3.2 Anticancer

Many sesquiterpene lactones have been tested against cancer cell lines. Helenalin **30** was extracted from *Arnica montana* L. Helenalin **30** showed inhibitory activity against the growth of murine P–388 lymphocytic leukaemia, Walker 256 carcinoma, and Ehrlich ascites carcinoma.[16] In addition, compound **30** inhibited the growth of Ehrlich ascites tumour by 99% in mice when injected twice in 33 mg/kg/day.[24] Costunolide **20** was isolated from the stem bark of Korean *Magnolia sieboldii*.[25] Furthermore costunolide **20** exhibited inhibition effects on many of human cancer cells; [25,26,27] it inhibited the growth of MCF-7 and MDA-MB-231 cells *in vivo* and *in vitro* and the half maximal inhibition (IC_{50}) for these cells was 90 μM and 50 μM respectively.[25]



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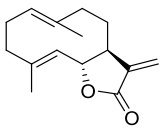

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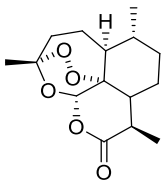
Figure 1.8: Structures of helenalin **30** and costunolide **20**

1.3.3 Antimalarial

Artemisinin **31** (qinghaosu) has been used in Chinese traditional medicine as an anti-pyretic and antimalarial from ancient time.[28] Its modern day use as an antimalarial by Youyou Tu led to the Noble Prize for Medicine in 2015. Sesquiterpene **31** was isolated from *Artemisia annua* (Asteraceae), in 1970s and is the most famous sesquiterpene lactone antimalarial drug isolated. It has high activity against the most virulent strain of malaria caused by *Plasmodium falciparum* which causes 1–2 million deaths every year in Africa.[15, 29] *P. falciparum* has developed resistance to the common antimalarial drug chloroquine. The synthesised derivatives of artemisinin, artenimol **32**, artemether **33**, arteether **34**, and artesunate **35** also exhibited high antimalarial activity. These derivatives clear parasites from the blood after 2 days of treatment. The peroxide bridge is the key structural feature of these molecules and is essential for the observed activity (Table 1.3).

Table 1.3: In vitro values of chloroquine resistant *P. falciparum* strains



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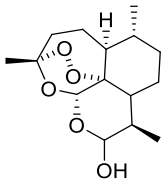

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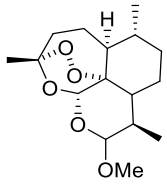

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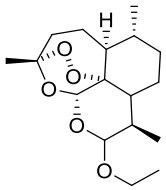

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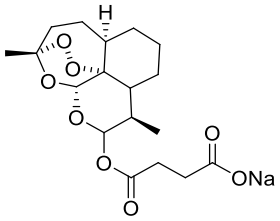
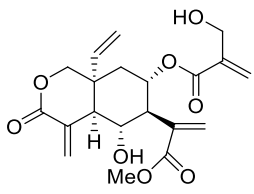


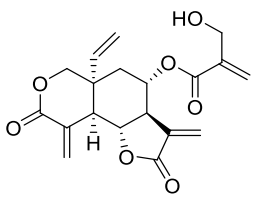
35

Drug	IC_{50} *P. falciparum*
Artemisinin	10^{-8} mol/L- 10^{-7} mol/L (3 – 30 µg/L)
Dihydrodroartemisinin	0.36 nmol/L- 7.00 nmol/L (0.1 – 2 µg/L)
Artesunate	1.66 nmol/L- 2.18 nmol/L
Artemether	0.57 nmol/L- 6.10 nmol/L
Arteether	1.74 nmol/L- 3.44 nmol/L

Many sesquiterpenes other than artemisinin have been isolated and showed anti-malarial activity. Vernolides **36** and **37** have been isolated from *Vernonia colorata* LESS (Asteraceae), and showed good antiplasmodial activity IC_{50} = 4.8 and 1.1 µg/ml respectively.[30]



36

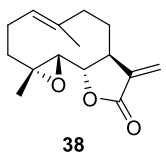


37

Figure 1.9: Structures of antimalarial Vernolides **36** and **37**

1.3.4 Anti-inflammatory

Costunolide **20** was isolated from *Magnolia ovata* and the compound exhibited potent anti-inflammatory activity in mice models. Costunolide **20** at 30 nmol/paw reduced the oedema after 1, 2, 4, and 24 hours from induced paw inflammation by carrageenan (300 μg/paw) by 39.2%, 37.3%, 39.6%, and 47.2% respectively.[31] The similar sesquiterpene parthenolide **38** was isolated from *Magnolia species* in America and isolated also from feverfew (*Tanacetum parthenium*) in Europe. Parthenolide **38** inhibited the expression of both cyclooxygenase 2 (COX-2) and pro-inflammatory cytokines in macrophages.[32] The activity of parthenolide is related to the α-methylene-γ-lactone and to the presence of an epoxide group.[33] This compound has been also reported as an inhibitor for the transcription factor IκB phosphorylation.[34] Wang and Li have reported that parthenolide **39** could be a promising anti-inflammatory drug. In *in vivo* experiments on mice, the parthenolide **39** at 8 mg/kg/day inhibited the oedema in paw mice by 73.4%.[35]

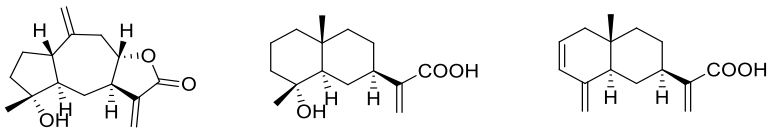


38

Figure 1.10: Structure of anti-inflammatory parthenolide **38**

Sesquiterpenes inuviscolide **39** and ilicic acid **40** were isolated from aerial parts of *Inula viscosa*. Inuviscolide **39** has inhibited the release of human leukocyte elastase by 51% at 100 μM. and showed 70% inhibition after 2 min against bee venom secretory PLA2 with an IC_{50} = 80.5 μM (r^2 = 0.96, P = 0.022). In addition, it significantly inhibits COX-1 at 50 μM (40%).[36] Ilicic acid **40** has shown high anti-inflammatory activity in the mouse ear edema that was induced by TPA, by inhibiting the mouse ear swelling by 72%.[37] A similar sesquiterpene dehydrocostic acid **41**, isolated from *I. viscosa* (L), showed anti-inflammatory effects in the mouse model.[38] The inhibition activity of the acid **41** has been studied on the mouse ear edema produced by TPA, paw edema produced by phospholipase A2 and on human polymorphonuclear leukocytes. Acid **41** inhibited the generation of LTB_4 *in vitro* with an IC_{50} = 22 μM (r^2 = 0.9966, P =

0.0017). The dehydrocostic acid **41** totally inhibited the elastase enzyme activity in human cell with an IC_{50} = 17 μM (r^2 = 0.9218; P = 0.0399). [38]



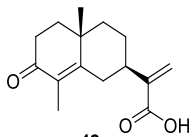
39 **40** **41**

Figure 1.11: Structures of some anti-inflammatory sesquiterpenes

1.3.5 Antibacterial

Some sesquiterpenes show moderate inhibition against a range of bacteria. 3-Oxocostusic acid **42** was isolated from *Varthemia iphionoides* of the Compositae family. The acid **42** showed potent antibacterial activity against six bacterial strains (Table 1.4).[39]

Table 1.4: Minimum inhibition concentration of acid **42**.[39]

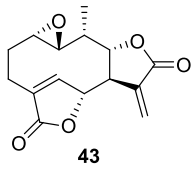


42

Bacterial strains	**MIC μg/ml**
Bacillus subtilis	450
Escherichia coli	250
Staphylococcus aureus	500
Salmonella enteritides	350
Bacillus cereus	450
Micrococcus luteus	350

Deoxymikanolide **43** has been isolated from the aerial parts of *Mikania micrantha*, and showed antibacterial activity. Deoxymikanolide **43** was investigated for antibacterial activity against 8 bacterial species (Table 1.5). [40,41,42,43]

Table 1.5: Effects of deoxymikanolide **43** against tested bacteria strains.[42]



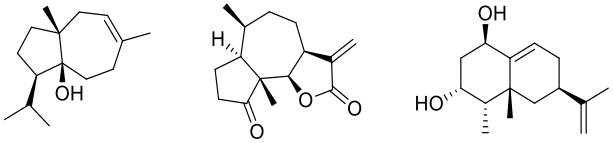
43

Bacteria strains	**MIC mg/L**	**MBC mg/L**
Staphyloccocus	62.5	125
Bacillus subtillis	62.5	250
Micrococcus luteus	125	250
Bacillus cereus	125	250
Ralstonia dolaanacearum	62.5	125
Xanthomonas oryzae pv. Oryzae	125	250
Xanthomonas Campestris pv. Vesicatoria	62.5	125
Xanthomonas campestris pv. Citri	62.5	125

1.3.6 Antifungal activity

Many sesquiterpenes exhibit high activity against pathogenic fungi. The cyclic sesquiterpenes carotol **44** was extracted from carrot seed essential oil. Carotol **44** was shown to be a high potent inhibitor against the growth of *Alternaria alternate* fungi by (65%) at a concentration of 150 mg/L.[44] Carotol **44** showed activity nearly as potent as the commercial antifungal Fonaben T (85%).[44] The sesquiterpene costunolide **20** showed potent activity against many types of fungi *Nigrospora spp., Rhizoctonia solani* and *Helminthosporium spp.* with EC_{50} = 0.48, 2.92 and 2.96 µg/ml respectively.[45] Neoambrosin **45** sesquiterpene was isolated from *Ambrosia maritime L,* and showed high inhibition activity against the plant pathogenic fungi *Botrytis cinerea* and *Fusarium oxysporum* with EC_{50} 332.5 mg/L and 230.2 mg/L respectively.[46] Capsidiol **46** was isolated from tobacco, jimson weed, and sweet pepper.[47] The capsidiol **46** exhibits a high biological activity against several type of

fungi (Table 1.6).[47] Arreola-Cortes, *et al.,* have reported that the capsidiol **46** at 5 µM showed 100% inhibition activity against the *Aspergillus niger* germination.[48]



44 **45** **46**

Figure 1.12: Structures of some antifungal sesquiterpenes

Table 1.6: Effect of capsidiol **46** on the germination of fungal spores

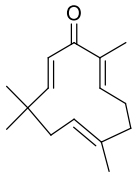
Fungal species	**MID**[a]	**ED_{50}**[b]
Alternaria brassicicola	100	10
Alternaria longipes IMI105920	50	25
Ascochyta fabae	50	10
Aspergillus niger	25	10
Botrytis cinerea	200	50
Botrytis fabae	50	30
Cladosporium cucumerinum	25	< 10
Colletotrichum lagenarium	25	10
Colletotrichum lindemuthianum	12	10
Clomerella cingulate	100	50
Septoria nodorum	50	15

[a] Minimum inhibitory dose (µg/mL) and [b] the concentration caused 50% inhibition

1.3.7 Anti-hyperlipidemic activity

Natural products have been used as a remedy to treat many diseases and used to treat obesity in humans. Hall *et al*., have reported that the sesquiterpenes helenalin **30** showed high activity by lowering blood serum triacylglyceride (TAG) and cholesterol.[49] Helenalin **30** showed high activity by lowering serum TAG in mice by 24% at 6 mg/kg/day for 2 weeks treatment and reduced the serum cholesterol by 38% at dose of 6 mg/kg/day for 16 days.[49] Sesquiterpene zerumbone **47** showed high activity on the serum lipid in mice model. HFD fed hamsters were treated with 25, 50,

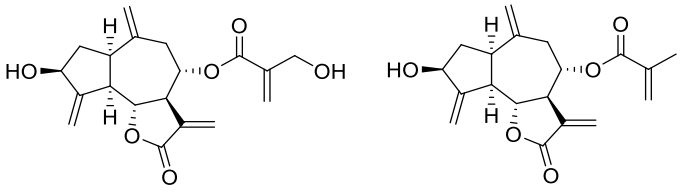
and 100 mg/kg/day zerumbone for 8 weeks. Zerumbone **47** at 100 mg/kg/day showed significant lowering in plasma of TC, TG and LDL–C concentration which were lower by 23.7%, 27.5% and 36.2% respectively.[50,51]



47

Figure 1.13: Structure of Anti-hyperlipidemic zerumbone **447**

Shimoda *et al*., have isolated the sesquiterpenes cynaropicrin **48** and aguerin B **49** from artichoke (*Cynara scolymus* L.).[52] The compounds **48** and **49** significantly reduced mice serum TG at 50 and 100 mg/kg after olive oil administration after two hours (Figure 1.15).[52]



48 **49**

Figure 1.14: Structures of Anti-hyperlipidemic cynaropicrin **48** and aguerin B **49**

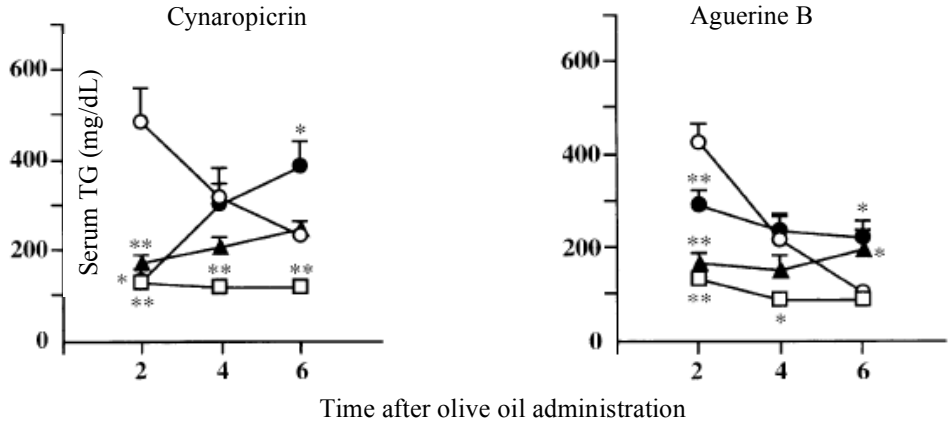


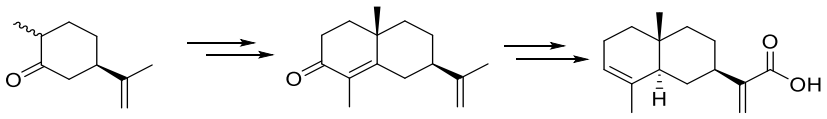
Figure 1.15: Inhibitory effect of cynaropicrin and aguerine B on serum TG elevation in olive oil in mice. Where: □: non olive oil treatment, ○: control, ●: 50 mg/kg, ▲: 100 mg/kg, and each value represents the ± SEM of 4-7 mice. Asterisks shows the significant differences from control group *: $P < 0.05$, **: $P < 0.01$, respectively.[52]

1. 4 **Total synthesis of bicyclic sesquiterpenes**

The ranges of biological activities associated with sesquiterpenes have made them attractive in both the pharmaceutical and agrochemical industries. They also provide challenging targets for synthetic chemists. The intricate structure of these compounds is also a feature that encourages chemists in the endeavour of total synthesis. The total synthesis of the bicyclic sesquiterpenes has proved to be a formidable challenge. The most significant synthetic challenge is to obtain the correct stereochemistry of the sesquiterpenes. Many total syntheses of bicyclic sesquiterpenes have been published and have been reviewed and some of these endeavours are highlighted here.[53-55]

1.4.1 Total synthesis of isocostic acid

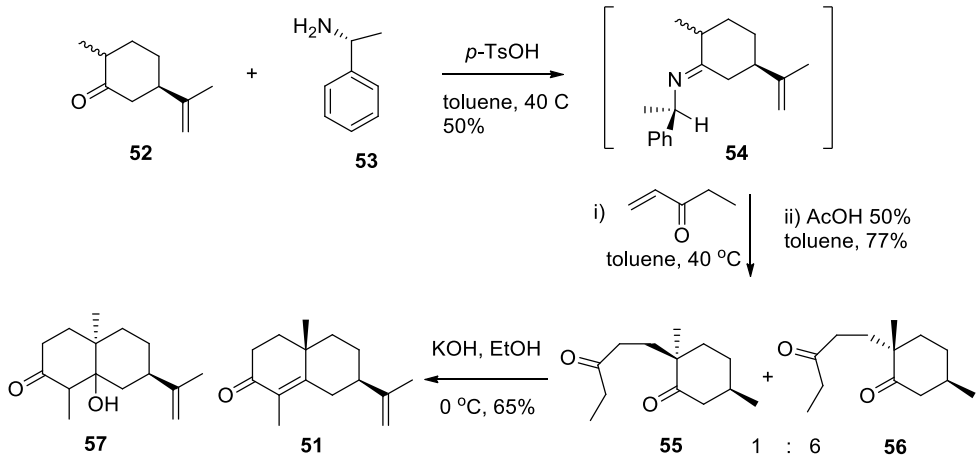
Isocostic acid **50** is a natural product isolated from *Inula viscosa Ait* that exhibited antibacterial and antifungal activity.[56,57] The total synthesis of this compound has been reported by Chen *et al.*, who prepared isocostic acid from (+)-dihydrocarvone **52** in 10 steps (Scheme 1.6).[58] The key intermediate in the synthesis is compound **51** which is made through a Robinson annulation of **52** using an activated amine (Scheme 1.7).[53]



52 **51** **50**

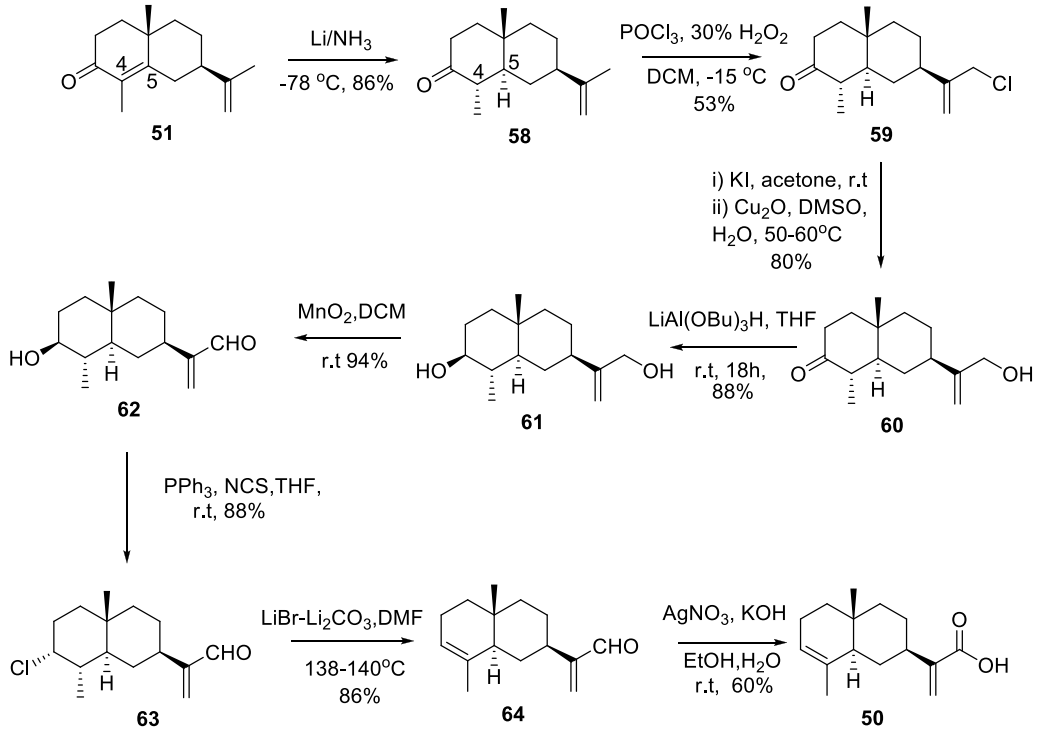
Scheme 1.6: Synthesis of isocostic acid **51**.[58]

The *α,β*-unsaturated carbonyl **51** was synthesised in 3 steps (Scheme 1.7). The cyclohexanone **52** was treated with the amine **53**, *p*-TsOH and heated at 40 ˚C to give the intermediate imine **54**. The reaction between the vinyl ketone and the imine **54** followed by hydrolysis produced two isomers of the Michael addition products **56** and **55** (1:6) in 71% yield. Intramolecular aldol condensation of the mixture of **56** and **55** was performed using potassium hydroxide in ethanol at 0 ˚C, to give the *α,β*-unsaturated carbonyl compound **51** and hydroxyl carbonyl **57** (5:1) in 65% yield.



Scheme 1.7: Synthesis of *α,β*-unsaturated carbonyl **52**.[53]

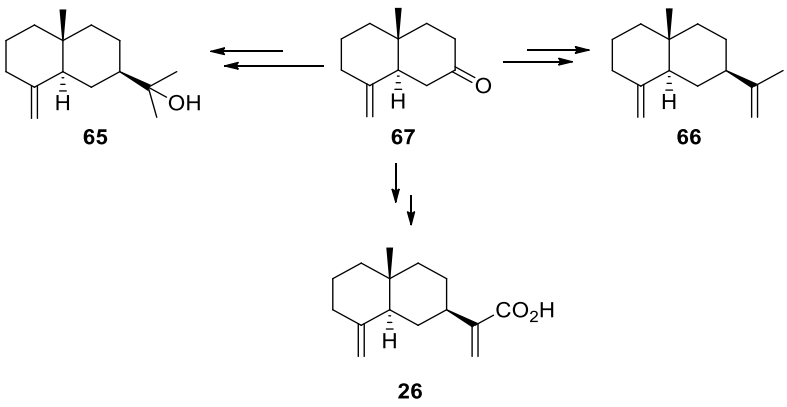
Reduction of the *α,β*-unsaturated carbonyl **51** using Birch conditions gave the hydrogenated product **58** (Scheme 1.8). The hydrogen at C5 is *anti* with respect to bridgehead methyl and also *anti* with hydrogen at C4 allowing the methyl group at C4 to be in an equatorial position. Allylic chlorination of **59** with phosphoryl chloride and 30% hydrogen peroxide in dichloromethane afforded the chloride **59**. Conversion of chloride **59** to allylic alcohol **60** was performed in two steps. A Fincklestein reaction converted the chloride to an iodide using potassium iodide in acetone. Substitution of the intermediate iodide using copper oxide in dimethyl sulfoxide and water gave the allylic alcohol **60** in 80% overall yield. The ketoalcohol **60** was reduced with lithium tri(*tert*-butoxy)aluminium hydride in tetrahydrofuran to produce the diol **61** with hydroxyl group *anti* to the methyl group. Oxidation of the allylic alcohol **61** with manganese dioxide in dichloromethane gave the aldehyde **62**. Conversion of the hydroxyl group in **62** to a chloride with *N*-chlorosuccinimide and triphenylphosphine gave the chloride **63** in good yield (88%). Elimination of the chloride **63** with lithium bromide and lithium carbonate in dimethylformamide gave the aldehyde **64**. The aldehyde **64** which was oxidized with silver nitrate to give the isocostic acid **50** in 60% yield.



Scheme 1.8: Synthesis of isocostic acid **50**.[58]

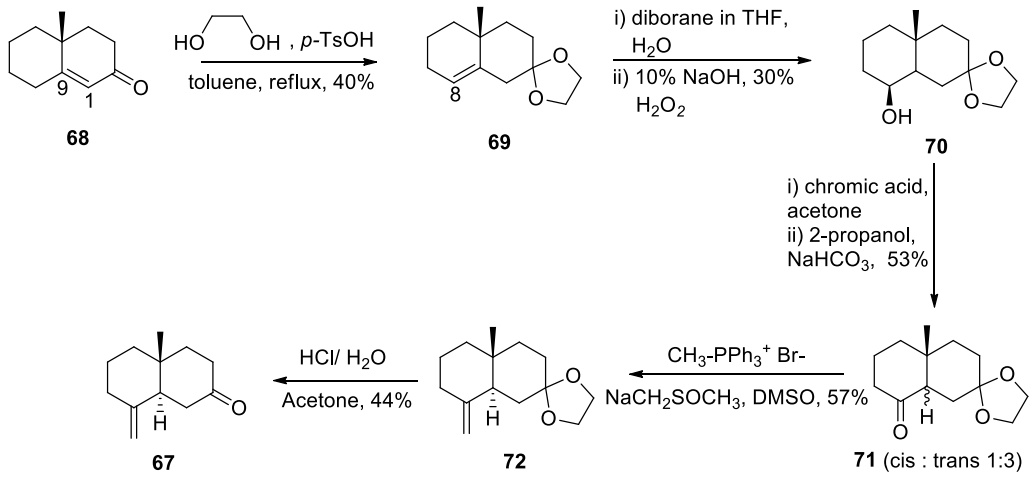
1.4.2 Total synthesis of *β*-selinene, costol and costic acid

The natural sesquiterpenes costol **65,** *β*-selinene **66** and costic acid **26** were synthesised from the decalone **67** (Scheme 1.9).[55] The decalone **67** is a good intermediate as it contains a decalin ring, methyl group at the ring junction C10 and an exocyclic methylene group at C4.



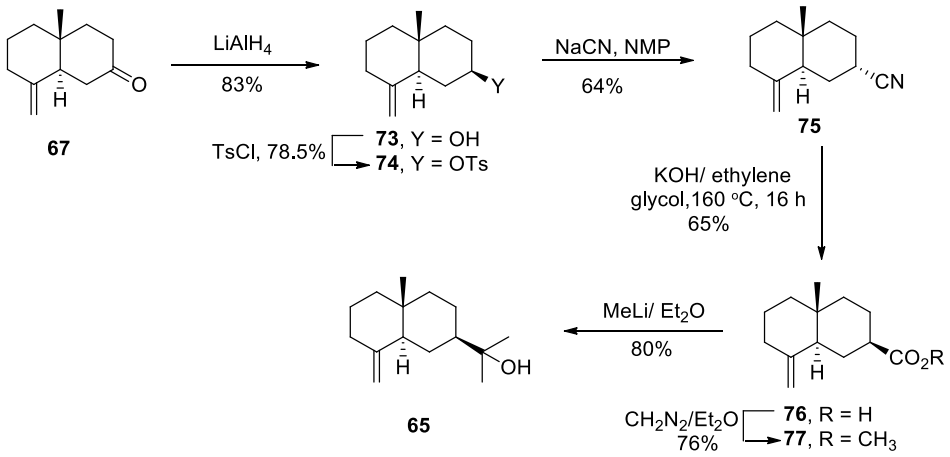
Scheme 1.9: Synthesis of costol **65**, *β*-selinene **66** and costic acid **26**.[55]

The decalone **67** has been synthesised in 7 steps starting from enone **68** which contained the core structure (Scheme 1.10). The ketone **68** was protected by converting it to the 1,3-dioxolane under standard conditions. Upon treatment of **68** with ethylene glycol, *p*-TsOH in refluxing toluene, **69** was formed with migration of the double bond. An *anti* Markovnikov hydration of the double bond of **69** using diborane with hydrogen peroxide gave the alcohol **70**. Marshall and his group postulated the *cis*-hydroxyl ketal is predominant since the underside double bond is blocked with an axial ketal group. The alcohol **70** oxidized with chromic acid in acetone to give ketone **71** as a mixture of two epimers. Treatment of **71** with the ylide derived from methyl-triphenylphosphonium bromide formed the *exo* cyclic methylene **72**. Deprotection of this compound afforded the intermediate decalone **67** in 44% yield.



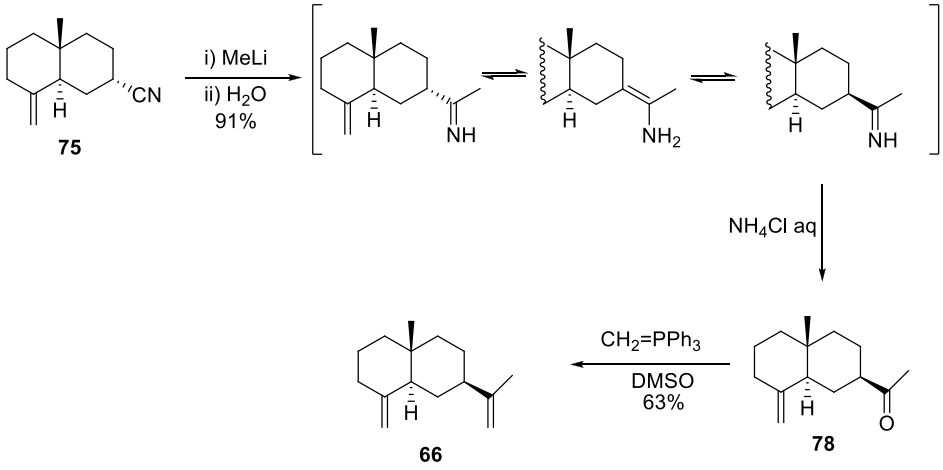
Scheme 1.10: Synthesis of decalone **67**.[55]

Marshall *et al*., undertook the total synthesis of costic acid and related sesquiterpenes using the decalone **67** (Scheme 1.11).[55] Reduction of the decalone **67** with $LiAlH_4$ afforded equatorial alcohol **73** which was converted to a tosyl group. Substitution of the tosylate **74** with sodium cyanide in *N*-methylpyrrolidine produced the nitrile **75** with inversion of stereochemistry. Hydrolysis of the nitrile with KOH gave the carboxylic acid with epimerization of C2 to give the more stable *cis* product **76** in 65% yield. The acid **76** was esterified using ethereal diazomethane to give the ester **77** which was treated with methyllithium to afford racemic *β*-eudesmol **65** in 80% yield.



Scheme 1.11: Synthesis of *β*-eudesmol **65**.[55]

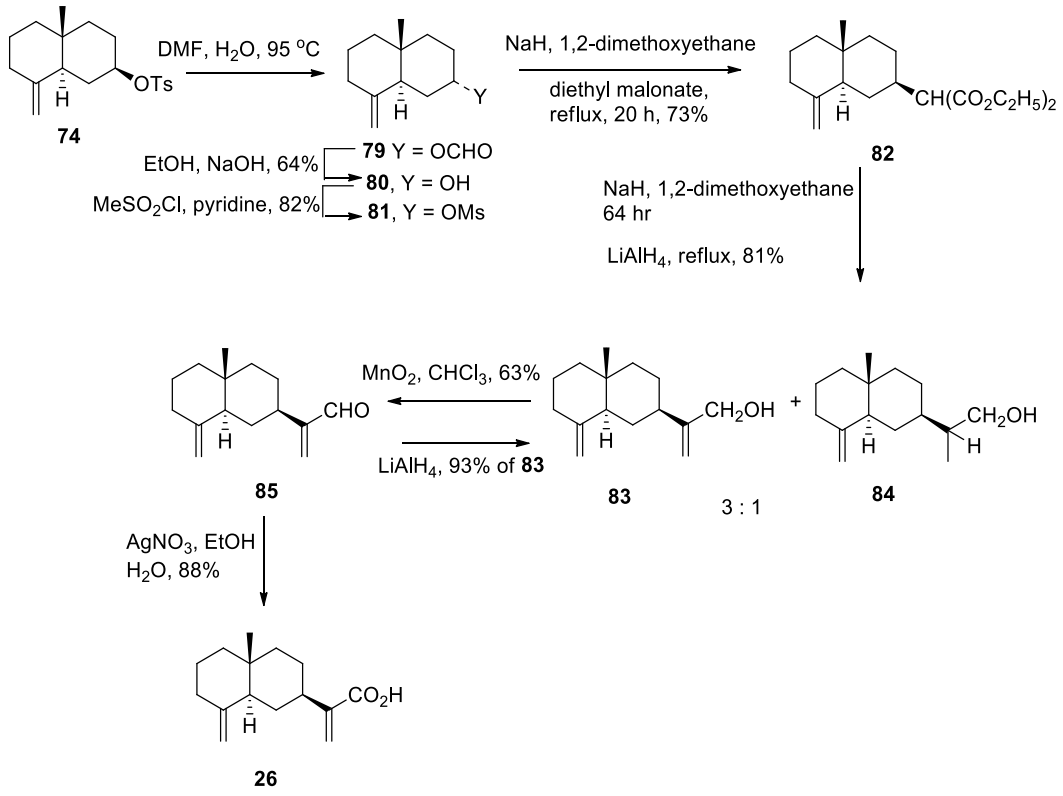
β-Selinene **66** was made from the nitrile **75** in 3 steps (Scheme 1.12). The nitrile **75** was reacted with methyllithium followed by hydrolysis with ammonium chloride to afford ketone **78** with inversion of stereochemistry through forming imine and enamine interconversion. *β*-selinene **66** was obtained by treatment of **78** with methylene triphenylphosphorane in DMSO.



Scheme 1.12: Synthesis of *β*-selinene **66**.[55]

Costic acid **26** was made from the tosylate **74** in 7 steps (Scheme 1.13). The tosylate **74** was hydrolysed with water in *N,N*-dimethylformamide at 95% to afford the formate **79**. Hydrolysis of the formate **79** with sodium hydroxide in ethanol was formed alcohol

80. Alcohol **80** was converted to mesylate **81** under standard conditions. Substitution of the mesylate **81** with the sodium salt of diethyl malonate introduced all the carbons for costic acid in compound **82**. The malonate **82** was reduced with $LiAlH_4$ to give a mixture of costol **83** and dihydrocostol **84** (3:1 respectively). Oxidation of costol **83** with MnO_2 in $CHCl_3$ gave the costal **85** which was oxidized with silver nitrate and sodium hydroxide in ethanol to give costic acid **26** in 88% yield.

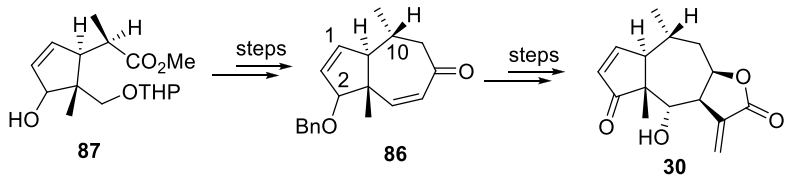


Scheme 1.13: Synthesis of costic acid **26**.[55]

1.4.3 Total synthesis of helenalin

Helenalin **30** is a sesquiterpene lactone isolated from *Helenium autumnale*.[54] Helenalin **30** as described earlier exhibited anticancer and anti-hyperlipidemic activity. This property and the complex structure has attracted the attention of many chemists.[59,60] In 1978, the first total synthesis of helenalin was accomplish by Yasufumi and co-

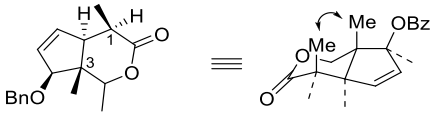
workers.[54] They started with the cyclopentanol **87** with *β*-methyl group at C10 which, previously reported was made in 6 steps in 25% yield,[61] and the main challenge was to construct the intermediate hydroazulenone **86** containing the α-methyl group at C10 (Scheme 1.14).[61]



87 **86** **30**

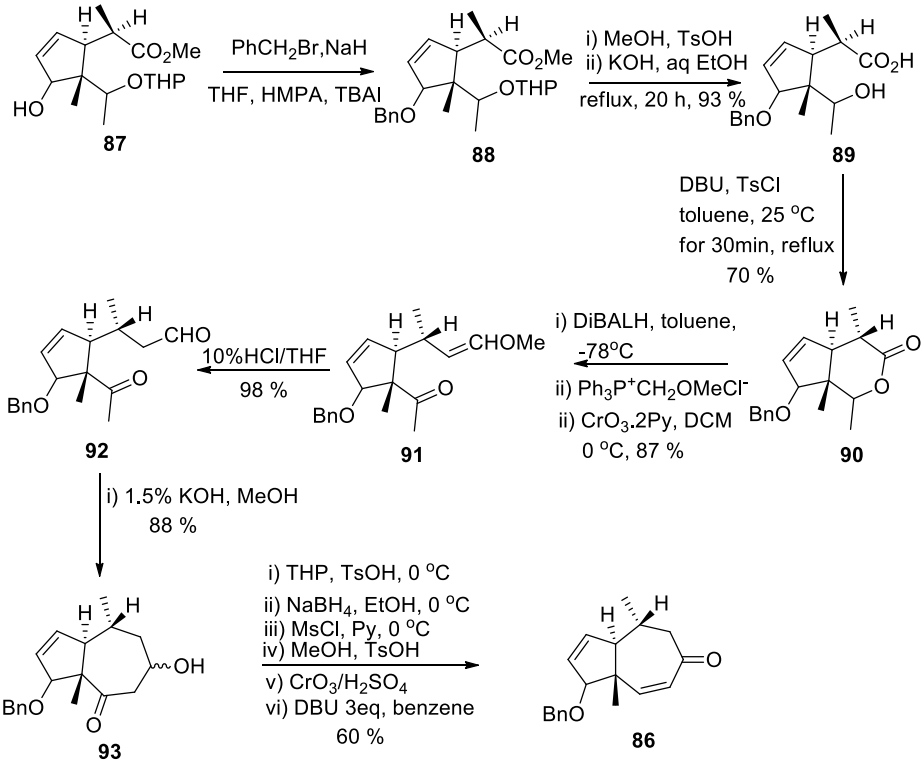
Scheme 1.14

The key intermediate hydroazulenone **86** was made from the cyclopentanol **87** in 7 steps (Scheme 1.15). Protection of the allylic alcohol **87** as a benzyl ether was performed using sodium hydride, benzyl bromide, HMPA and *t*-butylammonium iodide gave **88** in 81% yield. Removal of the THP group using toluenesulfonic acid in methanol and subsequent hydrolysis of the ester **88** with potassium hydroxide produced the hydroxyl carboxylic acid **89**. Lactonization of the hydroxyacid **89** was conducted using *p*-toluenesulfonyl chloride and DBU in toluene at 25°C to produce the lactone **90** with isomerisation of the methyl group to the α position in 70% yield. The epimerization occurs due to the unfavourable interaction between the 1,3-diaxial methyl groups (Figure 1.16). Reduction of the lactone **90** using DiBALH in toluene gave a lactol which was treated with the ylide derived from methoxymethyltriphenyl-phosphonium bromide. Collins oxidation of the free alcohol gave the enol ether **91**. Hydrolysis of the enol ether **91** followed by an intramolecular aldol condensation using 1.5% potassium hydroxide in methanol gave the intramolecular aldol product **93**. Through a 6 step sequence this aldol product **93** was converted to the enone **86**. Protection of the alcohol in **93** with tetrahydropyran followed by reduction with sodium borohydride ethanol at 0 °C liberated the alcohol which converted to mesylate group with methanesulfonyl chloride. Subsequent deprotection with *p*-toluenesulfonic acid in methanol gave the hydroazulenol that was oxidized using Jones reagent followed by treatment with DBU in benzene to give the hydroazulenone **86** in 60% yield over 6 steps.



90

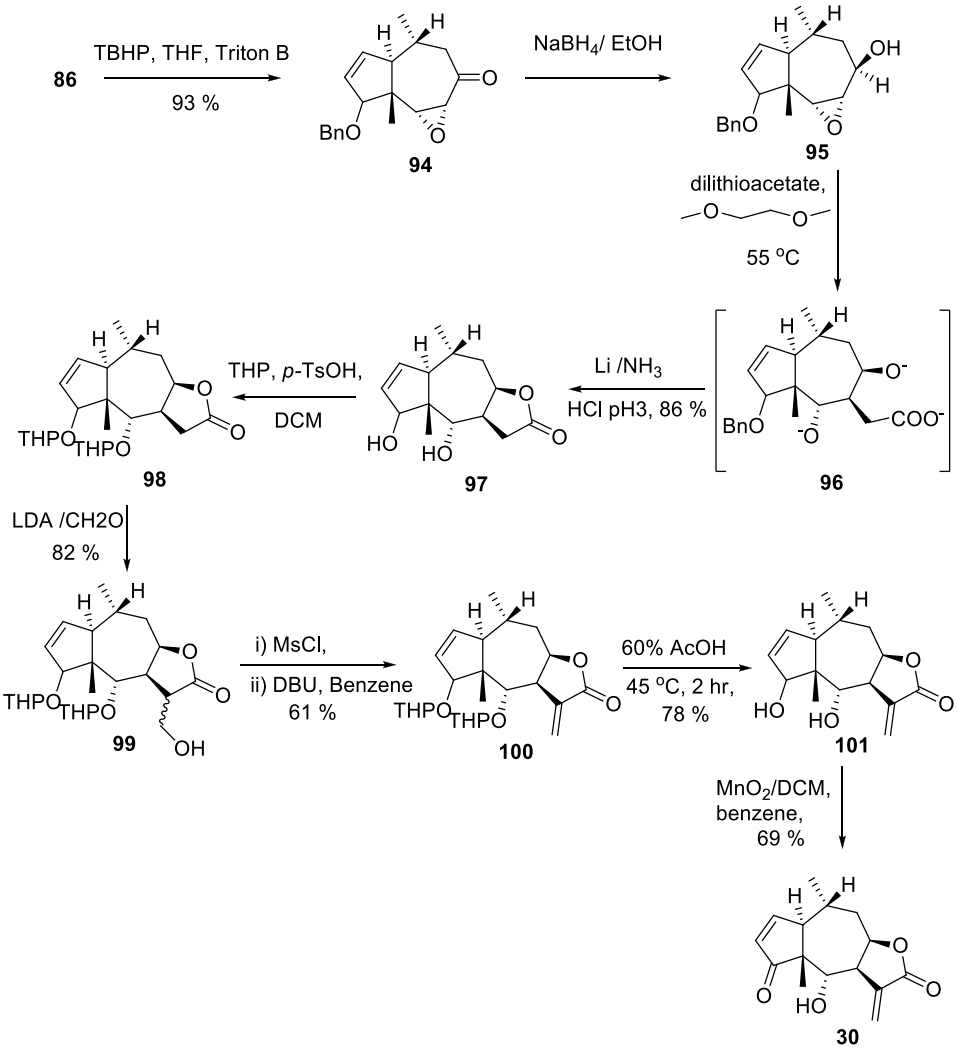
Figure 1.16



Scheme 1.15: Synthesis of hydroazulenone **86**.[54]

Epoxidation of enone in the hydroazulenone **86** using *tert*-butyl hydroperoxide and Triton B generated the epoxide **94** in 93% yield as a single stereoisomer (Scheme 1.16). The bulky alkyl hydroperoxide obstructed the approach from hindered side of the methyl group at C5 and generated the *β*-face epoxide. Reduction of the ketone **94** using sodium borohydride in ethanol at 0 °C gave the epoxy alcohol **95**. Treatment of the epoxide alcohol **95** with 14.7 equivalents of dilithioacetate in dimethyl glycol at 55°C yielded the trianion **96**. The benzyl group was deprotected with lithium in liquid ammonia and acidified to give the hydroxyl lactone **97** in 86% yield. The diol in **97** was protected with tetrahydropyranyl group to give **98** in 82% yield. Hydroxymethylation of the lactone **98** was accomplished using LDA and formaldehyde. The primary alcohol **99** was eliminated in a two step process using methanesulfonyl chloride and DBU to produce the γ-butenolide **100** in 61% yield. Hydrolysis of the protecting groups under acid conditions using 60% acetic acid at 45

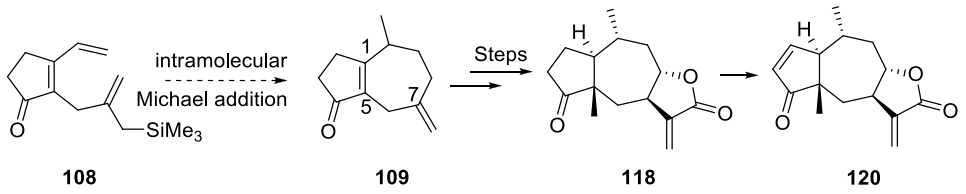
°C gave the diol lactone **101** in 78% yield. Finally, oxidation of diol lactone **101** with manganese dioxide in ethanol and benzene (1:2) gave the *dl*-helenalin **30** in 69% yield.



Scheme 1.16: Synthesis of helenalin **30**.[54]

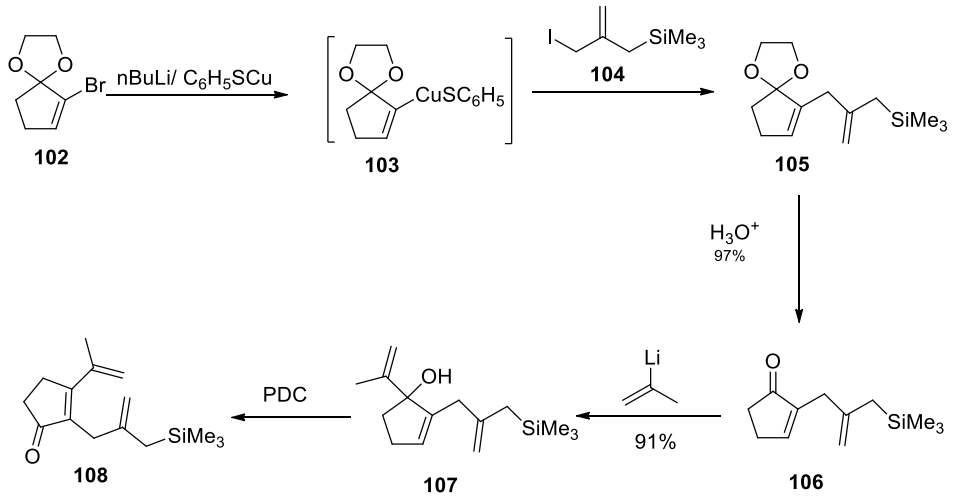
1.4.4 Total syntheses of (±)-Graveolide and (±)-Aromaticin

Majetich *et al.,* have demonstrated an elegant approach to the azulenic framework of graveolide and aromaticin using allylsilanes.[62] The allylsilanes such as **108** are used in both inter- and intramolecular Michael-like addition reactions and are good precursors to synthesis of the azulenic framework of graveolide and aromaticin (Scheme 1.17).



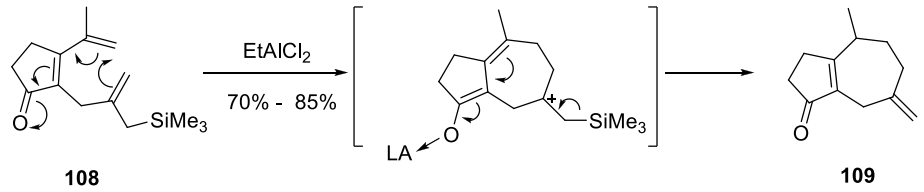
Scheme 1.17: Proposed synthesis of graveolide **118** and aromaticin **120**.[62]

The allylsilane **108** was made in 4 steps (Scheme 1.18) from the ketal **102** in an overall yield of 52%. A lithium-bromide exchange with butyllithium followed by transmetallation of ketal **102** with copper thiophenolate gave **103** *in situ*. The cuprate **103** underwent a substitution reaction with the iodide **104** to give the 1,4-diene **105** in 85% yield. Hydrolysis of **105** with acid liberated ketone **106**. Addition of 2-propenyllithum to ketone **106** gave the allylic alcohol **107**. Oxidation of the alcohol **107** with pyridinium dichromate gave the conjugated **108** with transposition of the ketone.



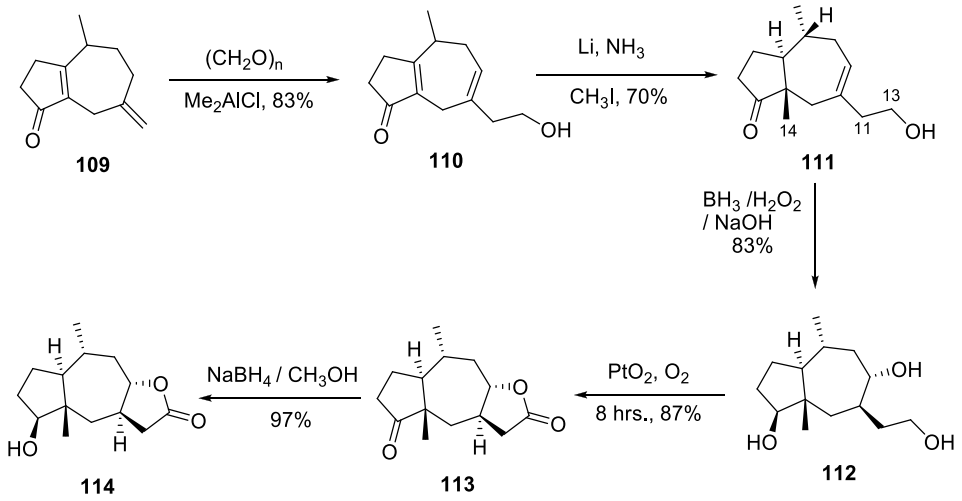
Scheme 1.18: Synthesis of allylsilane **108**.[62]

The key cyclisation step was performed with two or more equivalents of ethylaluminium dichloride. The reaction initially promoted by coordination of $EtAlCl_2$ on to the ketone. This produces a cyclisation reminiscent of an extended Michael addition. The newly generated carbocation is stabilised by the silicon atom (*β*-silicon effect). Elimination of silicon gives the exocyclic alkene **109** in 70% yield (Scheme 1.19).



Scheme 1.19: Cyclisation of **108**.[62]

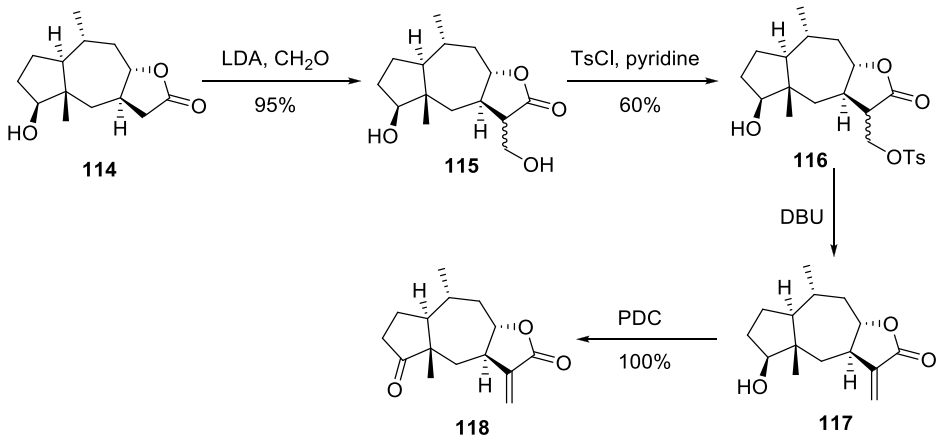
An electrophilic addition of formaldehyde to the exocyclic alkene **109** promoted by Me_2AlCl, gave **110** with transposition of the alkene into the ring. The bridgehead methyl group was introduced by reductive alkylation under Birch conditions followed by addition of iodomethane to give ketone **111**. The hydrogen at C1 is *anti* with respect to the hydrogen at C10 and is also *anti* with methyl group at C5. The methyl group at C10 in equatorial position and this simplified addition of the hydrogen at C1 from the bottom face. An anti-Markovnikov hydration of the remaining alkene in **111** afforded a hydroxyl group at C8 and reduces the ketone at C4 to give the diol **112** in 83% yield. Oxidation of **112** was performed with equal weight of PtO_2 in dilute acetone, and the oxygen was then bubbled through the reaction at 55°C producing the ketone lactone **113** in 87% yield. Reduction of **113** at C5 using borohydride afforded the hydroxylactone **114** in 97% yield.



Scheme 1.20: Synthesis of hydroxylactone **114**.[62]

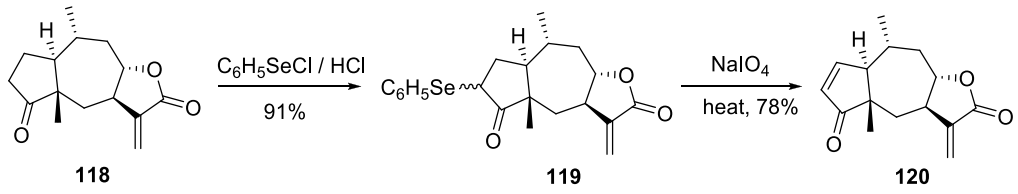
The α-methylene-δ-lactone **118** was made in 4 steps in 57% overall yield as shown in (Scheme 1.21). Treatment of hydroxyl lactone **114** with LDA and formaldehyde gave the primary alcohol **115** which was converted to tosylate **116** using toluenesulfonyl

chloride and eliminated with DBU to form α-methylene-δ-lactone **117**. Oxidation of cyclopentanol **117** using PDC gave the (±)-graveolide **118** in 100% yield.



Scheme 1.21: Synthesis of graveolide **118**.[62]

Finally, aromaticin was made from graveolide **118** in two steps through selenide elimination, (Scheme 1.22). Treatment of graveolide **118** with benzeneselenyl chloride in presence of anhydrous hydrogen chloride afforded the selenide **119**. Elimination of the selenide **120** using sodium periodate produced racemic aromaticin **120** in 78% yield.



Scheme 1.22: Synthesis of aromaticin **120**.[62]

1. 5 **Chemistry of isolated costic acid and its related compounds**

The chemistry of costic acid and related compounds isolated from natural sources has been reported. The chemistry of these compounds has been investigated for either structural elucidation or to prepare related compounds. Many isomers of costic acid

have been isolated, α-costic acid **50** (isocostic acid), *β*-costic acid **26**, and γ-costic acid **121** (Figure 1.17).

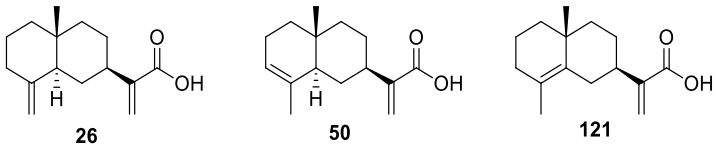
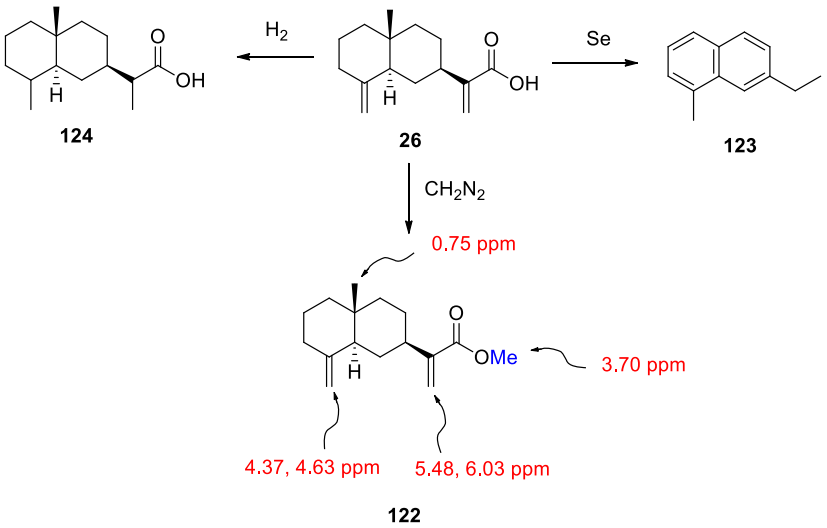


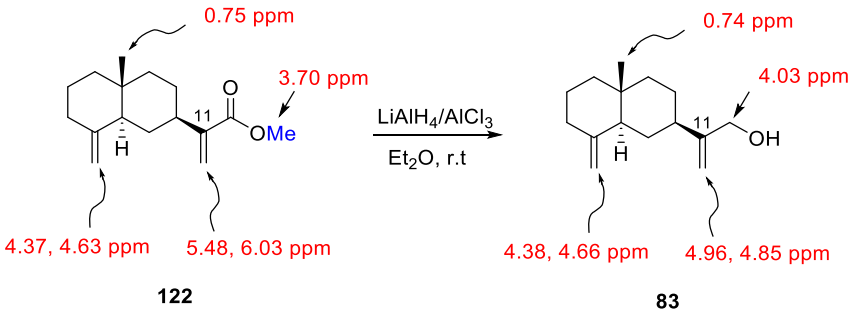
Figure 1.17: Costic acid isomers

Bawdekar and Kelkar have isolated *β*-costic acid **26** from the *costus* root oil in 1965.[63] At this stage NMR techniques were not powerful enough for elucidation of the structure. The acid was subjected to degradation reactions to elucidate the absolute structure (Scheme 1.23). The acid with a molecular formula of $C_{15}H_{22}O_2$ under catalytic hydrogenation showed the presence of two double bonds (Compound **124)**, and evidence of a bicyclic structure. Esterification of **26** produced **122** which also confirmed the *α,β*-unsaturated carboxyl group. The acid was dehydrogenated with selenium to form the naphthalene **123** in 30% yield. This compound contains 13 of the 15 expected carbon atoms of the acid. The loss of 2 carbons can be rationalized by the elimination of the bridge-head methyl group during the dehydrogenation and decarboxylation of the carboxylic acid. Based on the evidence it was postulated that the carboxylic acid group was part of the isopropyl sidechain.



Scheme 1.23: Degradation reactions of costic acid **26**.[63]

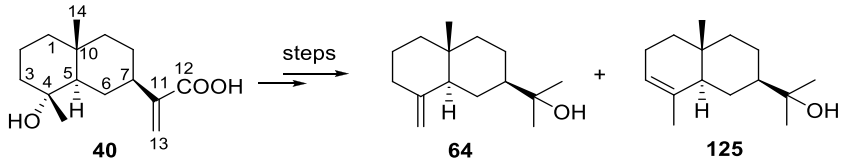
Reduction of the methyl ester of costic acid **122** with lithium aluminium hydride with aluminium chloride gave the allylic alcohol **83** (Scheme 1.24). From the ^{1}H NMR spectrum it was found that the new multiplet signal at 4.03 ppm related to the methylene of the primary alcohol (-CH_2OH), which was shielded by the alkene at C11. Alcohol **83** was identical to the natural product alcohol that was previously isolated and this completely proves the structure of the costic acid. It is interesting that no conjugate reduction at the alkene was observed in this reaction



122 **83**

Scheme 1.24: Reduction of costic acid methyl ester **122** to an allylic alcohol **83**.

Ilicic acid **40** a hydrated derivative of costic acid has been isolated from the aerial parts of *Inula viscosa* and its chemistry was investigated.[64] Transformation of ilicic acid to the bioactive compounds *β*-eudesmol **65** and α-eudesmol **125**, natural products which have anti-angiogenic and anti-Alzheimer properties. *β*-Eudesmol **65** was synthesised in six steps and α-eudesmol **125** seven steps.

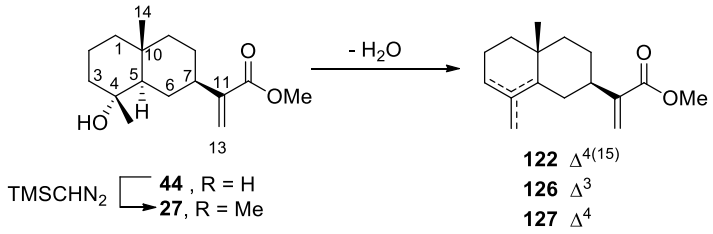


40 **64** **125**

Scheme 1.25: Proposed synthesis of Eudesmol.[64]

Esterification of ilicic acid **40** with trimethylsilyldiazomethane afforded the methyl ester **27** (Scheme 1.26). Elimination of the tertiary hydroxyl group at C4 under different conditions produced varying mixture of isomers from costic acid methyl ester (Table 1.7). *β*-Costic acid methyl ester **122** with an exocyclic double bond (C4- C15)

was produced in 86% yield by using $POCl_3$ in pyridine (Table 1.7, entry 1**)**. Whereas the α-costic acid methyl ester **126** with double bond (C3-C4) was formed in 35% yield by using *ρ*-TsOH in benzene (Table 1.7, entry 5). The *γ*-costic acid methyl ester **127** with a double bond (C4-C6) was made in 81% yield by using iodine in benzene at room temperature (Entry 7)



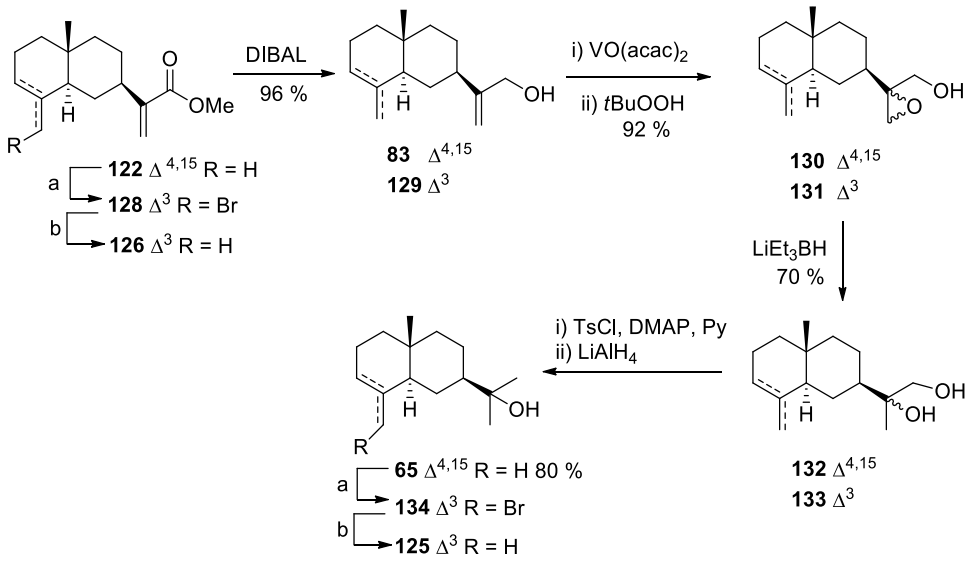
Scheme 1.26: Esterification and elimination reactions of ilicic acid **40**

Table 1.7: Conditions for dehydration of ilicic acid and the yield of products.[64]

Entry	Condition	**122**	**126**	**127**	Start material
1	$POCl_3$, pyridine, -20°C	86%	2%	2%	-
2	$POCl_3$, pyridine, 100°C	57%	17%	16%	-
3	I_2, Ph_3P, r.t.	10%	-	85%	-
4	I_2, Ph_3P, 0°C	27%	-	63%	-
5	TsOH, benzene, 80°C	15%	35%	43%	-
6	TsOH, benzene, r.t.	7%	3%	5%	73%
7	I_2, benzene, r.t.	-	-	81%	-
8	$SOCl_2$, pyridine, -40°C	48%	23%	24%	-

Reduction of *β*-costic acid methyl ester **122** or/and α-costic acid methyl ester **127** with DIBAL in toluene gave the allylic alcohol **83** in 96% yield and **129**. Epoxidation of allylic alcohol **83** with *t*-BuOOH/$VO(acac)_2$ in benzene gave a 1 : 1 mixture of epoxide epimers **130** and **131** in 92% yield. The epoxidation proportion was increased to 6 : 1 under asymmetric Sharpless epoxidation conditions using diethyl L-(-)-tartrate as a chiral auxiliary. Reduction of the mixture **130** and **131** with $LiEt_3BH$ in THF gave a mixture of diol epimers **132** and **133** in 70% yield. The primary alcohol in epimers **132**

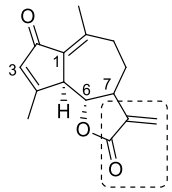
was selectively tosylated and subsequent reduction with lithium aluminiumhydride gave *β*-eudesmol **65**. α-Eudesmol **125** was synthesised with same reaction procedure from α-costic acid methyl ester **126**. However, the dehydration of ilicic acid ester produced a mixture of alkenes, with the desired alkene **126** obtained in low yields. Isomerisation of the alkene in **122** to **126** was achieved by bromination and immediate reduction of the intermediate bromide **128** to give alkene **126** in 10% yield. The process described above was then used to convert **126** to **125** via the intermediates **129**, **131**, **133** and **134**.



reaction and conditions: a) $Br_2/FeCl_3$; b) Bu_3SnH/ AIBN, 10%

Scheme 1.27: Synthesis of *β* and *α*-eudesmol **65** and **125**.[64]

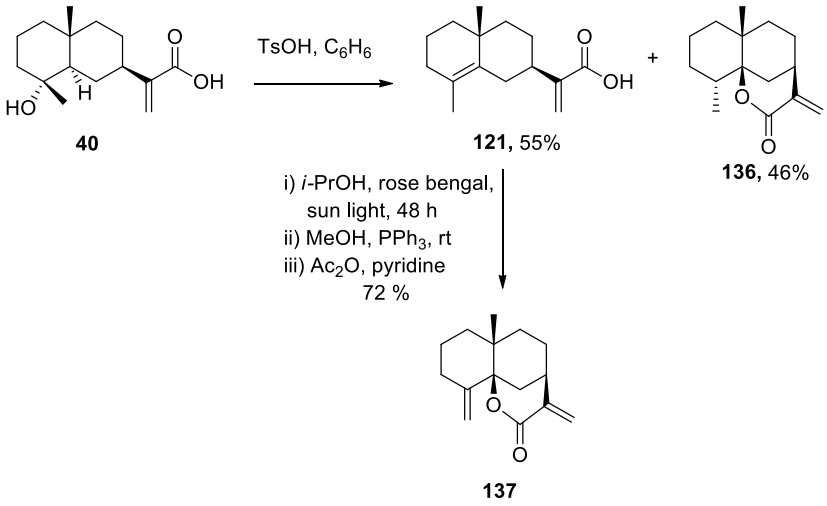
In a previous study, dehydroleucodine **135** (Figure 1.18) was isolated from *Artemisia douglasiana* Besser, and exhibited inhibitory activity against formation of gastric lesions produced in male Wistar rats by necrotizing agents.[65] The *α,β*-unsaturated carbonyl group at C7 in the lactone **135** was reported as responsible for this activity which can react with SH-containing compunds of the mucosa to form stable adducts by Michael addition reaction.



135

Figure 1.18: Dehydroleucodine

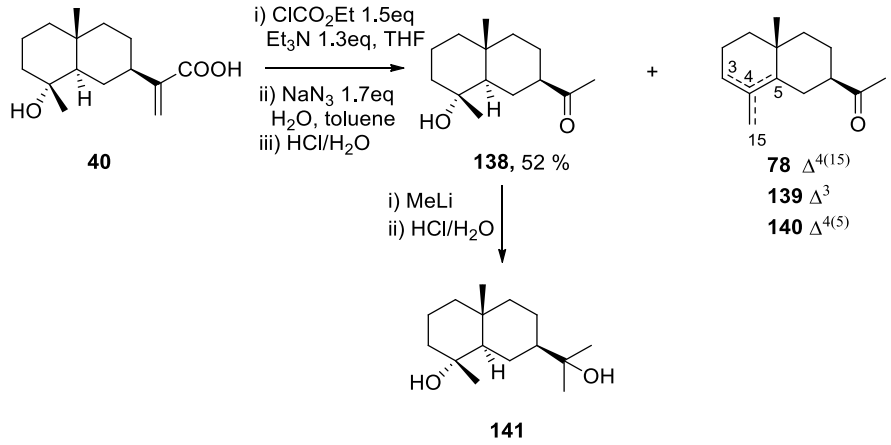
Donadel *et al*., isolated ilicic acid **40** from aerial parts of *Flourensia oolepis,*[66] and used it as starting material to produce new compounds with cytoprotective activity. Dehydration of ilicic acid **40** with *p*-TsOH in benzene gave *γ*-costic acid **121** and lactone **136**. The lactone **136** arises from an acid catalysed addition to the alkene of *γ*-costic acid **121**. Oxidation of costic acid **121** with singlet oxygen generated the hydroperoxide and exocyclic double bond. The hydroperoxide was then reduced using triphenylphosphine to give hydroxyl group which was treated with acetic anhydride to give the lactone **137** in 72% yield (Scheme 1.28).



Scheme 1.28: Synthesis of lactone **137**.[66]

Tebbaa *et al*., isolated ilicic acid **40** and α-costic acid **50** from *Dittrichia viscosa* L.[67] They synthesised cryptomeridiol **141** which is the active principle in Proximol®, a renal antispasmodic starting from the ilicic acid **40**.[67] They started from *α*-costic acid (isocostic acid) **50** for the synthesis of *α*-eudesmol **125**. The key reaction in the synthesis was a decarboxylation of the carboxylic acid using a Curtius rearrangement. Acylation of ilicic acid **40** with $ClCO_2Et$ and Et_3N in THF at – 10 °C formed an acyl

chloride which was treated with NaN_3 to produce an acyl azide. Curtius rearrangement of the azide gave the intermediate isocyanate, which was hydrolysed with 10% hydrochloric acid solution to give the ketone **138** in 52% yield and the by products **72**, **139**, and **140** in 45% yield. The ketone **138** was treated with methyllithium in ether to afford cryptomeridiol **141** (Scheme 1.29). The synthesis of *α*-eudesmol **125** from α-costic acid **50** was subjected to the same procedure in Scheme 1.29. In this experiment the ketone **139** was synthesised in 70% yield. Then the ketone **138** treated with MeLi in ether gave the α-eudesmol **125** in 70% yield (Scheme 1.30).



Scheme 1.29: Synthesis of cryptomeridiol **141**.[67]

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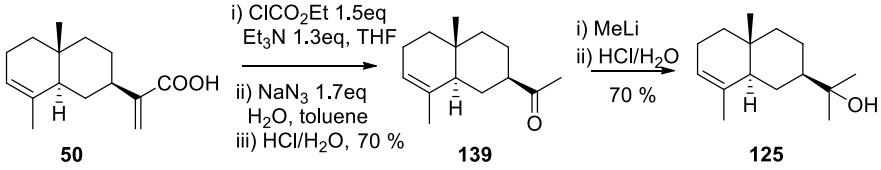
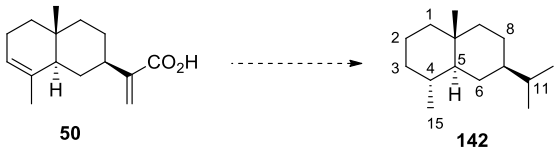


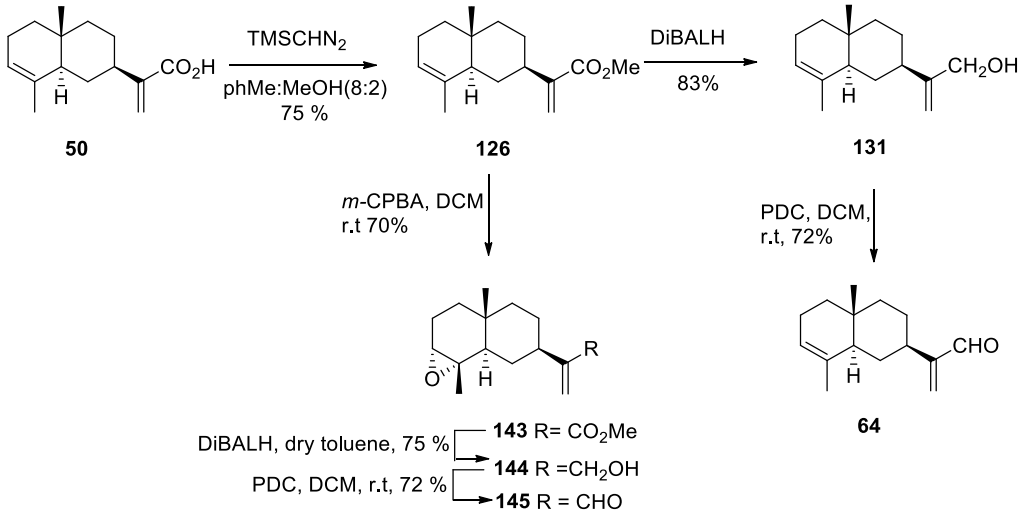
Scheme 1.30: Synthesis of α-eudesmol **125**.[67]

Zaki *et al.* isolated the main components of *Dittrichia viscosa*.[68] Many new eudesmane derivatives have been synthesised by functional group manipulation of α-costic acid **50**.



Scheme 1.31: Proposed tranformation of α-costic acid (isocostic acid)

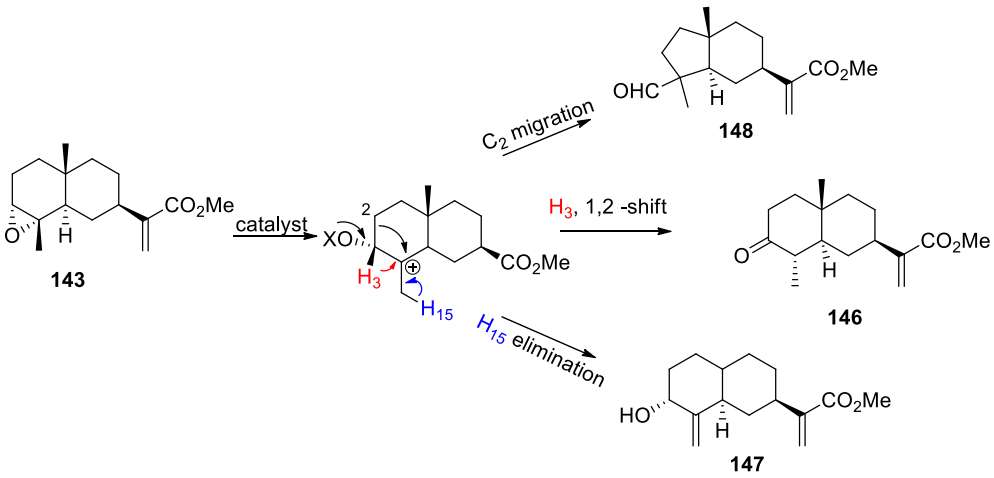
Esterification of α-costic acid **50** with trimethylsilyldiazomethane gave the methyl ester **126** in 75% yield. Reduction of ester **126** with diisobutylaluminium hydride afforded the allylic alcohol **129** in 83% yield. Oxidation of the alcohol **129** with pyridinium dichromate gave the aldehyde **64** in 72% yield. Whereas the epoxidation of ester **126**, using *m*CPBA produced the epoxide **143** in 70% yield. Reduction of the epoxide ester **143** with diisobutylaluminium hydride in anhydrous toluene gave allylic alcohol **144**, followed by oxidation using pyridinium dichromate in dichloromethane to give the aldehyde **145** in 72% yield.



Scheme 1.32: Synthesis of aldehyde **64** and epoxy ester **143**.[68]

The activity of epoxide **143** was investigated with a variety of Lewis and Brönsted acids at room temperature (Scheme 1.33). The compounds **146**, **147**, and **148** were synthesised in different yields as shown in Table 1.8. These transformations were explained as shown in Scheme 1.33. Cleavage of the epoxide in **143** forms the carbocation on C4. Then a 1,2-shift of the hydrogen from C3 delivers the ketone **146**. The formation of the alcohol **147** was due to the H_{15}-elimination from the carbocation. Ring resizing due to C2 migration formed the aldehyde **148**.

Examination of the epoxide **143** under Lewis and Brönsted acids produced three new compounds (Table 1.8). Treatment of **143** with a strong Lewis acid $BF_3.Et_2O$ gave selectively ketone **146** in 70% yield (Table 1.8, entry 1). The rearrangement of **143** using weak and moderate Lewis acids (Entry 2, 3, and 4) gave a mixture of two compounds. However, using very strong Brönsted acids like TfOH gave the ketone **146** in 63% (Entry 7). Use of moderate Brönsted acids PTSA and TFA, gave selectively alcohol **147** in 72% and 63% yield respectively (Entry 5, 6).

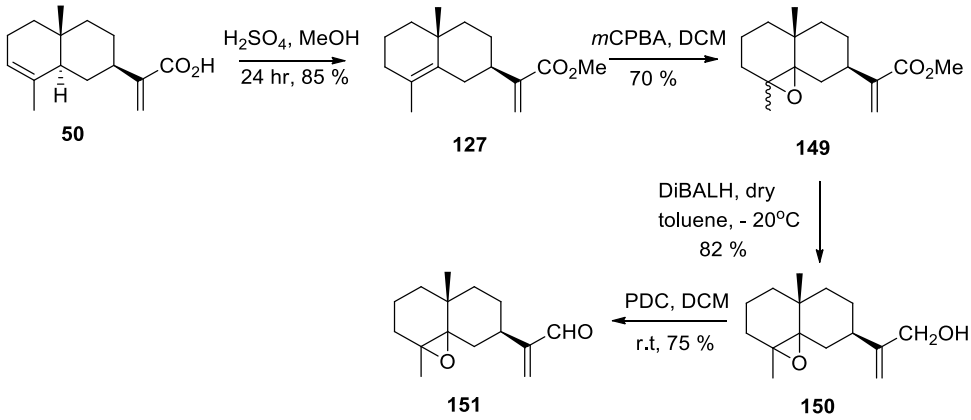


Scheme 1.33: Reaction of **143** with Lewis and Brönsted acids.[68]

Table 1.8: Reaction of **143** with different acids in DCM, for 30 min at room temperature.[68]

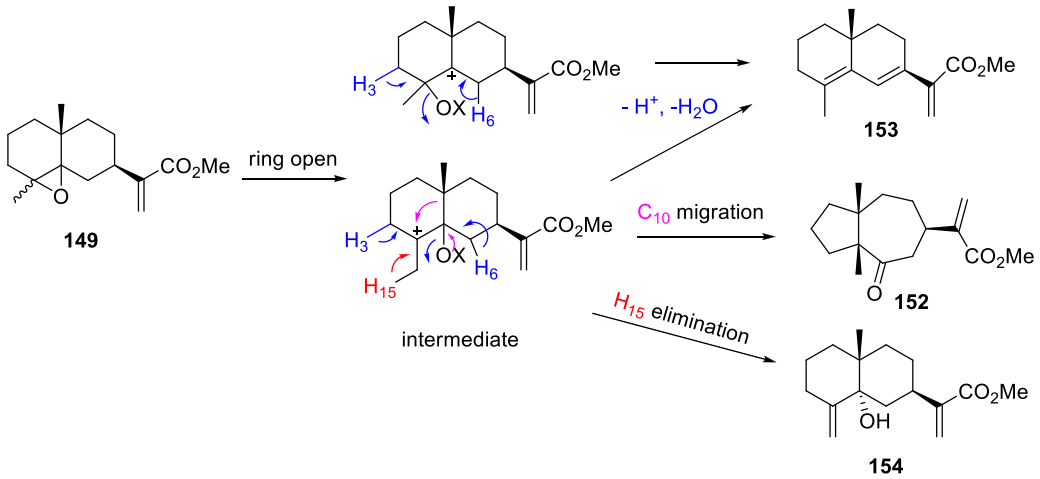
Entry	Catalyst	146	147	148
1	$BF_3.Et_2O$	70%	-	-
2	$InCl_3$	71%	-	3%
3	$ZnBr_2$	48%	38%	-
4	$Bi(OTf)_3$	-	56%	35%
5	PTSA	-	72%	-
6	TFA	-	52%	-
7	TfOH	63%	-	-

The epoxide of **149** was made in a 2 steps from α-costic acid **50** (Scheme 1.34). α-Costic acid **50** was treated with sulfuric acid in methanol to esterify the acid and isomerise the alkene to γ-costic acid methyl ester **127** in 85% yield. Epoxidation of γ-costic acid methyl ester **127** using *m*CPBA gave the epoxide **149** in 70% yield. The epoxide ester **149** was reduced with diisobutylaluminium to give allylic alcohol **150** in 82% yield. Subsequent oxidation of the alcohol **150** with pyridinium dichromate yielded the aldehyde **151** in 75% yield.



Scheme 1.34: Synthesis of epoxide.[68]

The chemistry of the epoxide **149** was investigated using similar acid catalysts given in Table 1.8. Treatment of epoxide **149** with a range of Brönsted and Lewis acids in dichloromethane at room temperature for 30 minutes gave three new products **152**, **153** and **154** in different yield (Table 1.9). Formation of the products was explained in Scheme 1.36. Cleavage of the epoxide **149** formed the stable carbocation intermediate at C4 and/or C5. A C10 migration in the intermediate cation at C4 formed a new C10-C4 bond and resized the ring to give the ketone **152**. H-6 elimination and dehydration yielded conjugated costic methyl ester **153**. Finally hydroxy costic acid methyl ester **154** was liberated elimination of H-15



Scheme 1.35: Chemistry of **149** with Lewis and Brönsted acids.[68]

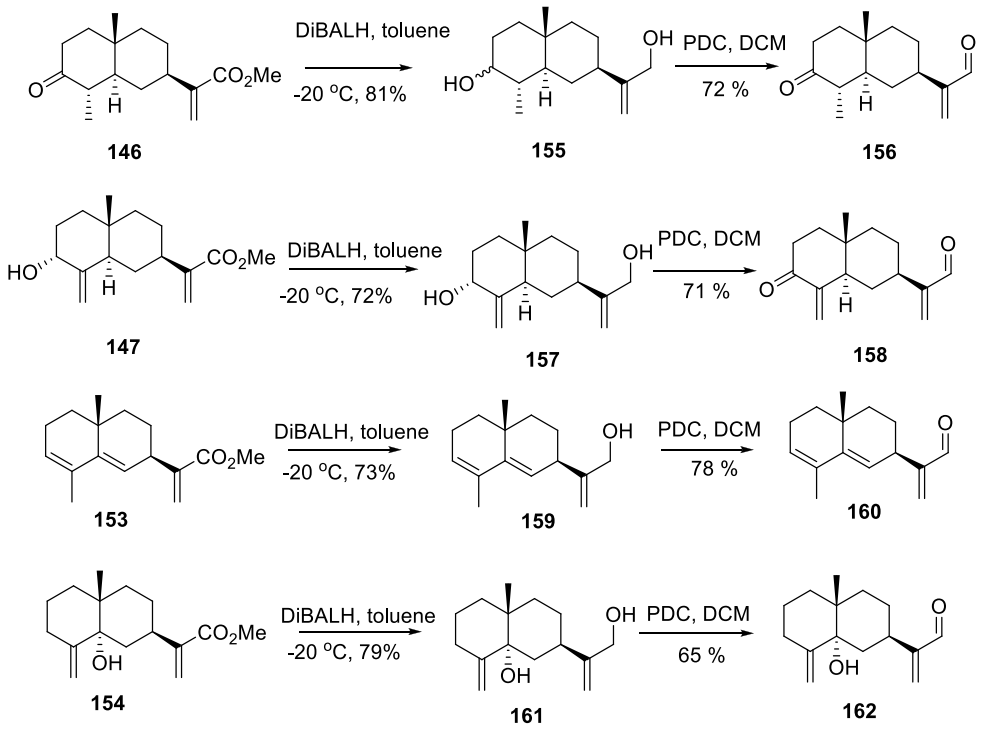
The best selectivity was observed when the strong Lewis acid, $BF_3.Et_2O$, was used as the catalyst to give the ketone **152** in 76% yield (Table 1.9, entry 1). Additionally using the moderate and weak Lewis acids gave mixtures of **152** and **153**. However, when the epoxide was treated with the Brönsted acids, PTSA and TFA, it gave the mixture of conjugated costic acid **153** and hydroxyl costic acid methyl ester **154** in 38, 60% and 36, 28% yield respectively. Whereas using the strong Brönsted acid like TfOH gave a mixture of ketone **152** and conjugated costic acid **153** in 46 and 43% yield respectively.

Table 1.9: Reaction of **150** with different acids in DCM, for 30 min at room temperature.[68]

Entry	Catalyst	152	153	154
1	$BF_3.Et_2O$	76%	-	-
2	$InCl_3$	47%	47%	-
3	$ZnBr_2$	25%	42%	-
4	$Bi(OTf)_3$	50%	48%	-
5	TfOH	46%	43%	-
6	PTSA	-	38%	60%
7	TFA	-	36%	28%

The above results show that α-costic acid **50** can be transformed into four new eudesmane structures **146**, **147**, **153**, and **154**. These compounds were reduced using

diisobutylaluminium hydride and oxidised with pyridine dichromate to make a library of the both alcohol and aldehyde analogues (Scheme 1.36).



Scheme 1.36: Synthesis of new eudesmanes.[68]

1. 6 ***Dittrichia graveolens***.

Dittrichia graveolens or stinkwort is a member of the Asteraceae family (sunflower family) (Figure 1.19).[69] Its scientific name was *Inula graveolens* until 1973 when the plant was reclassified by Greuter. It is commonly called stinkwort in Western Australia. *D. graveolens* is native to the Mediterranean basin.[11] Stinkwort has been introduced to many countries around the world including the United States and Australia. The plant is an annual, 20 – 50 cm tall, with a pleasant aromatic smell, is late-flowering and its fluffy seeds help it to spread so quickly along roads.

Figure 1.19: *Dittrichia graveolens*

1. 7 **Plant uses**

The fishermen in south Italy used *Dittrichia graveolens* to help them capture fresh fish.[6] The leaves of the plant are macerated in water and then steeped into the fishing place causing a definite sedative effect on the fish of the surrounding area. Furthermore the plant has been used externally to treat chickens from lice.[70] Many problems have been associated with this weed. The inhibition of seed germination of other plants has been observed. It has been reported that the seeds of *D. graveolens* caused pyogranulomatous enteritis in sheep.[3] In addition, Thong *et al.,* have reported that *D. graveolens* causes contact dermatitis in some people.[71]

1. 8 **Sesquiterpenes isolated from *Dittrichia graveolens***

1.8.1 Essential oil of *D. graveolens*

The composition of the essential oil of *Dittrichia graveolens* growing in Turkey, Greece, Italy, Tunisia, Morocco, France, Lebanon, Egypt, Algeria and Iran have been reported (Table 1.10). Many Monoterpenes and sesquiterpenes have been isolated from the *D. graveolens* (Figure 1.20). A study of *D. graveolens* in Greece found that crude oil 0.68% was isolated from the aerial parts contained bornyl acetate **165** (25.4%), borneol **164** (12.8%) and epi-α-cadinol (T-cadinol) **163** (30.2%) (Table 1.10). Another study on the compounds in the aerial parts of *D. graveolens* was performed in Iran and three major main compounds have been isolated which are borneol **164** (60.7%), β-caryophyllene **170** (8.3%) and bornyl acetate **165 (**6.8%).[72]

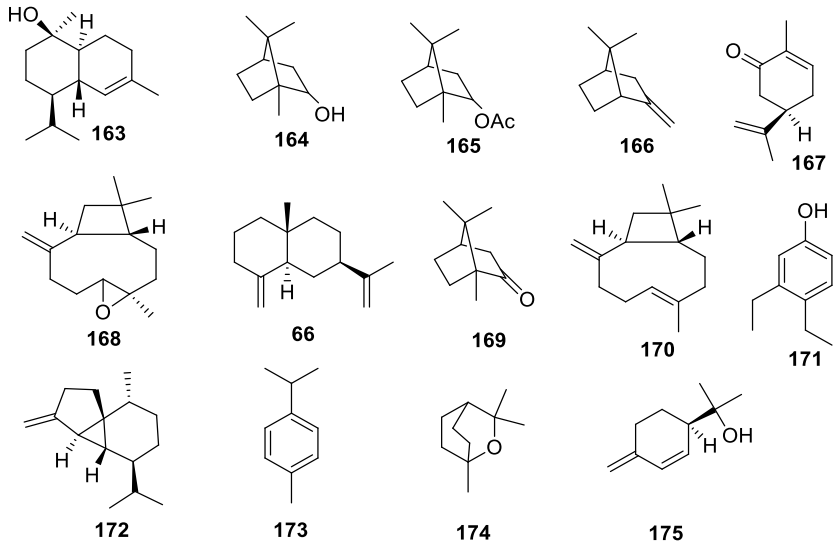


Figure 1.20: Essential oil major products of *D. graveolens*

Table 1.10: Major components of essential oil of *Dittrichia graveolens* from different location

Location	Plant parts	Extraction	Major components
Monastir, Tunisia[73]	Aerial parts without flowers	Distillation	**163** (9.2%), **164** (21.4%), **165** (33.4%)
	Flowers		**166** (5.5%), **163**(11.3%), **164** (5%), **165** (39.6%)
	Oil roots		**167** (5%), **165** (5.3%), **175** (5.3%), **66** (11.5%)
Morocco[74]	NA		**165** (63.9%), **164** (25.6%), **166** (5%), **169** (3.6%)
Attiki, Greece[75]	Aerial parts	Hydrodistillation	**163** (30.2%), **165** (25.4%)
Tahran, Iran[72]	Aerial parts	Distillation	**164** (60.7%), **170** (8.3%), **165** (6.8%), **168** (4.3).
Kashan, Iran	Aerial parts	Hydrodistillation	**164** (38.2%), **165** (14.86%), **168** (4.0%), **171** (7.5%), **172** (7.1%),
Shush, Iran[76]	Aerial parts	Hydrodistillation	**174** (54.89%), **173** (16.2%), **166** (6.94%), **164** (5.44%)
Corsica, France[77]	Aerial parts	Vapour distillation	**163** (23.8), **164** (3.7-32.2%), **165** (16.6-73.1%)
Bekaa, Lebanese[69]	Aerial parts		**163** (1.4-13.4%), **164** (2.7-12.4%), **165** (70.6-72.3%), **168** (1.9-2.3%).
Bingol Turkey[78]	Aerial parts	Microextraction	**164** (20.4%), **165** (21.3%), **173** (16.6%)

1.8.2 Non-volatile natural products isolated from *D. graveolens.*

Many natural products have been isolated from the *D. graveolens*. Oksun and Topcu studied the natural product of *D. graveolens* growing in Turkey, and they isolated 27 components (Figure 1.21).[4] They isolated the sesquiterpene lactones **176-181** and sesquiterpene acids **26**, **40**, **50**, **182**, **183**, and **184** as the main components and the major compound was costic acid **40,** and lactone **176** was isolated as a new compound.[4] In addition, they isolated flavonols **186** in a large amount, while the other flavonols **192** and **193**, flavonoids **185, 194-197** and the aromatic compounds **198** and **199** were isolated in small quantity. Similar sesquiterpene lactones and acids have been isolated by Abou-Douha, from the *D. graveolens* growing in Egypt (Figure 1.22).[5] Sesquiterpene ester **200** and sesquiterpene acid **202** were isolated as new compounds.

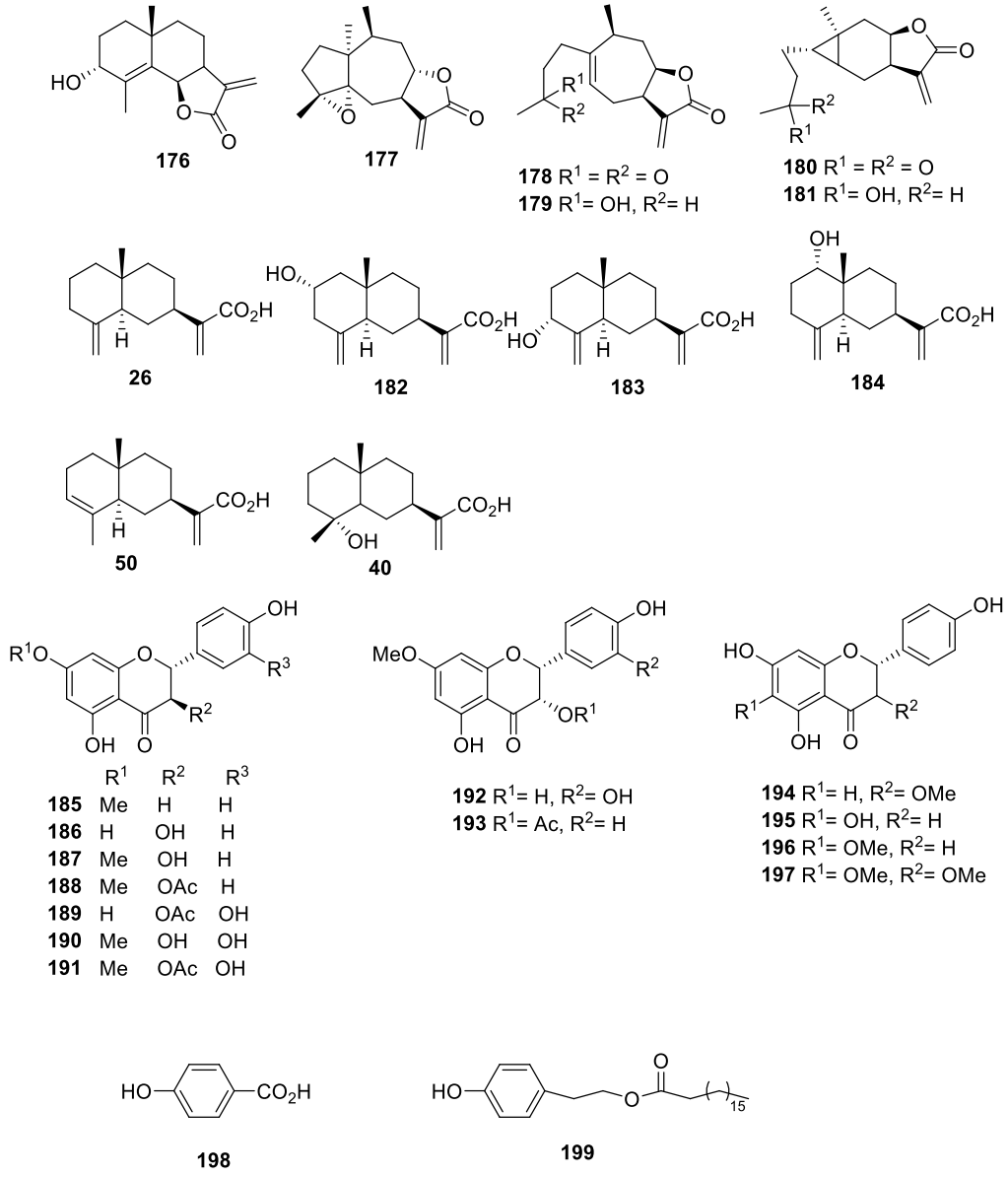


Figure 1.21: Non-volatile compounds isolated from *D. graveolens* growing in Turkey

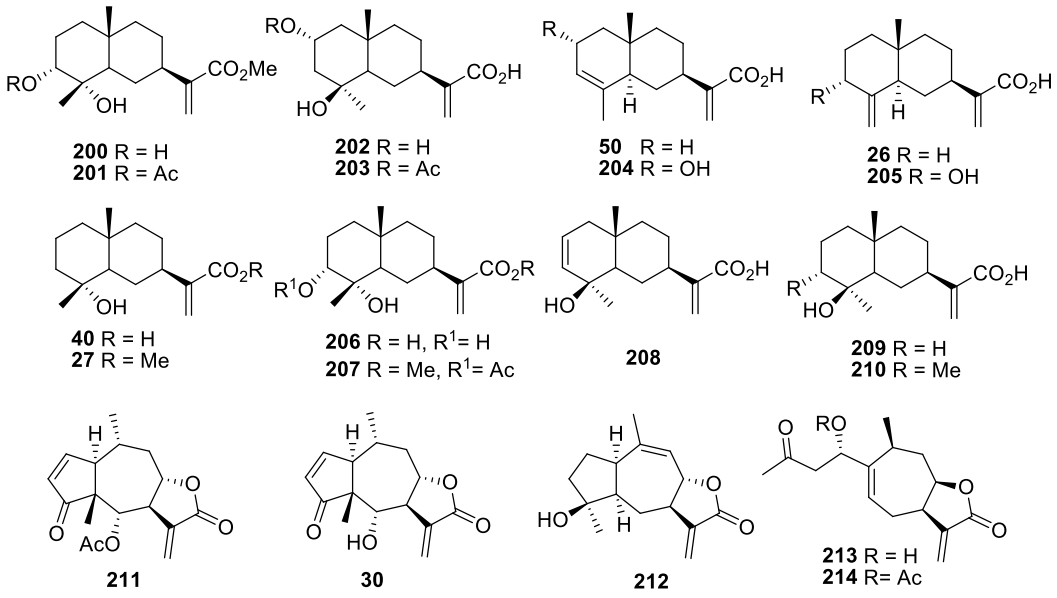


Figure 1.22: Non-volatile compounds isolated from *D. graveolens* growing in Egypt

1. 9 **Project Aims**

The progression of the project is illustrated in Figure 1.23. First, isolation and identification of the main components of aerial parts of *Dittrichia graveolens* growing in Western Australia. Determine the seed germination inhibition of the isolated components. Then investigate the chemistry of the isolated natural products to develop new feedstocks for the pharmaceutical industry. The synthesis of new natural products from the isolated sesquiterpenes will be undertaken, followed by a compound which could mimic lovastatin. Lovastatin is cholesterol reducing drug which acts on the 3-hydroxyl-3-methylglutaryl Co A (HMG–Co A) reductase.[79]

D. graveolens

Extraction

Crude Extract ⇒ Biological Activity

Elucidation

Natural Products ⇒ Biological Activity

Chemistry of the Natural Products

Semisynthesis

Figure 1.23: The key goals of the study

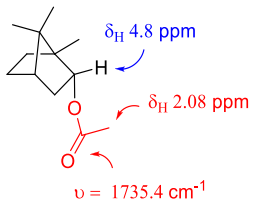
Chapter 2

2. 1 **Isolation of compounds from *Dittrichia graveolens***

D. graveolens (L.) W. Greuter (Figure 2.1) was collected from roads and pastures around Balingup Western Australia over the period of 2012-2015. The plant has copious amounts of sticky resin at the end of summer and was harvested in February/March. The aerial parts were dried in a well ventilated area in the shade. The dried plant was then stored in cardboard boxes until use. The dried aerial parts of the plant were roughly chopped and steeped in ethanol for only 30 minutes at room temperature to extract the resinous coating. Ethanol was chosen as a solvent as it was polar enough to extract most compounds, but fats/fatty acids are generally not extracted making isolation simpler. The resulting ethanol extract was gravity filtered to remove solid particulates. Removal of the solvent gave a brown oil which accounted for 5.2% of the total weight of the dry plant. The ^{1}H NMR spectrum of the crude resin indicated that mixture contained multiple components. A portion of the crude extract was subjected to flash chromatography and five main components were isolated. Each compound was isolated in about 1% w/w of the dried plant. Over the period of this study (2012 - 2015) the components and quantity of these 5 compounds did not vary.

Figure 2.1

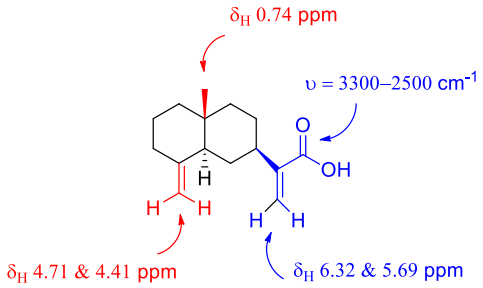
The first compound **165** was isolated as a colourless oil with strong camphor-like smell reminiscent of the smell of the plant. ^{13}C NMR spectroscopy indicated that the compound had 12 carbons. A peak at δ 171.5 ppm suggested a carbonyl group of acid or ester. The IR spectrum of **165** showed an absorbance at 1735 cm^{-1}, and no absorbance in the OH region to confirm that the carbonyl group was an ester. In addition, the ^{1}H NMR spectrum showed a singlet at δ 2.08 ppm, which integrated to 3 hydrogens that suggested the presence of a methyl next to a carbonyl group. The ^{1}H NMR spectrum also displayed three singlets at δ 0.80, 0.75 and 0.70 ppm integrating for 3 hydrogens and indicated the presence of three isolated methyl groups. The multiplet signal at δ 4.80-4.90 ppm suggested a proton attached to the carbon that is attached to the acetate group. Based upon the data, the compound is a bicyclic monoterpene with an acetate group similar to bornyl acetate. The compound **165** was confirmed as bornyl acetate as it matched ^{1}H NMR and ^{13}C NMR that was reported.[80] The specific rotation was $[\alpha]_D^{23}$ -16 (c = 2 , MeOH). The reported specific rotation of compound **165** isolated from different plants ranges from +44.23 to -40.0˚, and it differed from the measured –16 ˚ which was suggested isolated compound **165** was a mixture of enantiomers. Enantiomeric mixtures of (±)-borneol/ (±)-bornyl acetate have been observed in plant essential oils.[81]



165

Figure 2.2: Bornyl acetate **165** isolated from *D. graveolens*

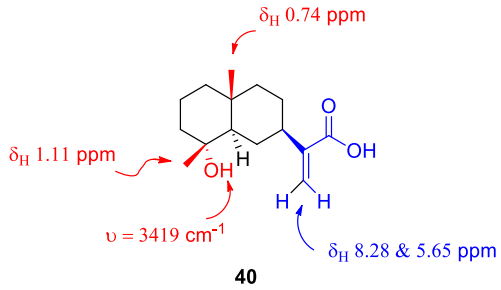
The second compound **26** was isolated as colourless needles. The compound had 15 carbons in the ^{13}C NMR spectrum. The presence of the peak at 172.3 ppm suggested a carbonyl group which was confirmed by the IR spectrum as a carboxylic acid due to absorbance at 1735 cm^{-1} and broad peak of OH stretching at 2800 cm^{-1}. The ^{1}H NMR spectrum showed two singlets at δ 6.31 ppm and 5.68 ppm which indicated the presence of a geminally substituted double bond conjugated to the carboxylic acid. A second set of two singlets at δ 4.71 and 4.41 ppm with integration one hydrogen each suggested the compound contain an exocyclic double bond in its structure. A singlet at δ 0.76 ppm integrating for 3 hydrogens suggested a methyl group connected to quaternary carbon. The ^{13}C NMR spectrum showed four peaks at δ 105.5, 124.8, 145.1, and 150.6 ppm which confirmed the presence of the two double bonds. Based upon the data, the compound is a bicyclic sesquiterpene carboxylic acid. The spectra obtained were consistent with costic acid **26**. Garcez *et al.,* isolated costic acid from *Nectandra cissiflora*[82] and their ^{1}H NMR spectrum data was comparable with compound **26**. Costic acid is commonly found in many types of Asteraceae, Compositae and other plant families. The ^{1}H and ^{13}C NMR spectra of compound **26** matched to costic acid that was isolated previously from Compositae and other plant families. [14, 63, 82, 4] The specific rotation was $[\alpha]_D^{23}$ +22.00 (c = 1, $CDCl_3$) which was similar to the reported specific rotation of isolated costic acid + 23.42°.[63]



26

Figure 2.3: Costic acid **26** isolated from *D. graveolens*

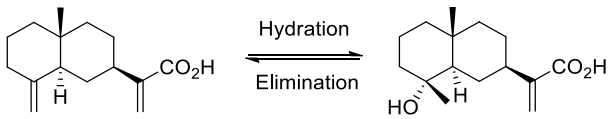
The third compound **40** was isolated as a colourless solid. Like costic acid the ^{13}C NMR spectrum showed that the compound also had 15 carbon atoms. A signal at 172 ppm in ^{13}C NMR spectrum suggested the presence of carbonyl group, which was confirmed by an IR to be due to a carboxylic acid with a peak at 1693.7 cm^{-1} and a broad absorbance at 2800 cm^{-1}. In addition, the IR spectrum showed a characteristic absorbance at 3419 cm^{-1} which indicated the presence of hydroxyl group. The hydroxyl group most likely is a tertiary hydroxyl group since there were no signals present at δ 3.4 - 4.0 ppm in ^{1}H NMR spectrum. The ^{1}H NMR spectrum showed two singlets at 6.28 and 5.65 ppm which indicated a 1,1-disubstituted double bond conjugated to a carboxylic group. The ^{13}C NMR spectrum confirmed the presence of the double bond by two peaks at δ 145.2 and 124.2 ppm. A singlet at δ 0.90 ppm in ^{1}H NMR spectrum corresponded to a CH_3 group attached to a quaternary carbon. A singlet at δ 1.11 ppm in ^{1}H NMR spectrum suggested a CH_3 at C4 connected to the C-OH. Based on these combined spectra, compound **40** was identified as the bicyclic sesquiterpene hydroxyacid, ilicic acid **40**. This compound was previously isolated from *Inula viscosa* and the ^{1}H and ^{13}C NMR spectra reported matched compound **40**.[83,4] The specific rotation was $[\alpha]_D^{23}$ –39 (c = 1, $CDCl_3$) which was close to the reported value for ilicic acid –40˚.



40

Figure 2.4: Ilicic acid **40** isolated *D. graveolens*

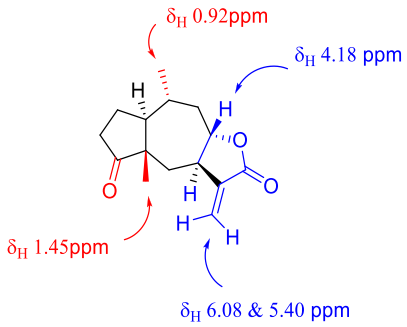
The structure of costic acid **26** and ilicic acid **40** are similar. The acids could be related to each other through the biosynthetic pathway (Scheme 2.1). Costic acid **26** could be hydrated by action of selective enzyme at C4 to form ilicic acid. On the other hand ilicic acid **40** could be eliminated to produce the costic acid **26**.



26 **40**

Scheme 2.1: Structure of costic and ilicic acid

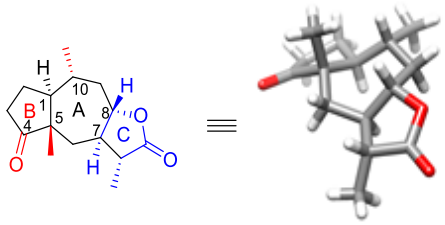
The fourth compound **215** was isolated as a colourless solid. The compound **215** also had 15 signals in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed two quaternary signals at 222.5 and 170 ppm suggested that the compound has two carbonyl groups. IR spectrum had the absorbance at 1764.6 and 1736.2 $cm^{-1}$and none at 3500 cm^{-1} which suggested the one carbonyl group was an ester and the other was a ketone. The ^{1}H NMR spectrum showed singlets at δ 6.08 and 5.40 ppm suggesting the presence of a double bond conjugated to carbonyl group. In addition, the compound was characterised by the presence of two singlet methyl signals at 1.45 and 0.92 ppm. Based upon the spectroscopic data, the compound is a sesquiterpene lactone. The spectroscopic spectra obtained were consistent with graveolide in literature.[62, 84,85,86]



215

Figure 2.5: α-methyl-δ-lactone (graveolide) **215** isolated from the aerial parts resin of *D. graveolens*

Finally, the last compound **216** was isolated as a colourless solid. The compound **216** also had 15 carbon signals in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed two quaternary signals at 222.5 and 177.0 ppm suggested that the compound has two carbonyl groups. IR spectrum had the absorbance at 1764.6 and 1736.2 cm^{-1} and none at 3500 cm^{-1} which suggested the one carbonyl group was an ester and the other was ketone. The ^{1}H NMR spectrum showed a multiplet signal at 4.20 ppm with integration of one hydrogen which is suggested the hydrogen shares the carbon with an oxygen atom. In addition, the compound was characterised by the presence of three singlet methyl signals at 1.22, 1.14 and 1.06 ppm. The structure appear to be too difficult to determine by NMR, so a single crystal suitable for x-ray analysis was obtained. Structural determination was made by Professor Brian Skelton at the University of Western Australia (Figure 2.5). The x-ray crystallography indicated the compound contain three rings (cycolpentanone A, cycloheptane B, and γ-lactone C) The cyclopentanone ring fused on C1-C5 with cycloheptane and the γ-lactone fused on C7-C8. The ring junction of the sesquiterpene lactone is *trans*. The hydrogen at C1 is *anti* to the methyl group at C5 on the bridgehead between rings A and B. In addition the hydrogen on C7 is *trans* to the hydrogen at C8 on the bridgehead between rings B and C. Whereas, the methyl group at C10 *cis* with hydrogen at C1. The structure proved to be a new sesquiterpene at the time of the study. It was a hydrogenated form of graveolide **215** and at this time was a new compound.



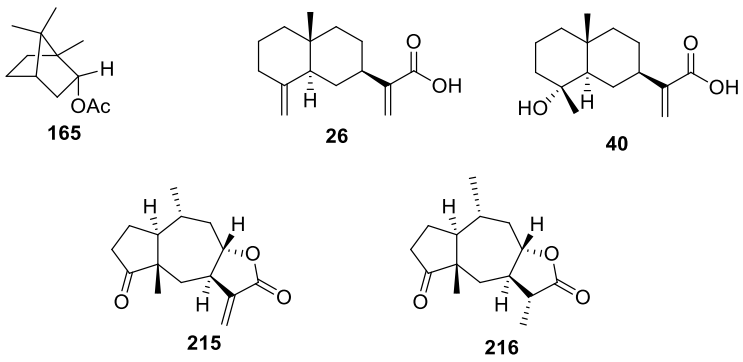
216

Figure 2.6: α-Methyl lactone **216** isolated from *D. graveolens*

Unfortunately during the course of this study the same compound was reported by Abu Irmaileh *et al*., from the *Inula graveolens* growing in Jordan. The spectra and x-ray data matched the data on this study.[83]

2. 2 **Extraction optimization**

As five interesting compounds could be isolated from *D. graveolens* in excellent yield of 0.5-1% (w/w) of dried plant, an alternative large scale isolation of these compounds was attempted. Considering the 5 compounds, the two carboxylic acids **26** and **40** could be isolated from the other compounds by using acid base chemistry.



165 **26** **40**

215 **216**

Although ethanol is reasonably cheap, the volumes required for large scale extraction become problematic. There are many hazards in using ethanol, which carry health and safety hazards, including its flammability. Therefore use of an aqueous solvent was investigated instead of organic solvents which may provide a safe and cheap large scale extraction.

Roughly cut *D. graveolens* was steeped in water for 5 days. The aqueous extract was filtered and acidified with concentrated hydrochloric acid to pH≈2 then extracted with dichloromethane. The crude organic extract was concentrated under reduced pressure. The ^{1}H NMR spectra of the crude oil contained ilicic acid **40**, varying amounts of costic acid **26** and the other 3 compounds **165**, **215** and **216** (Figure 3.3). To find a more efficient extraction method a range of aqueous solvent were tested. A number of different aqueous solution (10% EtOH, 10% Na_2CO_3, 10% $NaHCO_3$, 5% Na_2CO_3, 1% Na_2CO_3 and 5% $NaHCO_3$, 2% $NaHCO_3$) were tested to optimise the extraction process, and all gave the expected five compounds. The optimal conditions were extraction with the 2-5% of sodium carbonate or sodium bicarbonate for 5 days depending on the amount of the plant material (Figure 4.3). A typical extraction is as follows, the aerial parts of *D. graveolens* was steeped in 5% sodium bicarbonate for five days. The extract was filtered, acidified with concentrated hydrochloric acid to pH ≈ 1- 2. The extracted solution was extracted with dichloromethane. The organic extract was concentrated under reduced pressure to give a brown oil. The ^{1}H NMR for the crude extract showed the presence of the same five main components as in the ethanol extraction (Figure 2.2).

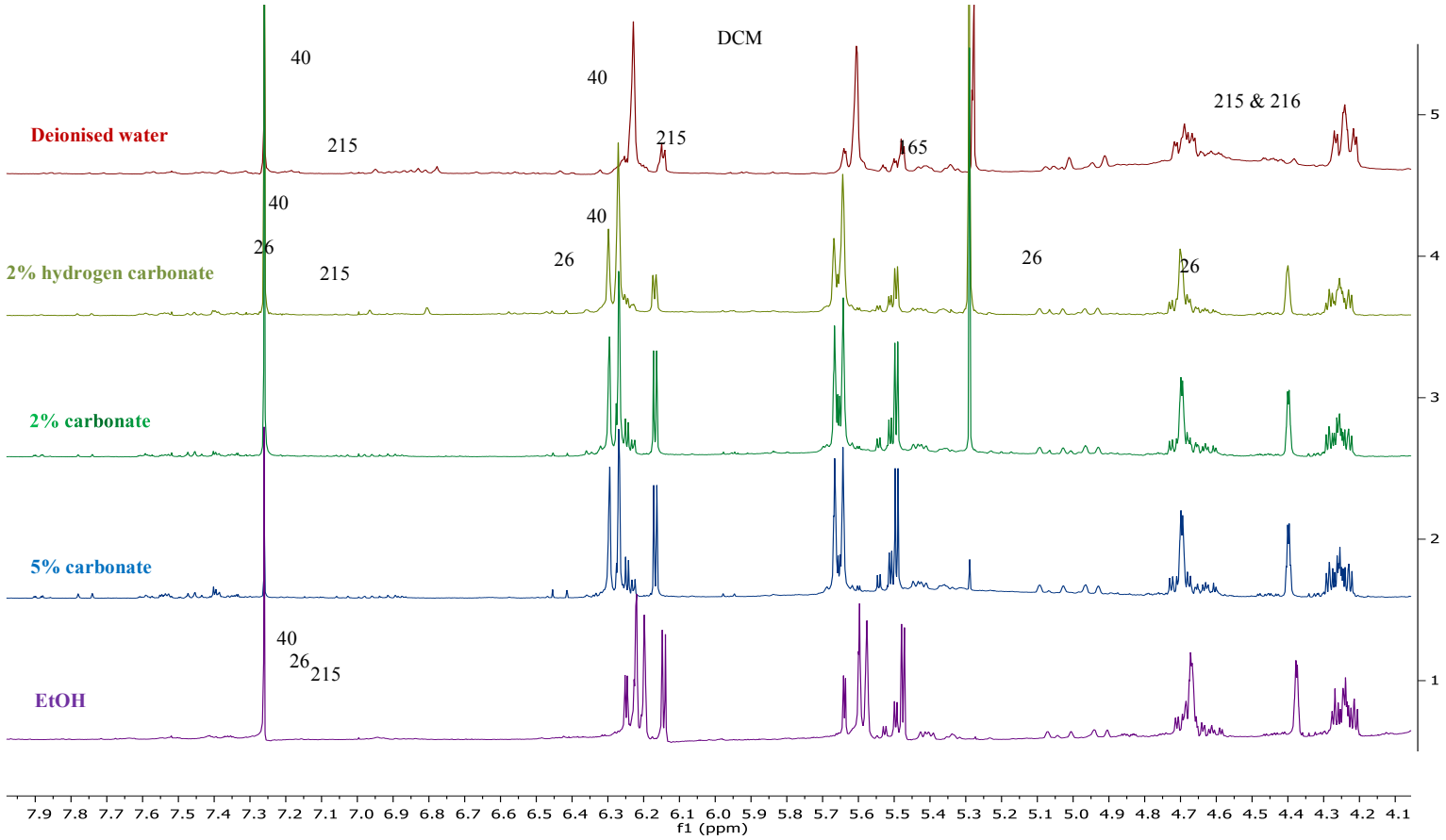


Figure 2.7: Crude ^{1}H NMR spectrum ($CDCl_3$) of *D. graveolens,* aerial parts resin using different solvents (ppm)

The project then focused on the sesquiterpene carboxylic acids since they could be easily extracted from the crude extract using acid/base chemistry. The crude extract was washed with 5% aqueous sodium hydroxide to deprotonate isolated sesquiterpenes acids (Figure 2.2). The aqueous extract was acidified to pH≈2 using concentrated hydrochloric acid. During these extractions, it was noted that ilicic acid **40** was deprotonated more readily than costic acid **26**. Thus the choice of suitable weaker base (5% $NaHCO_3$) could selectively separate ilicic acid over costic acid. This technique was partially successful in isolating ilicic acid from costic acid affording ilicic acid containing 20% costic acid and pure costic acid. The two compounds could also be easily separated by column chromatography.

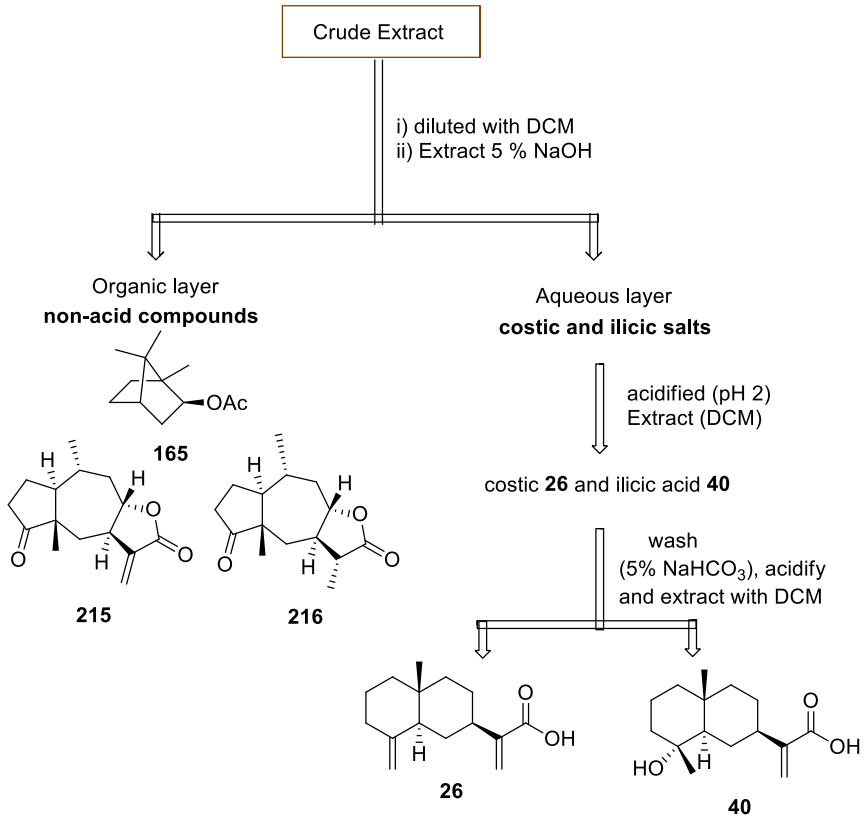


Figure 2.8: Acid/ base isolation of costic **26** and ilicic acid **40**

2. 3 **Large scale extraction with 2% $NaHCO_3$**

After optimising the extraction condition using different aqueous extraction methods, a large scale extraction was attempted on fresh plant material using a 60 L drum (see Figure 2.9). The plant material (10 kg) was soaked in 56 L of 2% $NaHCO_3$ for 5 days (Figure 2.10). The obtained solution was filtered through celite to remove solid particulates, and the extract was acidified with concentrated HCl to pH ≈ 1-2. The acidified solution was extracted with dichloromethane. The combined organic extracts were concentrated under reduced pressure to give a brown oil crude (40 g). ^{1}H NMR spectroscopy confirmed the presence of the five main resin components **26**, **40**, **165**, **215** and **216**. However, extraction of the acidified aqueous extract formed dispersing emulsions which slowed down the extraction process. This problem was overcome by filtration through the celite and saturating the aqueous solution with salt.

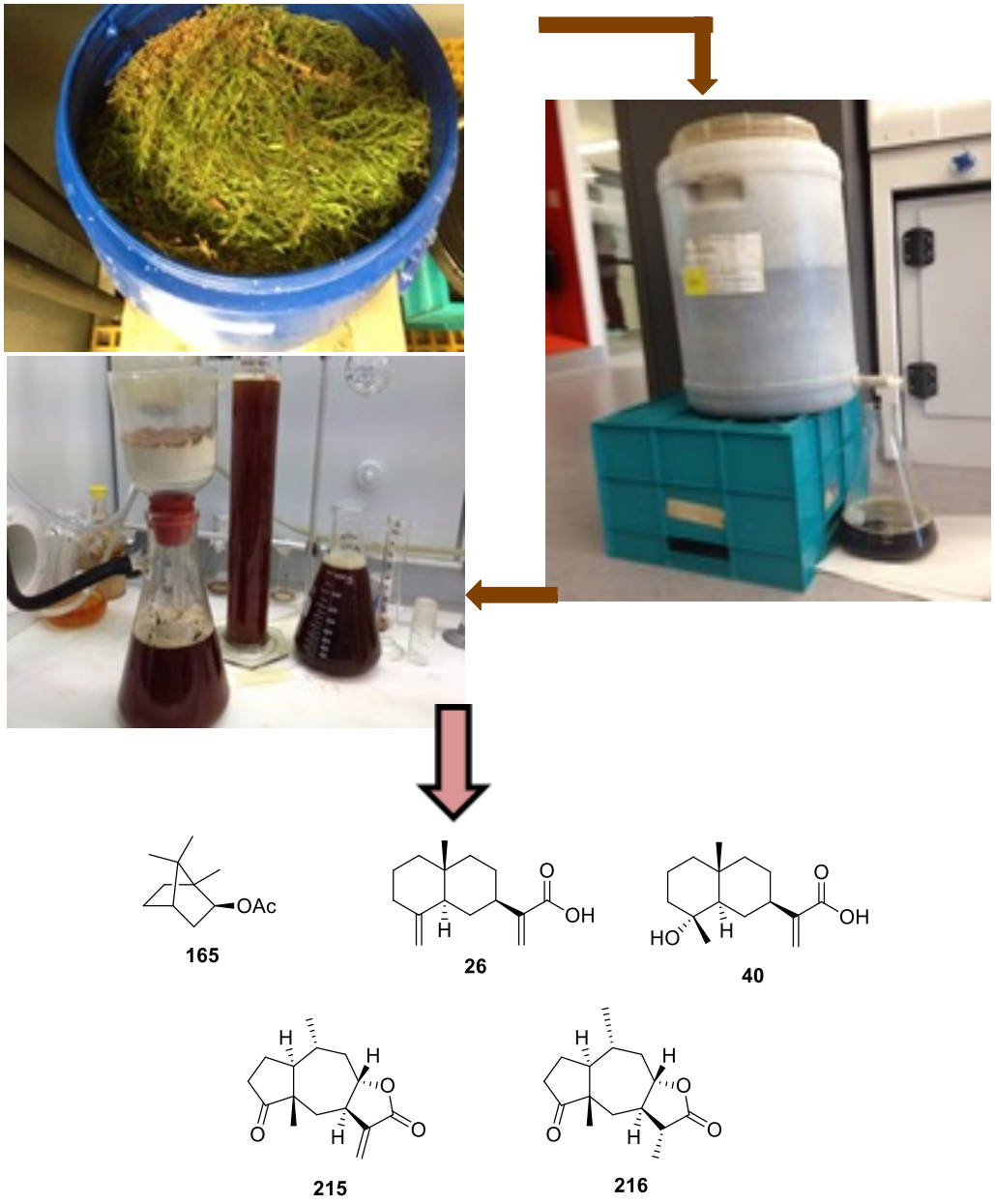


Figure 2.9: Large scale extraction

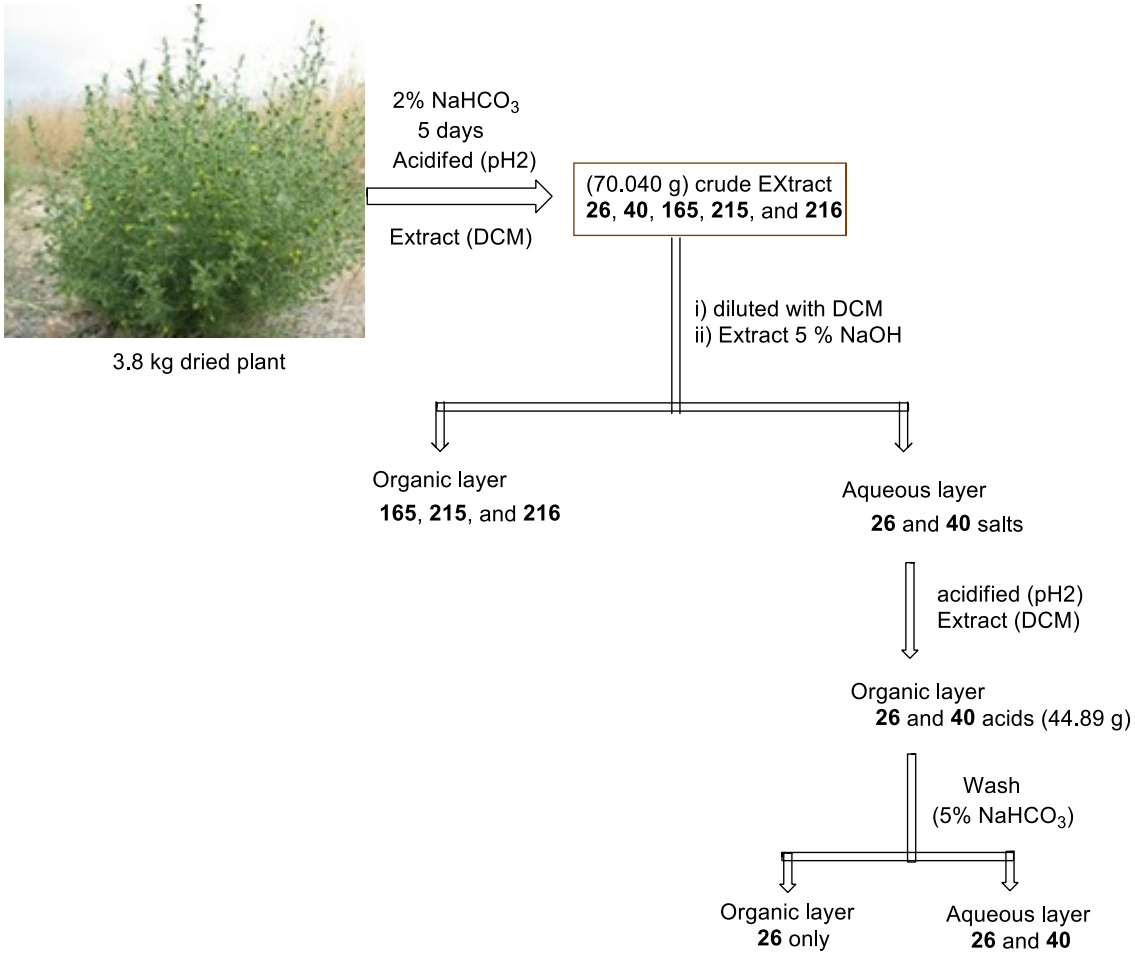


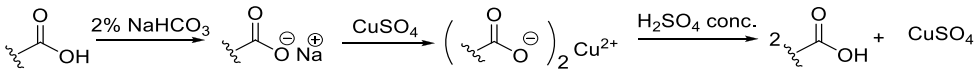
Figure 2.10: Summary of chemistry extraction method

2. 4 **Extraction of bicyclic sesquiterpene acids method**

Since the project focused on the sesquiterpene carboxylic acids, further optimisation of the large scale selective extraction of these acids was investigated. In Chapter 2.2, the carboxylic acids were extracted with 2% of sodium hydrogen carbonate to make the sesquiterpenes more water soluble. However, extraction of the acidified aqueous extract formed slowly dispersing emulsions which slowed down the extraction process. An alternative isolation of these acids was investigated. In particular, the precipitation of the extracted carboxylates as insoluble metal salts. To gauge potential metal salts, the solubility of acetate salts was use as a reference (Table 2.1). Generally all acetates are water soluble, however, both silver and copper have poor solubility 1.05 and 7.2 g/100 mL respectively (Table 2.1). Silver salts are most insoluble but they are very expensive. Copper salts appear to be the best compromise between solubility and cost. Copper sulfate is cheap and should form a reasonably insoluble salt, which could be isolated by filtration (Scheme 2.2). The carboxylic acids **26** and **40** can then be isolated using an acidic workup.

Table 2.1: water solubility of some acetate g/100 mL at 20 ˚C

Acetate	**Solubility g/100 mL at room temperature**
Potassium acetate	256
Barium acetate	72
Magnesium acetate	53.4
Sodium acetate	46.4
Calcium acetate	34.7
Zinc acetate	43
Copper acetate	7.2
Silver acetate	1.05



Scheme 2.2: extraction and isolation of the carboxylic acid compounds

The dried aerial parts of *D. graveolens* (5 Kg) were soaked in 50 L 1% Na_2CO_3 for 5 days at room temperature. The extract was filtered and $CuSO_4$ was added. The resulting mixture was left to settle down for 4 days. The green precipitate was filtered by vacuum filtration, suspended in water and acidified with H_2SO_4. The aqueous solution was extracted with DCM and the solvent was removed to afford a crude extract. The ^{1}H NMR spectrum of the crude mixture showed the presence of costic and ilicic acid (Figure 6). The crude extract was subjected to column chromatography (1:1 ethylacetate: petrol) to give pure ilicic and costic acid.

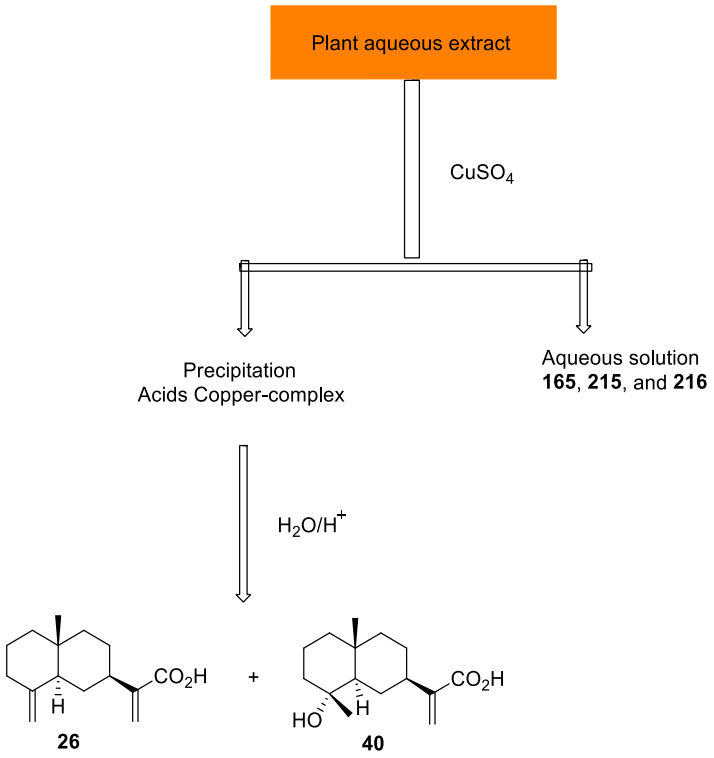


Figure 2.11: ^{1}H NMR spectrum of material obtained from the copper precipitate

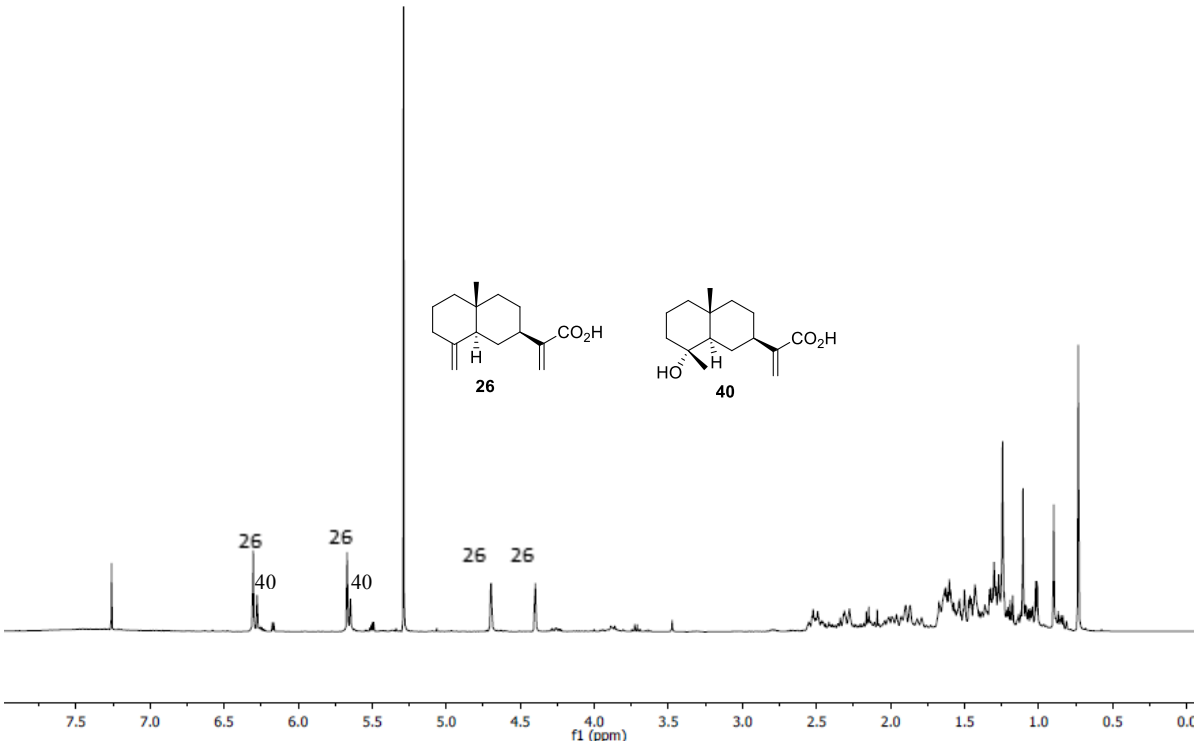


Figure 2.12: ^{1}H NMR spectrum ($CDCl_3$) of costic and ilicic acid isolated by 2% sodium hydrogen carbonate extract followed by Cu precipitation, acidification and extraction

Chapter 3

3. 1 **Biological activity of sesquiterpenes**

There have been several anecdotes of *D. graveolens* having a biological effect. Fishermen in northern Italy used an aqueous extract of the plant to sedate fish. It was later shown that compounds isolated from *D. graveolens* costic acid **26** and ilicic acid **40**, were ichytoxic. Contact with *D. graveolens* was reported to cause a rash, and further clinical investigation showed it cause contact dermatitis. The observation of the inhibition of growth around areas were *D. graveolens* grows (Figure 3.1) was of interest as it had not been tested. The working hypothesis was that the water soluble compounds present in the resin could be washed to the ground during a rain shower and inhibits the germination of competing plant seeds around the plant (Figure 3.2). At the outset of this work, there were no scientific reports on the inhibition of seed germination by compounds isolated from *D graveolens*. Traditionally, germination inhibition experiments are performed using lettuce seeds *Lactuca sativa*. A more user friendly germination experiment using alfalfa *Medicago sativa* was developed.

Figure 3.1

Initially, the reliability of germination of alfalfa was examined with deionised water. The alfalfa seeds were soaked on deionised water for two hours. Three replicates of each twenty seeds were placed on filter paper (9 cm diameter) in Petri dishes. Deionised water (4 mL) were added to each Petri dish. The Petri dishes were wrapped with aluminium foil to keep the seedling in the dark and kept at laboratory temperature (25˚C) for three days. The alfalfa seeds proved to be a reliable assay with excellent germination rates (> 98%) and taking only 3 days (Figure 3.2).

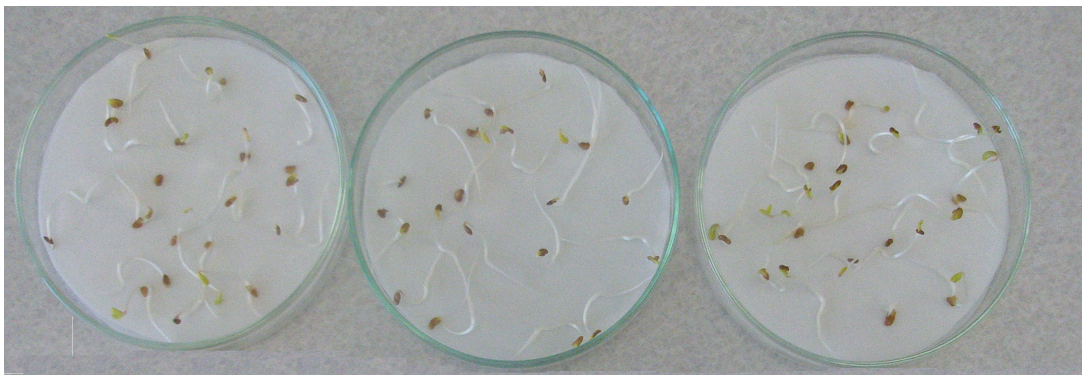


Figure 3.2: Alfalfa seed germination assay

The primary investigation of the inhibition germination effect of the *D. graveolens* was conducted using the crude extract. The crude extract was dissolved in ethanol to make a 5 g/L solution, serial dilutions of the solution gave 1/10 and 1/100 dilutions. The bioassay was carried out using the procedure provided by Wolf *et al.*[87, 88] Two pieces of (Whatman number 1) filter paper were set in 9 cm diameter Petri dishes. First, each test solution 4 mL (stock, 1/10, 1/100) were placed onto each plate, and controls were prepared identically using the pure ethanol. The plates were left overnight to evaporate the ethanol. Next, the filter papers were moistened with (4 mL) of deionized water. Finally, twenty seeds were placed on each plate with three replicates per treatment. The Petri dishes were covered from the light using the lid, sealed by aluminium foil and kept in the dark at 25˚C. After three days, the Petri dishes were frozen to kill the seedlings and to facilitate measuring the length. The fall lengths of each seedling for each set were measured (Figure 3.3), and mean values were calculated and converted to the percentage to ease expression this value in bar chart. Seed germination [SG %] was calculated from the equation SG = 100 × [T/C], where T and C are the numbers of the germinated seeds on the treated and control filter

papers, respectively. The *D. graveolens* showed inhibitory effect against the alfalfa seed germination (Figure 3.4 and 3.5)

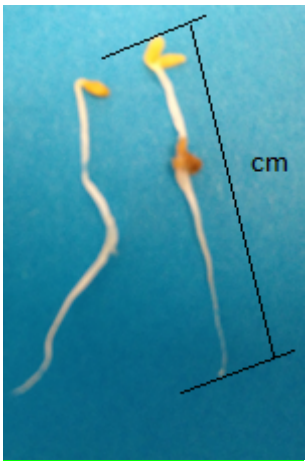


Figure 3.3: Measuring of seedling lengths

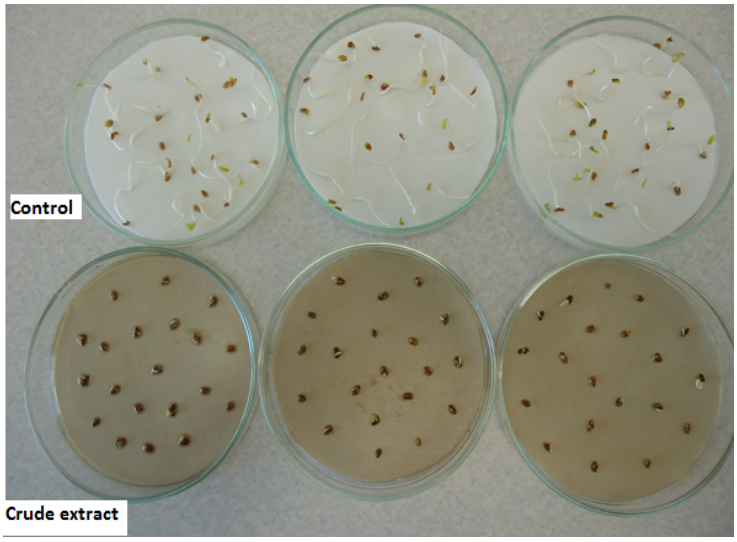


Figure 3.4: Effect (0.125 g) of crude extract on seed germination

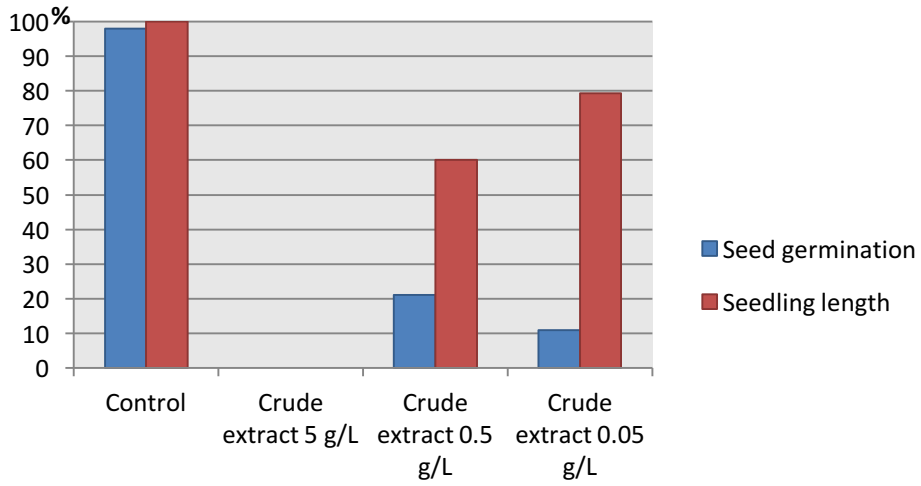


Figure 3.5: Effect of crude extract of *D. graveolens* on seed germination and seedling length

The crude extract of *Dittrichia graveolens* showed complete germination inhibition at 5 g/L compared with the control (Figure 3.4). This made the study more exciting and increased curiosity to identify the compound(s) that were responsible for this inhibition activity.

Extraction of the crude aqueous extract with DCM gave two fractions to test: The organic extract and the remaining aqueous solution. These two fractions were tested for their activity using the same procedure, the DCM extract completely inhibited the seed germination. The remaining aqueous solution had no significant inhibition compared to the control but showed slight shortening of seedling length (Figure 3.6 and 3.2). This observation suggests that the active compounds are organic soluble and are likely to be the sesquiterpenes.

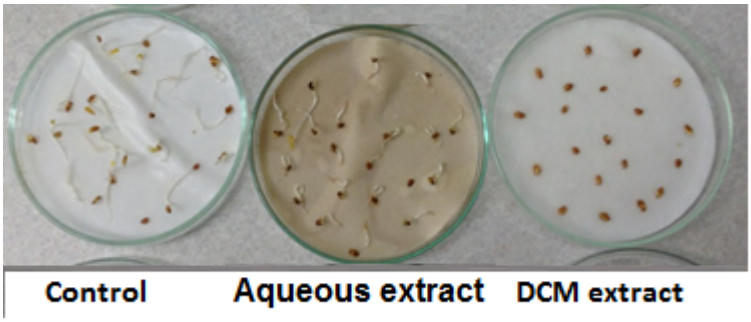


Figure 3.6: Seed inhibition germination

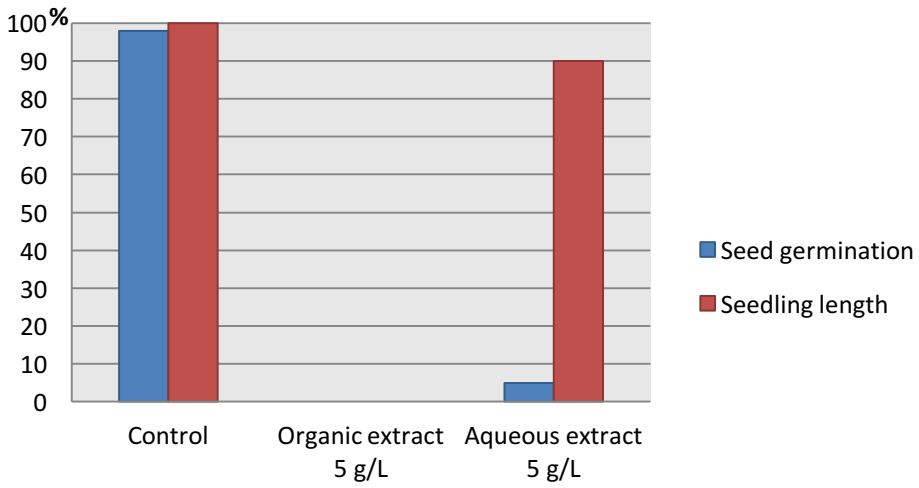
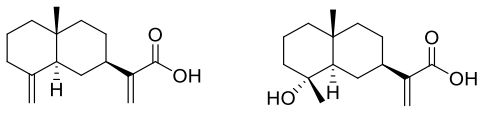


Figure 3.7: Effect of the organic and aqueous extracts of *D. graveolens* on seed germination seedling length

Finally, the sesquiterpene acids, costic acid **26** and ilicic acid **40** were investigated for their inhibition activity following the same procedure above. The inhibition activity for the acids were illustrated in Figure 3.9 and Table 3.10. Both costic acid **26** at 21 mM and ilicic acid **40** at 20 mM (5 g/L) caused significant inhibition of seed germination on Alfalfa (Figure 3.9). In general, the ilicic acid **40** showed 100% seed germination inhibition at 20 mM and 33% at 2.1 mM. Costic acid **26** at 21 mM showed less inhibition activity 78% and at 2.0 mM was 6%. Costic acid showed less seed

germination inhibition than ilicic acid and this may be due to the lower solubility of costic acid compared to ilicic acid. These results make the hypothesis that resin washed off the plant by rain inhibits the surrounding plant germination plausible.



26 **40**

Figure 3.8: Costic acid **26** and ilicic acid **40**

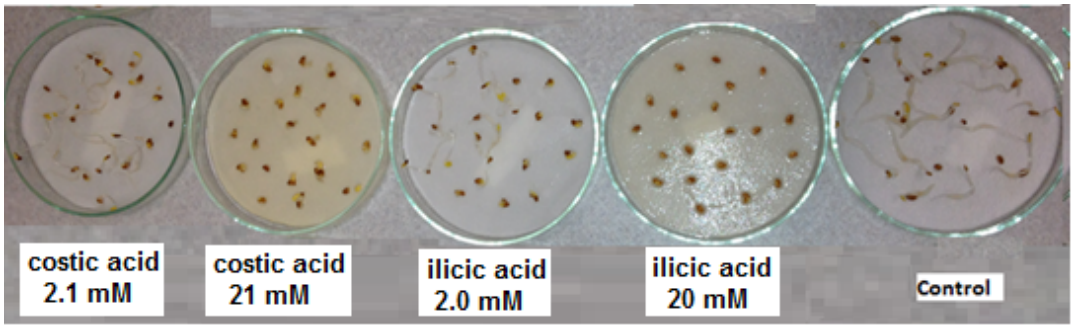


Figure 3.9: Effect of the costic and ilicic acid on seed germination

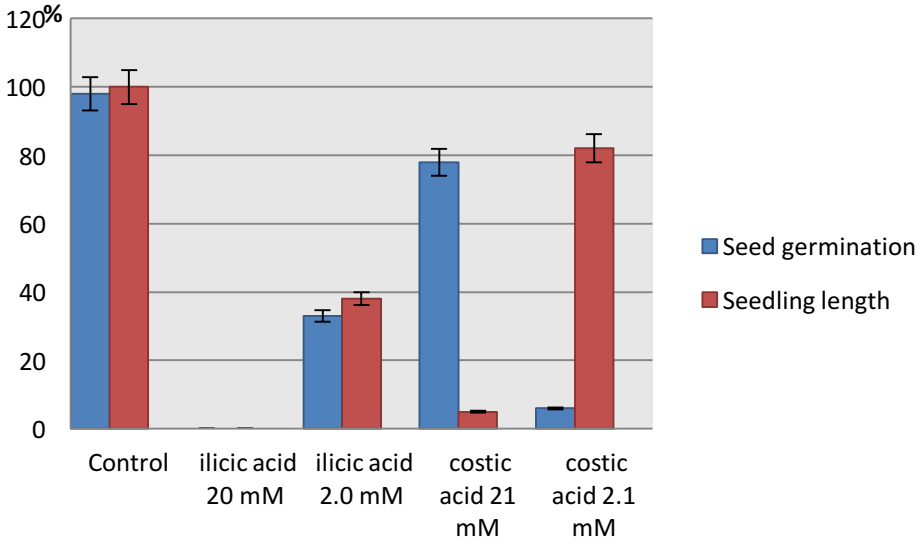
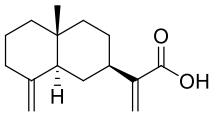


Figure 3.10: Effect of costic & ilicic acid on seed germination and seedling length

3. 2 **Other bioactivity assays**

Costic acid **26** was tested against the parasites that cause human African trypanosomiasis (HAT) and malaria by Dr. Sumalee Kamchonwongpaisan at BIOTEC, NSTDA in Thailand. Testing of costic acid against HAT was carried out with the parasite *Trypanosoma brucei rhodesiense,* which causes acute trypanosomiasis amongst the human population. Costic acid have high IC_{50} (Table 3.1). The antiplasmodial activity of costic acid was also tested against two strain of *Plasmodium falciparum*: *TM4/8.2* and *K1CB1*. The *TM4/8.2* type is the sensitive strain, whereas the *K1CB1* type is the multidrug resistant strain. Compound **26** showed no activity against antifolate and chloroquine sensitive strain *TM4*/*8.2* with $IC_{50} > 50$ and slight activity against the *K1CB1* IC_{50} 28 (Table 3.1). Costic acid cytotoxicity was investigated against Vero cells. Costic acid **26** was did not show any significant toxicity against Vero cells (Table 3.1).

Table 3.1: HAT, antimalarial and cytotoxicity results of costic acid **26**



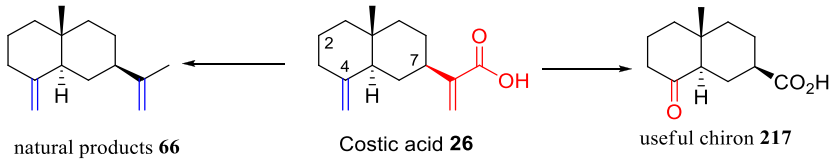
26

Type of test	**IC_{50} (µM)**
T. brucei rhodesiense	19.83±1.21
P. falciparum (*TM4*/*8.2*)	> 50
P. falciparum (K1CB1)	28.2
Cytotoxicity (Vero)	> 50

Chapter 4

4. 1 **Chemistry of costic acid**

Costic acid **26** was one of the sesquiterpenes from *Dittrichia graveolens* that could be isolated easily on a large scale. To determine if this compound is a useful starting material for synthesis, a range of reactions were attempted on this compound to make different natural products or useful enantiopure compounds (Scheme 4.1). Costic acid contains two key functional groups: an exocyclic double bond attached to C4 and an acrylic acid attached to C7. Only limited chemistry has been performed on costic acid (see Chapter 1).

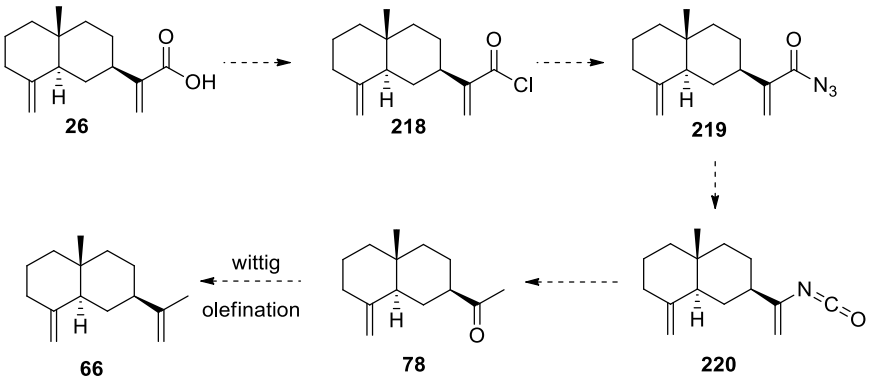


natural products **66** Costic acid **26** useful chiron **217**

Scheme 4.1:

4. 2 **Transformation of costic acid to *β*-selinene**

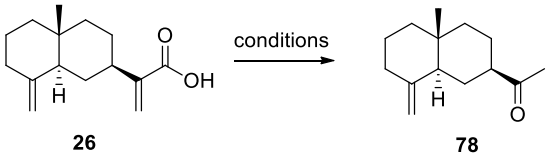
The natural product *β*-selinene **66** has been isolated from seeds of Celery (*Apium graveolens*) in 0.03% yield.[89] As direct isolation of *β*-selinene is not viable, a semisynthetic pathway was investigated (Scheme 4.2). *β*-Selinene could be synthesised from costic acid **26** which was isolated in significant quantities. The intermediate ketone **78** could be synthesised by following the procedure shown in Scheme 4.2. First, transformation of costic acid **26** to the acid chloride **218** and reaction with sodium azide would give the acyl azide **219**. A Curtius rearrangement of the acyl azide would give the ketone **78** upon hydrolysis. Finally, a Wittig olefination of the ketone **78** would give the desired compound *β*-selinene **66**.



Scheme 4.2: Proposed synthesis of the *β*-selinene

The ketone **78** was synthesised from costic acid **26** in three steps based on a method by Tebbaa *et al.,* with some modification.[67] Costic acid **26** was treated with 1.8 equivalents of methyl chloroformate in anhydrous THF at -10 ˚C to convert the acid to acid chloride (Table 4.1, entry 1). An aqueous solution of sodium azide (1.5 equivalents) was added to the reaction mixture. Then the reaction mixture was stirred overnight, and heated at reflux in toluene for 2 h. The reaction was cooled to room temperature, acidified with 10% hydrochloric acid and heated at reflux for another 2 h. The ^{1}H NMR spectrum of the crude reaction mixture indicated only recovered starting material. The reaction was revisited using oxalyl chloride (Table 4.1, entry 2). Costic acid **26** was treated with oxalyl chloride and dimethylformamide (catalyst) in anhydrous dichloromethane at -10 ˚C for 1 h. The same conditions were used for the rest of the reaction to give ketone **78** in 17% yield. The ^{1}H NMR spectrum of the **78** did not have the acrylic acid hydrogen signals at 6.31 and 5.68 ppm and indicated the consumption of the starting material. In addition, the presence of a new singlet at 2.16 ppm integrating for three hydrogens suggested an acetyl group. The ketone was detected and confirmed with IR and ^{13}C NMR spectrum at 1730 cm^{-1} and 210 ppm respectively. The ^{1}H NMR spectrum matched those data provided in the literature made by an alternative method.[90, 91]

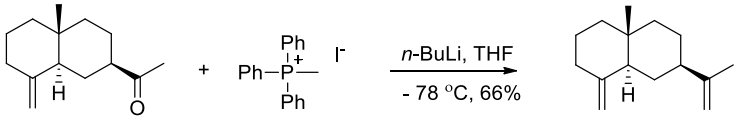
Table 4.1: Transformation of costic acid to ketone **78**



26 **78**

Entry	Conditions	Yield of **78**
1	$ClCO_2CH_3$, THF, NaN_3, Et_3N, 10% HCl, reflux 130 °C	0
2	Oxalyl chloride, DCM, NaN_3, 10% HCl, reflux 130 °C	17%

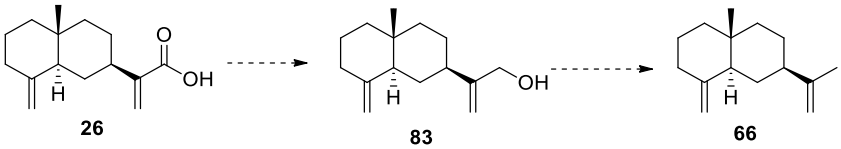
The ketone **78** is an intermediate in the synthesis of *β*-selinene and thus **78** could be converted to the *β*-selinene in one step. The phosphonium salt **221** was prepared in 82% yield by the reaction of triphenylphosphine with methyl iodide in anhydrous tetrahydrofuran at 0 ˚C to room temperature for 12 h. The ketone **78** was reacted with the Wittig reagent derived from the phosphonium salt **221** in anhydrous tetrahydrofuran at 0 ˚C to form an impure product of *β*-selinene **66** in 66% yield.



78 **221** **66**

Scheme 4.3: Synthesis of *β*-selinene **66** from ketone **78**.

Since the conversion of acrylic acid to the acetyl group was problematic an alternative approach was investigated. Costic acid **26** could be reduced to allylic alcohol **83**, and then a deoxygenation reaction would produce *β*-selinene (Scheme 4.4).

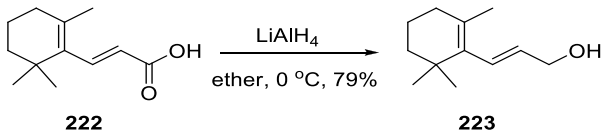


26 **83** **66**

Scheme 4.4: Proposed synthesis of *β*-selinene **66**

Costol **83** has been prepared in two steps from costic acid however direct reduction of costic acid may be possible since the allylic alcohol **223** was prepared in 79% yield

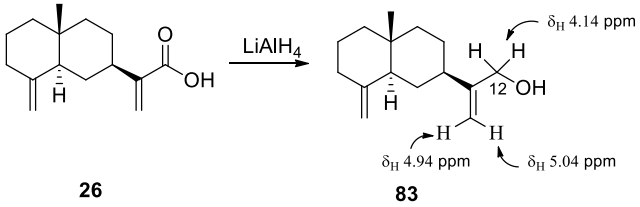
from acrylic acid **222** in one step using lithium aluminium hydride as reported by He and Wu (Scheme 4.5).[92]



222 **223**

Scheme 4.5: Reduction of the acid **222** to allylic alcohol **223**.

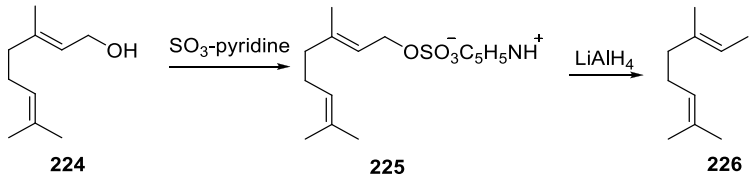
The reduction of costic acid **26** using 1.5 equivalents of $LiAlH_4$ at 0 ˚C gave the allylic alcohol **83** in 64% yield (Scheme 4.6). Interestingly no conjugate reduction was observed under these reaction conditions. The ^1H NMR spectrum has four vinylic singlets at 5.04, 4.94, 4.70 and 4.42 ppm, and a new multiplet at 4.14 ppm integrating for two hydrogens which suggested for CH_2 next to the OH group. The obtained spectra matched that provided by Bawdekar and Kalkar who synthesised the allylic alcohol from costic acid in 2 steps via the ester.[63] Thus, it is not necessary to esterify costic acid before the reduction as described in the literature.



26 **83**

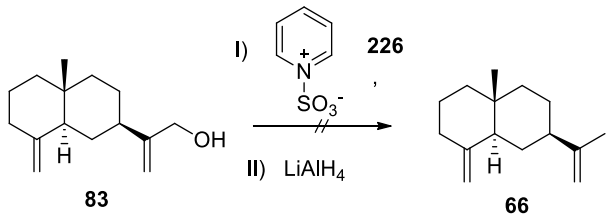
Scheme 4.6: Transformation of costic acid to allylic alcohol.

Allylic alcohols **83** can be deoxygenated using the procedure reported by Corey and Kazuo.[93] A range of alcohols such as geraniol **224** was treated with pyridine sulfur trioxide complex to give sulfate ester salt **225**. The salt was treated then with $LiAlH_4$ to give the desired deoxygenated compound **226** (Scheme 4.7) by a hydride substitution.



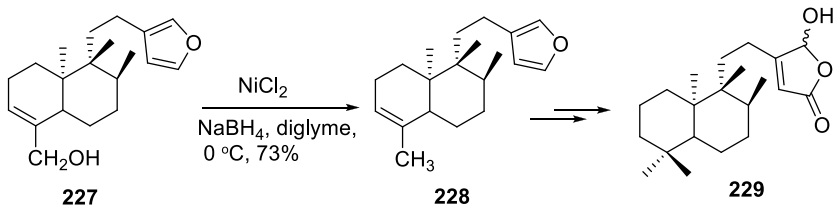
Scheme 4.7: Deoxygenation of allylic alcohols **224**.

The allylic alcohol **83** was stirred with sulphur trioxide pyridine complex **226** at 0 °C for 3 hours. (Scheme 4.8), until the reaction was complete (monitored by TLC). A solution of lithium aluminium hydride in anhydrous tetrahydrofuran was added to the reaction mixture at 0 °C for 1 h, and then allowed to warm to room temperature for 3 h. Unfortunately, only the starting material was recovered from the reaction.



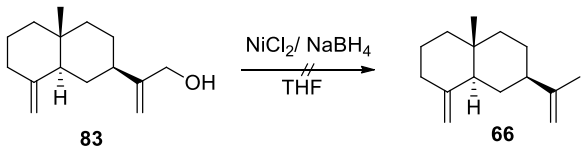
Scheme 4.8: Synthesis of *β*-selinene **66** from allylic alcohol **83**

An alternative attempt to deoxygenate the allylic alcohol was performed following the procedure provided by Imamura and Costa in the preparation of the clerodane **229** (Scheme 4.9).[94] Treatment of allylic alcohol **227** with anhydrous nickel chloride and sodium borohydride for 6 h at 0 ˚C gave the compound **228** in 73% yield in the presence of a furan ring.



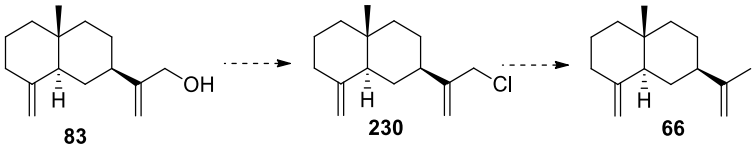
Scheme 4.9: Deoxygenation of clerodane.[94]

The allylic alcohol **83** was treated with anhydrous nickel chloride and sodium borohydride at 0 ˚C for 6 h (Scheme 4.10). Again, these conditions did not give the *β*-selinene **66** as the ^{1}H NMR showed only the start material was recovered.

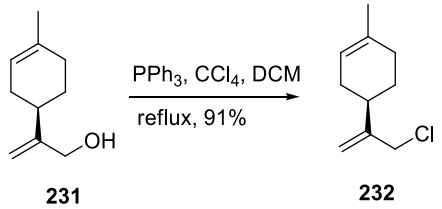


Scheme 4.10: Synthesis of *β-selinene* **66**

Since the all attempts to convert the allylic alcohol **83** in one step were unsuccessful. Another attempt to convert allylic alcohol to *β*-selinene was investigated through convert alcohol group to good leaving group chloride then elimination the last product to *β*-selinene (Scheme 4.11). Allylic alcohol **83** could be converted to allyl chloride **230** following the procedure presented by Kaufman and his group in the synthesis of 9-chloro-1,8-*p*-menthadiene **232** (Scheme 4.12).[95] Treatment of allylic alcohol **231** with triphenylphosphine, carbon tetrachloride gave the chloride **232** in 91% yield.

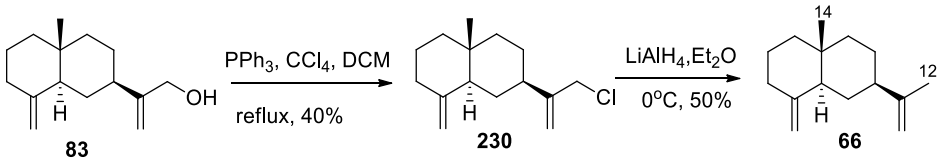


Scheme 4.11: Proposed synthesis of *β*-selinene **66**



Scheme 4.12: Synthesis of 9-chloro-1,8-p-menthadiene **232**.[95]

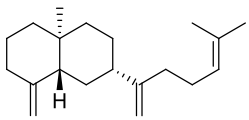
Allylic alcohol **83** was treated with triphenylphosphine and carbon tetrachloride at reflux gave the allyl chloride **230** in 40% yield (Scheme 4.13). The allyl chloride **230** then treated with lithium aluminium hydride to give *β*-selinene. The formed *β*-selinene was isolated as an inseparable mixture with unknown compound.



Scheme 4.13: Synthesis of *β*-selinene **66**

4. 3 **Synthesis of diterpene 230 isolated from *L. cristatum***

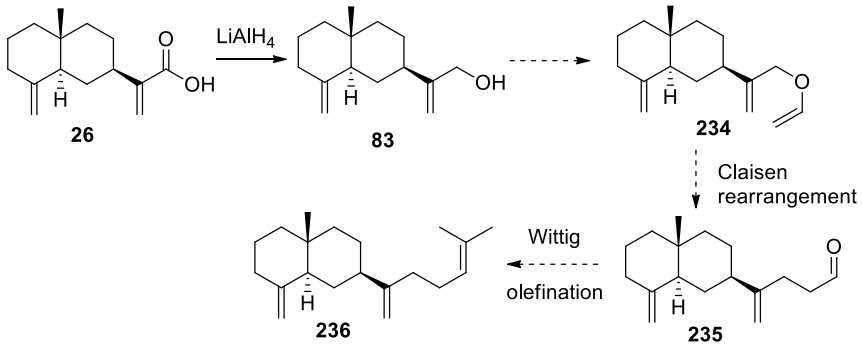
Having successfully converted costic acid to *β*-selinene, a synthesis of second natural product was investigated. The diterpene **233** was isolated from soft coral *Lobophytum cristatum*.[96] The absolute configuration of this compound is not known but is thought to be as shown in figure 4.1.[96,97] To establish the absolute stereochemistry of the isolated terpene, semisynthesis from a compound of known stereochemistry like costic acid could confirm the structure by the comparison of optical rotation. The diterpene has yet to be prepared by total synthesis.



233

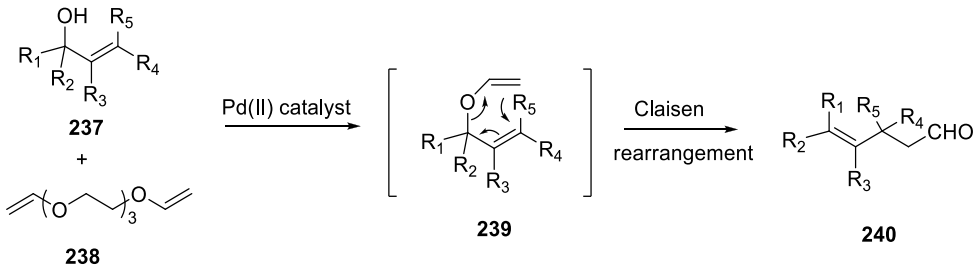
Figure 4.1: Diterpene isolated from *L. cristatum*

The aldehyde **235** could be synthesised in 2 steps starting from the allylic alcohol **83** (Scheme 4.14). Transetherification with butyl vinyl ether would convert the allylic alcohol to the **234**. This vinyl ether **234** is setup to undergo a Claisen rearrangement to give the aldehyde **235**. Wittig olefination using isopropyltriphenylphophonium bromide **241** would then afford **236** which is expected to be the opposite enantiomer based on literature reports.



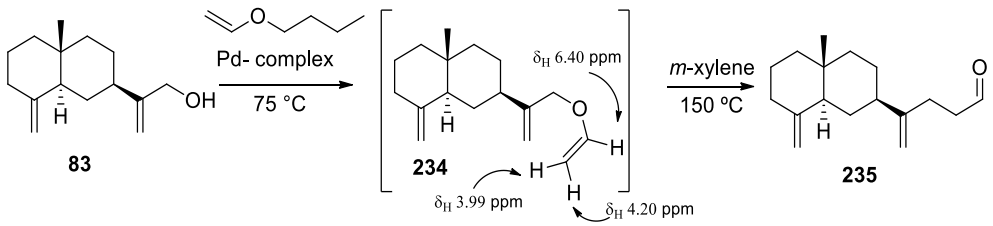
Scheme 4.14: Transformation of costic acid to diterpenes

Wei and co-workers converted many allylic alcohols to vinyl ethers using triethyleneglycol divinyl ether **238** and the catalyst $Pd(OAc)_2$-phenanthroline complex at reflux. The reaction was then heated at reflux 140 ˚C to promote the Claisen rearrangement and produce the corresponding aldehyde in good yields (Scheme 4.15).[98]



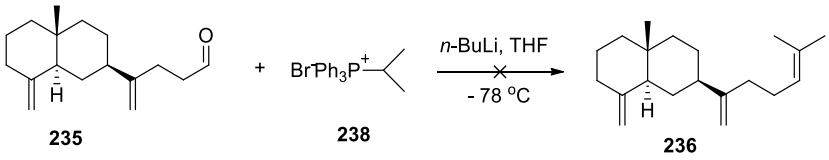
Scheme 4.15: Claisen rearrangement

The allylic alcohol **83** was treated with butyl vinyl ether and $Pd(OAc)_2$-phenanthroline complex (0.5 mol%) then heated at reflux 70 °C to give vinyl ether **234**. The ^{1}H NMR spectrum showed that the allylic alcohol was converted to vinyl ether due to the presence of a new multiplet at 6.10 ppm ascribed to the vinyl ether. This crude mixture was heated at 150 °C to promote a Claisen rearrangement and form the aldehyde **235** in 60% yield. The ^{1}H NMR spectrum of the aldehyde **235** showed three signals at 4.86, 4.71 and 4.42 ppm that were characteristic of the four vinyl hydrogens while the signal at 0.73 ppm was assigned to the bridgehead methyl group. In addition, signal at 9.79 ppm in ^{1}H NMR spectrum that was assigned to the hydrogen of the aldehyde.



Scheme 4.16: Synthesis of aldehyde **235**

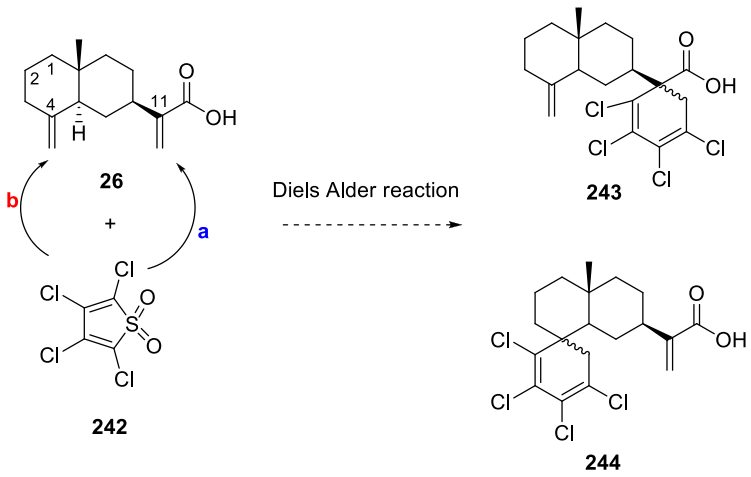
Finally, synthesis of diterpene **236** using Wittig olefination was investigated. Treatment of the phosphonium salt **241** with excess of the *n*-BuLi at -84 °C, the colour was changed to the orange as mention in the literature then followed by adding the aldehyde **235** in anhydrous tetrahydrofuran. The ^{1}H NMR spectrum of the isolated material from the reaction showed a complex mixture of products. This was an unfortunate result and further optimization reactions are required to obtain **236**.



Scheme 4.17: Synthesis of diterpene **236**

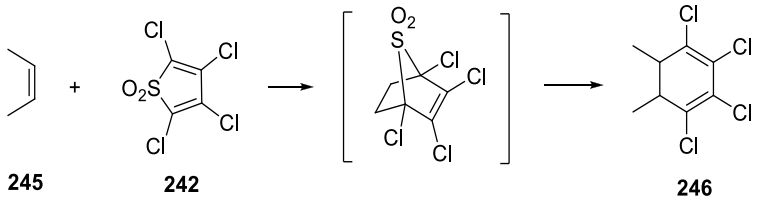
4. 4 **Diels-Alder reactions of costic acid derivative**

Costic acid **26** contains two vinyl groups C4 and C11 and their reactivity toward the Diels-Alder reaction was investigated using 2,3,4,5-tetrachlorothiophene-1,1-dioxide **242**. There are two possible products that can be formed. Addition to the acrylic acid would give **243** and addition to the exocyclic methylene would give **244** (Scheme 4.18).



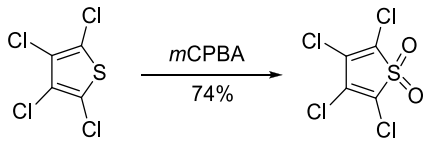
Scheme 4.18: Proposed possible pathways of Diels Alder reaction

Tetrachlorothiophene-1,1-dioxide **242** is a reactive inverse electron demand diene in the Diels-Alder reaction. It is a stable compound and is a useful reagent in organic synthesis.[99] Tetrachlorothiophen-1,1-dioxide is non-aromatic and can participate in a range of reactions including cycloaddition reactions and Michael additions. When tetrachlorothiophene-1,1-dioxide **242** reacts as a diene in a Diels-Alder reaction, the initial adduct spontaneously releases sulfur dioxide by a cheletropic elimination to regenerate a new diene **246** (Scheme 4.19).



Scheme 4.19

Tetrachlorothiophene-1,1-dioxide **242** was synthesised by the oxidation of the 2,3,4,5-tetrachlorothiophene **247** by the procedure described by Raasch (Scheme 4.20).[99] Oxidation of 2,3,4,5-tetrachlorothiophene **247** using *m*-CPBA in 1,2-dichloroethane heated under reflux gave the tetrachlorothiophene-1,1-dioxide **242** in 74% yield. The ^{13}C NMR spectrum shows two signals at 131.3 and 127.6 ppm.

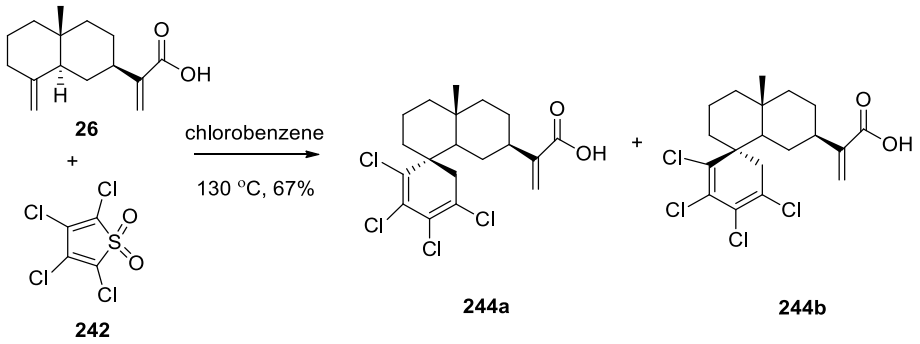


247 **242**

Scheme 4.20: Oxidation of the tetrachlorothiophene **247**

A solution of tetrachlorothiophene-1,1-dioxide **242** and costic acid **26** in toluene was heated under reflux for 1h. The ^{1}H NMR spectrum of the crude product showed only recovered starting material (Table 4.2, entry 1). The reaction was repeated by increasing the time of the reaction to 24 h, the same result was obtained (Table 4.2, entry **2**). Using chlorobenzene as a solvent gave a new product in 67% yield after heating the reaction under reflux for 24 h (Table 4.2, entry 3). The newly formed adduct as a mixture of stereoisomers of **244** since the two vinyl signals at 4.40 and 4.60 ppm in the ^{1}H NMR spectrum disappear. The signals of the acrylic acid remained at 5.30 and 6.48 ppm and this suggested that the addition has occurred on the C4-C14 alkene and not on C11-C13. The electron deficient nature of the C11-C13 alkene is likely to deter the inverse electron demanding cycloaddition at this position. In addition, the ^{1}H NMR spectrum showed presence of two isomers **244a** and **244b** and the intensity of the signals suggested the composition ratio is 90:10 respectively. Unfortunately, all attempts to separate the **244a** and **244b** isomers using either column chromatography or recrystallization were unsuccessful. The major product was tentatively assigned as **244a** based on the addition to the least hindered face of the alkene.

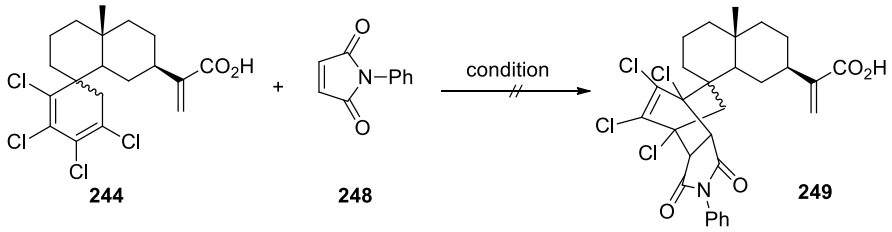
Table 4.2: Diels-Alder reaction condition



Entry	Solvent and condition	Yield **244**
1	Toluene, 1 h under reflux	N.R
2	Toluene, 24 h under reflux	N.R
3	chlorobenzene, 24 h under reflux	67%

A second Diels-Alder reaction on the tetrachlorothiophene adducted would generate a molecule of higher complexity **249** and may allow for the isolation of the two stereoisomers **244**. *N*-phenylmaleimide was used as it is a symmetrical dienophile with complete *endo* selectivity. A solution of **244** was stirred with 1.2 equivalents of *N*-phenylmaleimide **248** in dichloromethane at room temperature for 24 hours (Table 3.4, entry 1). The ^{1}H NMR spectrum of the crude showed only the starting material was recovered. Heat the mixture to reflux using toluene, chlorobenzene and dichlorobenzene respectively disappointingly only resulted in recovered starting material (Table 3.4, entry 2-5). The steric hindrance around the diene of **244** is likely to prevent this second the cycloaddition.

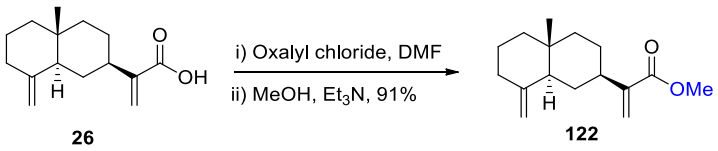
Table 4.3: Condition of synthesis of more complex compound **249**



Entry	Solvent and conditions	Results
1	DCM, at room temperature	N.R
2	Toluene, 24 h, reflux	N.R
3	Chlorobenzene, 24 h, reflux	N.R
4	Dichlorobenzene, 24 h, reflux	N.R

4. 5 **Esterification of costic acid**

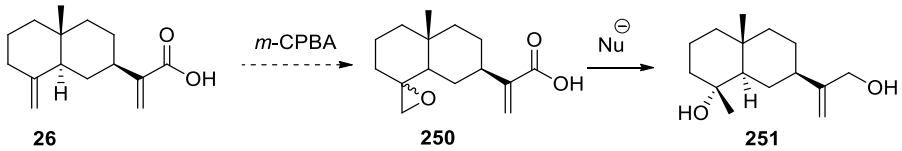
Although esterification is simple reaction, the formation of esters can be challenging. Bawdekar and Rao originally methylated costic acid with diazomethane.[63, 100] This was repeated in the laboratory to yield costic acid methyl ester in 83% yield. However, as large amounts of this ester were required, a safer method to esterify costic acid was investigated. Costic **26** was treated with oxalyl chloride (5 equiv), using a drop of dimethylformamide as a catalyst at 0 °C for one hour. The volatiles were removed from the reaction mixture and the residue was stirred in methanol and triethylamine to produce the costic acid methyl ester **122** in 91% yield (Scheme 4.21). The structure of methyl costate **122** was confirmed by its ^{1}H NMR spectrum as a new signal at 3.75 ppm integrating for three hydrogens due to the newly formed methyl ester.



Scheme 4.21: Esterification of costic acid **26**

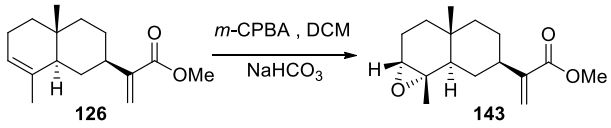
4. 6 **Epoxidation of costic acid 26**

Epoxides very useful intermediates in organic synthesis. The strain of the epoxide makes it reactive toward the nucleophilic addition (Scheme 4.22). Reacting the epoxide **250** with a good nucleophile can give new compounds. For example, treating **250** with hydride could give ilicol **251** which was isolated from *Fluorensia oolepis* by Guerreiro and co-workers which is related to ilicic acid.[101]



Scheme 4.22: Proposed synthesis of epoxide **250**

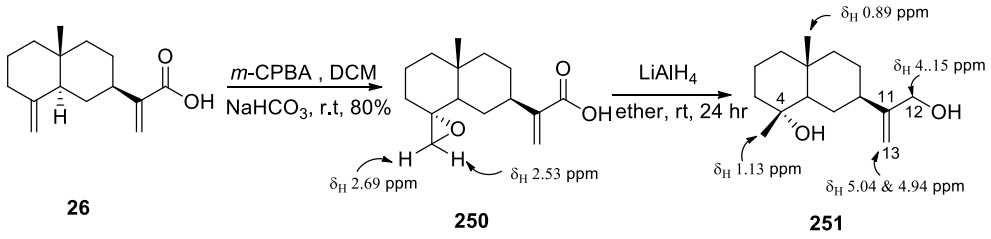
Epoxidation of costic acid **26** was investigated using *m*-CPBA following a procedure similar to that reported by Ulubelen and co-workers.[57] They treated isocostic acid methyl ester **126** with *m*-CPBA and $NaHCO_3$ at room temperature for one hour, the epoxide **143** was obtained as single stereoisomer (Scheme 4.23).



Scheme 4.23: Epoxidation of isocostic acid **126**

Treatment of costic acid **26** with 1.7 equivalents of *m*-CPBA and sodium bicarbonate afforded the epoxide **250** as one stereoisomer in 80% yield (Scheme 4.24). The epoxide **250** was identified by the disappearance of two vinylic signals in the ^{1}H NMR spectrum. The absence of signals at 4.40 and 4.60 ppm indicated the addition occurred at the exocyclic double bond. In addition, two new signals appeared at 2.69 and 2.53 ppm for hydrogens at C14. The removal of *m*-chlorobenzoic acid from the epoxide **247** using chromatography was unsuccessful due to their similar polarities, so the mixture was used directly in the next step. A solution of the crude epoxide in anhydrous ether was treated with lithium aluminium hydride at room temperature. The ^{1}H NMR spectrum of the diol **248** showed a new doublet at 4.15 ppm integrating for two hydrogens, which suggested an allylic alcohol **251**. In addition, presence of a new

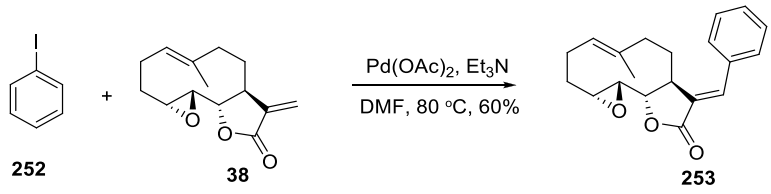
signal at 1.13 ppm indicated for methyl group at C4. The ^{1}H NMR spectrum of the diol matched the one that reported by Guerreiro and co-workers.[101]



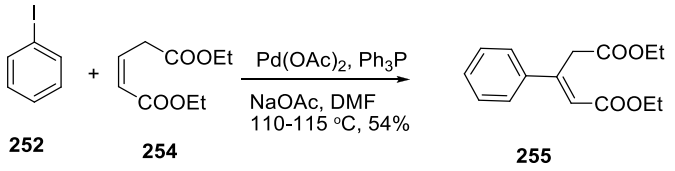
Scheme 4.24: Synthesis of ilicol **251**

4. 7 **Heck reaction with costic acid methyl ester 129**

Arylation of costic acid **26** was investigated with iodobenzene using condition reported by Han and co-workers.[102] Arylation of sesquiterpene lactone parthenolide **38** was conducted using a range of aryl iodides and its substituted, $Pd(OAc)_2$ and Et_3N in DMF at 80 ˚C give compound **253**. Iodobenzene **252** was also reacted with diethyl glutaconate **254**, $Pd(OAc)_2$ and triphenylphosphine at 115 ˚C for 12 h to give the strene **255**. [103]



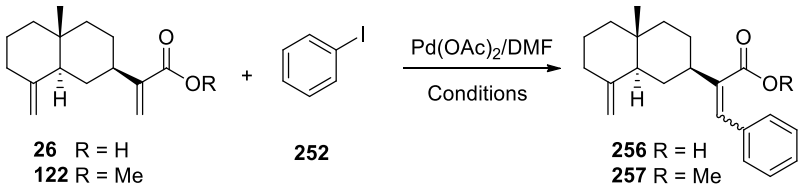
Scheme 4.25: Heck reaction



Scheme 4.26

Using the same conditions reported by Han,[102] a solution of costic acid methyl ester **122** was treated with 2.5 equivalents of aryl iodide **252**, palladium acetate, triethylamine in dimethylformamide (Table 4.4, entry 1). The reaction mixture was heated under reflux. The ^{1}H NMR spectrum of the crude product showed a complex mixture of products. The alternate method reported by Padmanabhan,[103] was then attempted. A solution of costic acid was treated with of 1.2 equivalents iodobenzene **252**, palladium acetate, triphenylphosphine and sodium acetate (Table 4.4, entry 2). The reaction mixture turned black after 2 h under reflux. The ^{1}H NMR spectrum of the crude product showed a complex mixture. Increasing iodobenzene equivalents to 2.5 gave the same result (Table 4.4, entry 3).

Table 4.4: Arylation of costic acid



26 R = H
122 R = Me

252

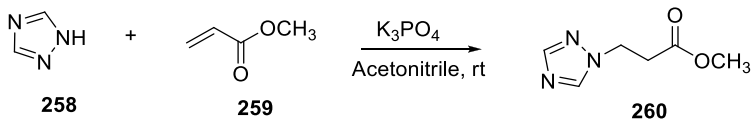
256 R = H
257 R = Me

Entry	R	Conditions	Results
1	Me	PhI 2.5 eq, Et_3N, DMF, 80 °C	Complex mixture
2	H	PhI 1.2 eq, Ph_3P, NaOAc, DMF, 100 °C	Complex mixture
3	Me	PhI 2.5 eq, Ph_3P, NaOAc, DMF, 100 °C	Complex mixture
3	H	PhI 2.5 eq, Ph_3P, NaOAc, DMF, 100 °C	Complex mixture

4.27

4. 8 **Addition of 1,2,4-triazole to costic acid methyl ester 122**

An attempt to introduce nitrogen to the costic acid was investigated. Hou and co-workers added a range of heterocyclic compounds (imidazole, 1,2,4-triazole, indole and benzotriazole) to the α,β-unsaturated carbonyl compounds through Michael addition using anhydrous K_3PO_4 at room temperature in excellent yield (Scheme 4.28).[104]

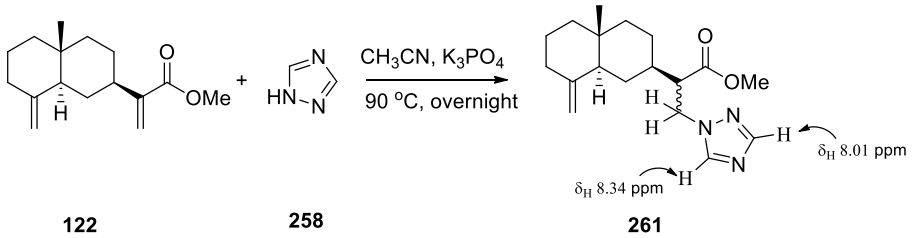


258 259 260

Scheme 4.28: Michael Addition

Using the same conditions reported by Hou, costic acid methyl ester **122** was treated with one equivalent of 1,2,4-triazole **258** and 25 mol% anhydrous potassium phosphate 25 mol% in acetonitrile at room temperature for 24 h. The ^{1}H NMR spectrum of the crude product showed only the starting material (Table 4.5, entry 1). The reaction mixture was heated under reflux for 24 h. (Table 4.5, entry 2), but no reaction was observed. Increasing the amount of the base K_3PO_4 to 0.50 equivalents and heating the reaction mixture under reflux for 24 h gave a yellow oil containing a new compound **261**. The ^{1}H NMR spectrum of this compound showed new signals at 8.07 and 7.92 ppm integrating to one hydrogen each, and absence of the two vinylic signals at 6.14 and 5.56 ppm were indicative of the Michael adduct **261**. The ^{1}H and ^{13}C NMR spectra of the product showed duplicate signals, which suggested formation of two isomers in a 1:1 ratio.

Table 4.5: Michael addition



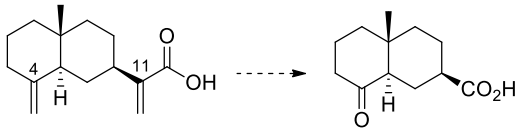
122 258 261

Entry	Conditions	Yield 261
1	K_3PO_4, 0.25 eq , CH_3CN, rt, 24h	N.R
2	K_3PO_4 0.25 eq, CH_3CN, reflux, 24h	N.R
3	K_3PO_4, 0.50 eq, CH_3CN, reflux, 24h	45%

4. 9 **Oxidation of costic acid**

A simplified decalin framework derived from costic acid may be more useful as starting material for medical chemistry programs. The ketoacid **217** is a useful synthon

as it has a ketone and a carboxylic acid attached to the decalin ring. This molecule has been made in racemic form by Carlson and Zey.[105] Ketoacid **217** was used as a key intermediate to make a several *β*-eudesmol derivatives. The ketoacid could be synthesised from the costic acid **26** by the oxidative cleavage of the alkenes at C4-C14 and/or C11-C13 positions.

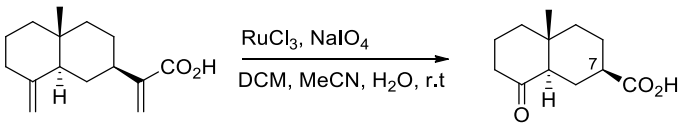


26 **217**

Scheme 4.29: oxidation of costic aicd **26**

The oxidative cleavage of the double bonds of the costic acid **26** was investigated using ruthenium tetroxide generated *in situ*.[106, 107] When a mixture of costic acid **26**, ruthenium chloride hydrate and sodium periodate in water, acetonitrile and dichloromethane (dichloromethane was used instead of carbon tetrachloride as reported) was stirred at room temperature for two hours (Table 4.6, entry 1), the ketoacid **217** was obtained in 46% yield. Extending the reaction time to 24 hours gave the ketoacid in 56% yield (Table 4.6, entry 2). The ^{1}H NMR spectrum of the ketoacid **217** shows absence of the vinylic hydrogens. In addition, a new signal existed at 9.88 ppm which indicated the carboxylic proton (COOH). The compound had 12 carbons in its skeleton based on the ^{13}C NMR spectrum. Signals at 211.5 ppm and 180.1 ppm in ^{13}C NMR spectrum indicated the presence of a ketone and carboxylic acid respectively. The carboxylic group was confirmed by IR spectra with a carbonyl stretching frequency at 1705 cm^{-1}, and a broad absorbance at 2500 – 3300 cm^{-1} indicating an OH. The ^{13}C and ^{1}H NMR spectra matched the literature racemic mixture.[108, 105, 109] The relative stereochemistry of the product could not be confirmed by NMR spectroscopy as the carboxylic acid at C7 could epimerise. Recrystallisation using dichloromethane and petrol afforded a single crystal suitable for x-ray analysis. Structural determination was performed by Professor Brian Skelton at the University of Western Australia. The X-ray structure confirmed the suspected structure (Figure 4.2). The decalin ring had a chair conformation with *anti* the bridgehead substituents. A carboxylic acid group at C7 was on the same face as the bridgehead methyl.

Table 4.6: Transformation of costic acid to ketoacid



26 **217**

Entry	Conditions	Yield
1	RuCl. xH_2O, $NaIO_4$, DCM:MeCN: H_2O (1:1:1.5), r.t, 2h	46%
2	RuCl. xH_2O, $NaIO_4$, DCM:MCN: H_2O (1:1:1.5), r.t, 24h	56%

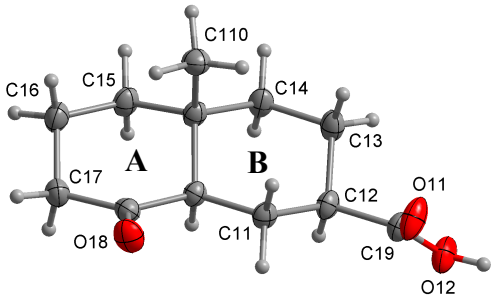
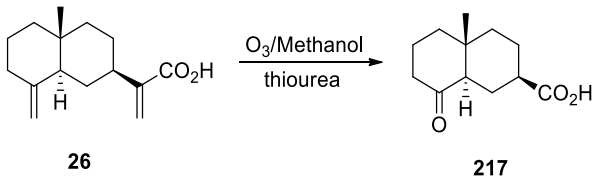


Figure 4.2: X-ray structure of the ketoacid **217**

4. 10 **Ozonolysis of costic acid**

As the ketoacid was difficult to purify from the oxidation of costic acid **26** using RuO_4, ozone was tested as an oxidant. Ozonolysis of costic acid **26** was performed followed the procedure reported by Payne and co-workers.[110] A solution of costic acid **26** in methanol and dichloromethane was treated with ozonised oxygen at – 78 ˚C until the reaction colour turned blue. The reaction mixture was stirred overnight with thiourea to reduce the peroxides produced during the reaction and gave the ketoacid **217** in 70% yield.

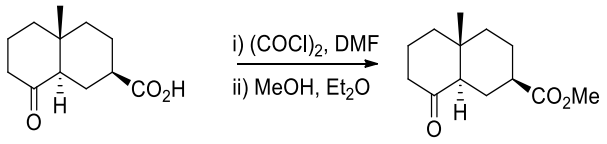


26 **217**

Scheme 4.30: Ozonolysis of the costic acid

4. 11 **Esterification of the ketoacid 217**

Esterification of ketoacid **217** was investigated using the same procedure as in Scheme 4.6. The ketoacid **217** was treated with oxalyl chloride (5 equiv), using a drop of dimethylformamide as a catalyst at 0 °C for one hour. The volatiles were removed from the reaction and the residue was stirred in methanol and triethylamine to produce the ketoester **262** in 85% yield (Scheme 4.31). The structure of ketoester **262** was confirmed by its ^{1}H NMR spectrum as a new signal at 3.66 ppm integrating for three hydrogens due to the newly formed methyl ester.

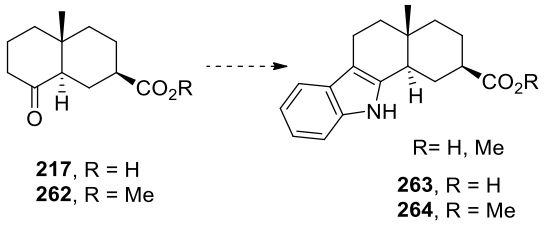


217 **262**

Scheme 4.31: Esterification of ketoacid **217**

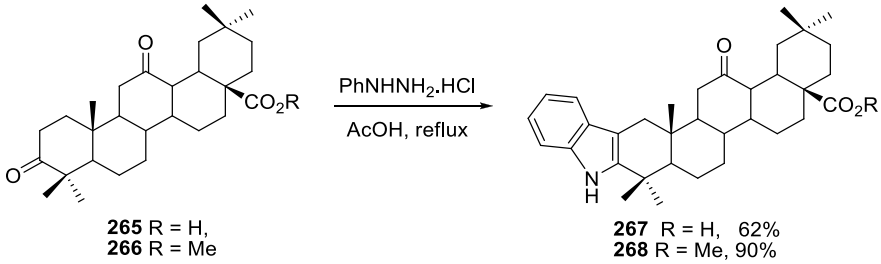
4. 12 **Synthesis of indole derivatives**

There are many indole derivatives that have important biological activity in human, animal and plants. The Fischer indole synthesis was discovered by Emil Fischer in 1883 and provides easy access to these compounds. The ketoacids **217** and **262** could be substrates for the Fisher indole synthesis to make the indole-terpene hybrids **263** and **264** (Scheme 4.32).



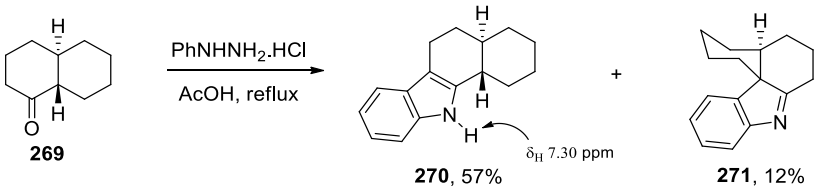
Scheme 4.32: Proposed synthesis of the indole **263** and **264**

Indoles **267** and **268** were synthesised in one step from the triterpene derivative **265/266**.[111] Treatment of triterpenes **265** or/and **266** with phenylhydrazine in acetic acid and heat under reflux gave the corresponding indole in good yield (Scheme 4.33).[111] The indole synthesis of the ester **266** gave high yield 90%, whereas the acid **265** gave modest 62% yields of the indole **267**.



Scheme 4.33: Synthesis of the indole **267** and **268**

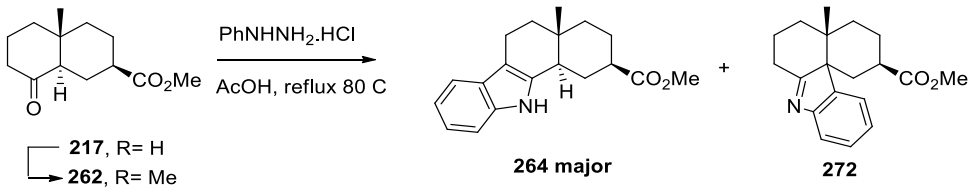
Indoles **270** and **271** were synthesised in one step from the *trans*-1-decalone **269**.[112] Treatment of *trans*-1-decalone **269** with substituted phenylhydrazine in acetic acid and heat under reflux gave two products (Scheme 4.34). Indole **270** was optained in a good yield 57% whereas indole **271** produced in 12%.



Scheme 4.34: Synthesis of indole **270** and **271**

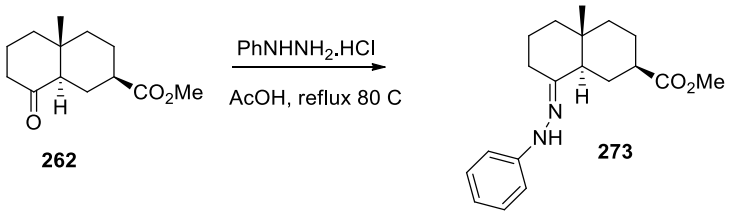
The ketoester **262** has a similar structure of 1-decalone and the treatment of the ketoester **262** with phenylhydrazine could give the two products. The ketoester **262**

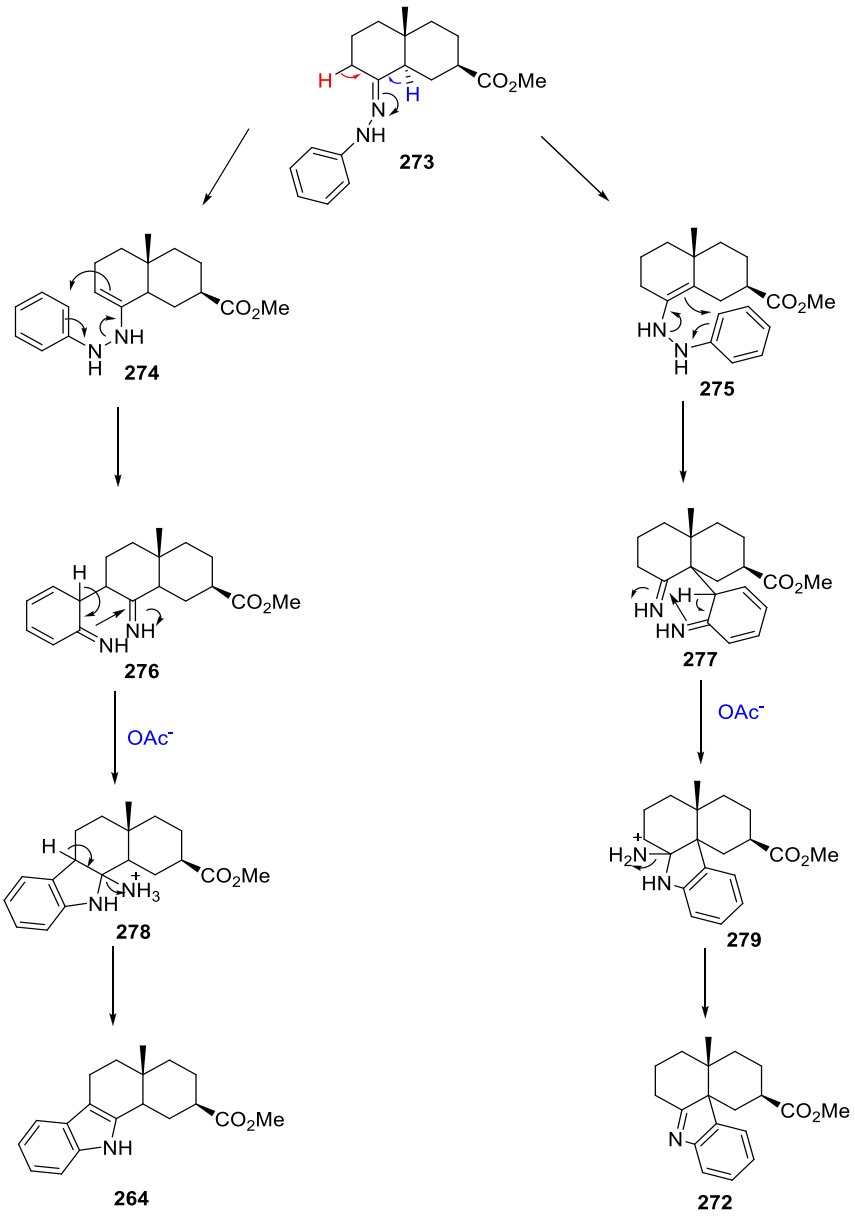
was treated with 2 equivalents of phenylhydrazine in glacial acetic acid and heated under reflux 80 ˚C for 30 minutes, two new products were obtained (Scheme 4.35) with similar spectra to **270** and **271**. The ^{1}H NMR of the indole **264** showed a broad signal at 7.81 ppm integrating for one hydrogen assigned to the indole NH; and two apparent doublets at 7.44 and 7.30 ppm integrating for one hydrogen each and multiplet at 7.09 ppm for two hydrogens associated with the benzene ring.



Scheme 4.35: Synthesis of the indole

The proposed mechanism for formation the indole **264** and **272** (Scheme 4.35) is described in Scheme 4.36. A condensation reaction between the hydrazine and the ketoester **262** in presence of acid form the ayrlhydrazones **273**. Rearrangement of the ayrlhydrazones **273** could give two intermediates **274** and/or **275**. These intermediates rearrange with a (3,3) sigmatropic shift to form new C-C bond. Then benzene ring restored and the five members ring cyclic. Finally, loss of ammonia to form a double bond in the five member ring and give the final products. The two compounds were not stable, indole **264** was the major compound, another indole **272** degraded during further purification

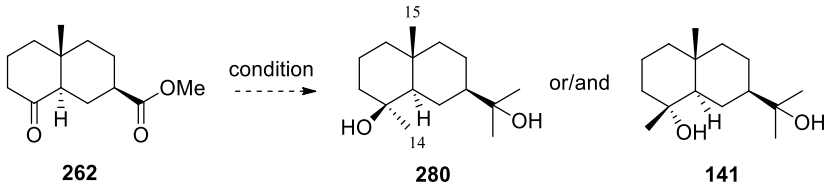




Scheme 4.36: Proposed mechanism for the synthesis of compound **264** and **272**

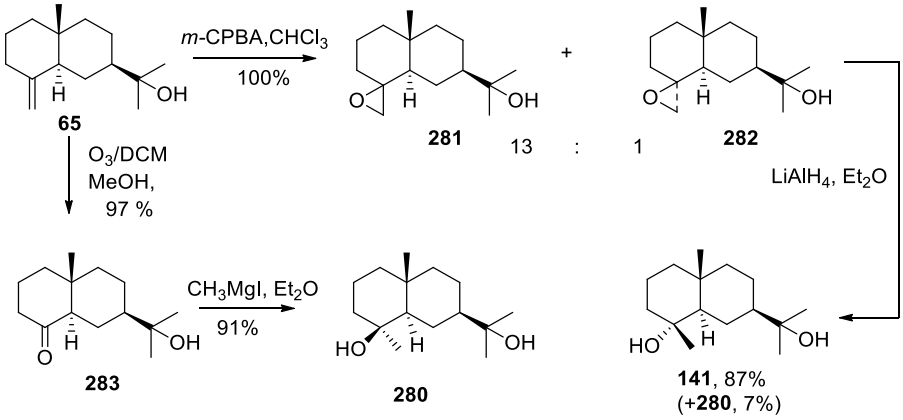
4. 13 **Addition of methylmagnesium iodide**

The reaction of the ketoester **262** with an excess of methylmagnesium iodide would give a product with three extra methyl groups. The two possible products from this reaction are the *β*-alcohol **280** or the other C-4 epimer, *α*-alcohol **141** (Scheme 4.37). These compounds **280** and **141** are effectively sesquiterpenes again.



Scheme 4.37: Proposed of addition Grignard reagent to the ketoester **262**

These alcohols **141** and **280** were synthesised from *β*–eudesmol **65** in two pathways as reported by Ando and co-workers (Scheme 4.38).[113] Epoxidation of *β*-eudesmol **65** using *m*-CPBA gave two isomers epoxide **281** and **282**, which were then treated with lithium aluminium hydride to give two epimers of diols **141** and **280** in 87% and 7% yield respectively. The synthesis of the alcohol **280** was improved, *β*–eudesmol was subjected to ozonised oxygen to give ketone **283** in 97% yield. Addition of methylmagnesium iodide to the ketone **283** gave exclusively the diol **280** in 88% overall yield.



Scheme 4.38: Synthesis of alcohols **280** and **141**

When an excess of methylmagnesium iodide was added dropwise to the ketoester **262** at 0 °C a single isomer of the diol **280** in 70% yield was obtained (Scheme 4.34). The ^{13}C and ^{1}H NMR spectra of the diol **280** matched the diol reported by Ando (Table 4.7).[113] The bottom face of the ketoester **262** is less hindered than the top face (Figure 4.4), and the addition proceeds via the bottom face to give diol **280**. The addition to the top face of the ketoester **262** is not observed as the Bürgi–Dunitz trajectory of Grignard would be hindered by the bridgehead methyl group (Figure 4.3).

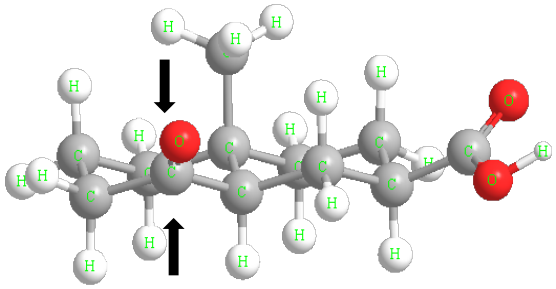
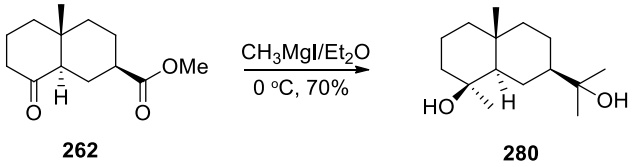
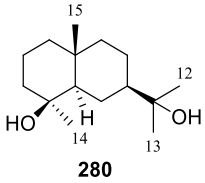


Figure 4.3: 3D of the ketoacid **217**



Scheme 4.39: Synthesis of eudesmol **280**

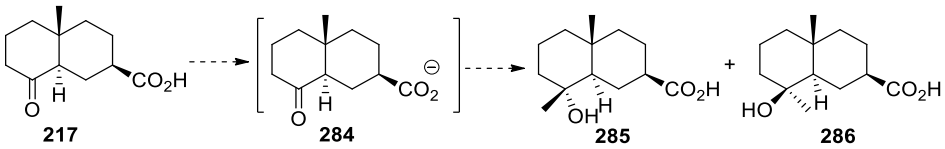
Table 4.7: NMR comparison of compound **280** (ppm)



	Diol **280** [113]*		Diol **280****	
position	^{1}H	^{13}C	^{1}H	^{13}C
12	1.22	26.84	1.21	26.81
13	1.22	27.48	1.21	27.48
14	1.18	30.32	1.17	30.29
15	1.03	18.66	1.02	18.66

*^{1}H 200 MHz, ^{13}C 50.3 MHz **^{1}H 400 MHz, ^{13}C 100 MHz.

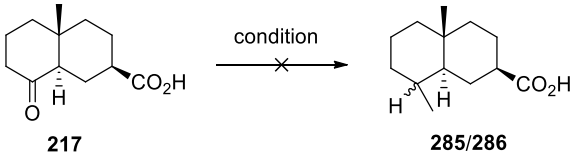
The exclusive selectivity in the reaction between methylmagnesium iodide and ketoester **262** in Scheme 4.39 encouraged further reactions. Addition of methylmagnesium iodide to the ketoacid **217** would first form a carboxylate **284** which would prevent further addition of the nucleophile at that position. This reaction could then produce two stereoisomers, *β*-alcohol **285** or the other C-4 epimer α-alcohol **286** (Scheme 4.40).



Scheme 4.40: Addition selectivity of Grignard reagent

To a solution of ketoacid **217** in anhydrous tetrahydrofuran was added three equivalents methylmagnesium iodide solution at -84 ˚C (Table 4.8, entry 1). The ^{1}H NMR spectrum of the crude mixture only showed starting material. Using more forcing conditions by increasing the temperature and the number of equivalents of methylmagnesium iodide gave the same result (Table 4.8, entry 2, 3). When the reaction was heated under reflux for three hours a complex mixture was obtained (Table 4.8, entry 4). This suppressing observation could be caused by the formation of an enolate (acid–base reaction) or the carboxylate may deter addition of the nucleophile by columbic repulsion.

Table 4.8: Grignard addition

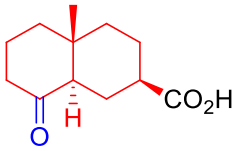


Entry	Conditions	Results
1	MeMgI 3 eq, THF, -84 ˚C	N.R
2	MeMgI 3 eq, THF, 0 ˚C to r.t.	N.R
3	MeMgI 10 eq, THF, -84 ˚C to r.t.	N.R
4	MeMgI 10 eq, THF, 0 ˚C to r.t then reflux	Complex mixture

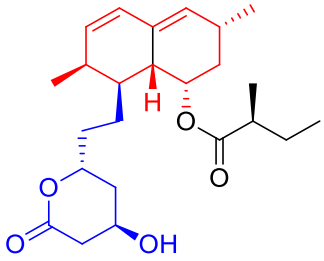
Approaches to lovastatin-like compound

The ultimate goal of this research was to prepare lovastatin-like compounds. Lovastatin belong to a group of cholesterol lowering drugs, called the statins, and are the highest selling class of drugs in the world. The ketoacid **217** contains a decalin ring similar to lovastatin (Figure 4.4). The key challenge to this lovastatin mimetic would

be to attach the essential feature of the statins, the hydroxylactone moiety, to the decalin core of **217**. Addition of substituent to ketone by a Wittig olefination may provide rapid access to the hydroxylactone.



217



Lovastatin **287**

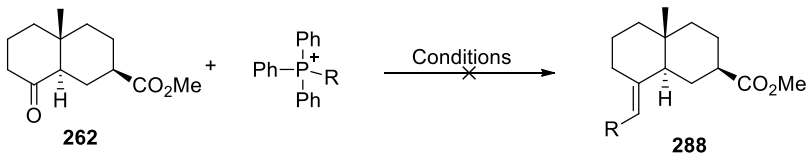
Figure 4.4: Lovastatin-like compound

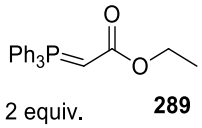
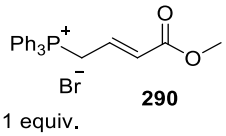
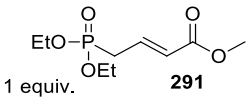
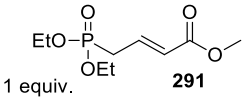
4. 14 **Wittig reaction**

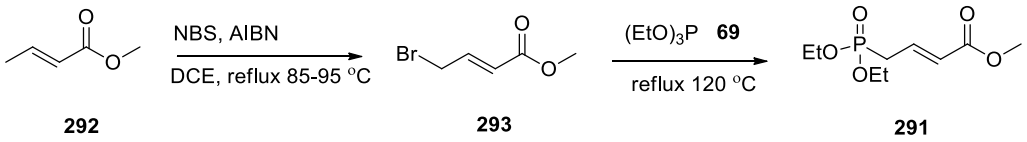
Wittig olefination of the ketoester **262** was initially tested using a stabilized ylide. The stabilize ylide **289** and the ketoester **262** were heated under reflux in chlorobenzene overnight (Table 4.9, entry 1). The ^{1}H NMR spectrum of the crude showed only recovered starting material. Since this reaction was unsuccessful, a more reactive ylide was used. The phosphonium salt **290** was performed, the salt **290** was treated with 2 equivalents of *n*-BuLi at 0 ˚C followed by addition of the ketoester **262** (Table 4.9, entry 2) but no reaction occurred. The phosphonate ester **291** is less hindered and would add to the ketoester **262** (Table 4.9, entry 4). Phosphonate ester **291** was prepared from methyl crotonate **292** following procedure reported by Banerji and Pal in two steps (Scheme 4.41).[114] Radical bromination of methyl crotonate **292** using the radical initiator AIBN and *N*-bromosuccinimide (1 equiv) gave the bromocrotonate **293** in 53% yield. The bromocrotonate **293** was then treated with triethylphosphite and heated under reflux for 4 hours to give the phosphonate ester **291** in 90% yield (Scheme 4.41). Then the phosphonate ester **291** was treated with 2 equivalents of *n*-BuLi in anhydrous tetrahydrofuran at -84 ˚C (Table 4.9, entry 4). The mixture allowed to warm to room temperature over 30 minutes, then cooled again and the ketoester **262** was added, but unfortunately, only recovered the starting material. The reaction was revisited by increasing the number of equivalents of the ylide, but this again also no result (Table 4.9, entry 5). Both the ketone in the ketoester **262** and the ylide are

sterically hindered and may prevent the Wittig olefination. A competing enolisation of the ketone of **262** under basic conditions may also a contributing factor (see later in the chapter).

Table 4.9: Wittig olefination Addition



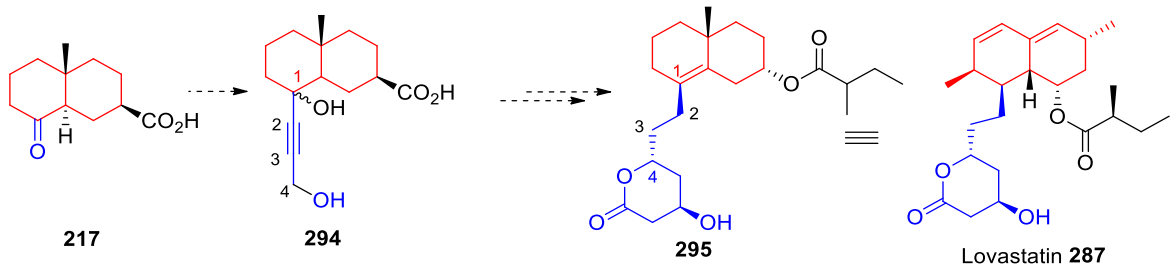
Entry	Phosphonium salts	Conditions	Result
1	 2 equiv. **289**	Chlorobenzene, reflux	N.R
2	 1 equiv. **290**	1.2 equiv. *n*-BuLi, THF , 0℃ - r.t.	N.R
3	 1 equiv. **291**	2 equiv. *n*-BuLi, THF, -8 4℃ - r.t.	N.R
4	 1 equiv. **291**	3 equiv. *n*-BuLi, THF, 0℃ - r.t.	N.R



Scheme 4.41: Synthesis of phosphonate ester **291**

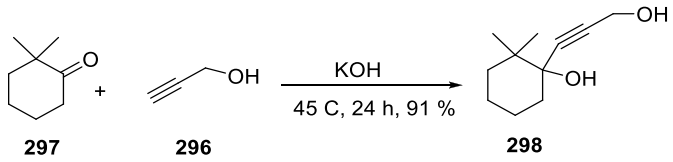
4. 15 **Addition of propargyl alcohol**

An attempt to the synthesis of a lovastatin-like compound **295** starting from the intermediate ketoacid **217** was investigated. Lovastatin could be synthesised from the ketoacid (Scheme 4.42). Addition of propargyl alcohol **296** to the ketone in ketoacid **217** would be produced **294** which is the first step toward the semisynthesis of the statin-like **295**.



Scheme 4.42

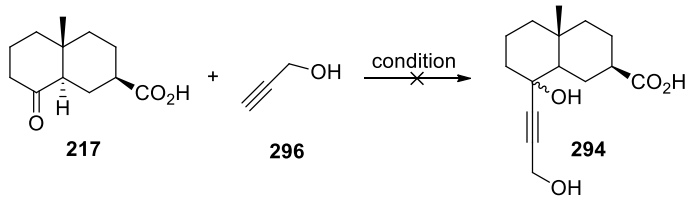
An example of addition of propargyl alcohol **296** to cyclohexanones such as **297** has been demonstrated by Ueno and co-workers (Scheme 4.43).[115] Treatment of cyclohexanone **297** with potassium hydroxide and propargyl alcohol **296** overnight at 45 ˚C gave **298** in 91% yield.



Scheme 4.43

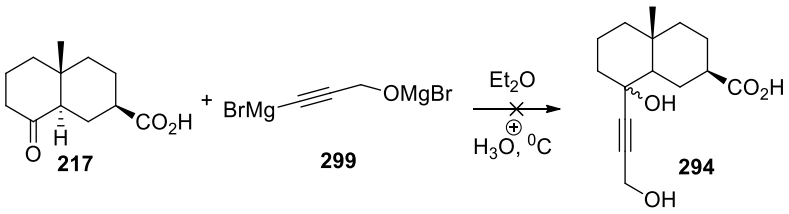
Addition of the propargyl alcohol **296** to the ketoacid **217** was investigated following the same condition reported by Ueno.[115] A solution of ketoacid **217** in dichloromethane was treated with potassium hydroxide and propargyl alcohol **296** for 24 hours at 45 ˚C (Table 4.10, entry 1). The ^{1}H NMR spectrum of the crude mixture showed only recovered starting material. The addition reaction was repeated using dilithiated propargyl alcohol. When *n*-BuLi was added to propargyl alcohol **296** in anhydrous tetrahydrofuran followed by addition of ketoacid **217** (Table 4.10, entry 2), only starting material was recovered. More forcing reaction conditions were used by increasing the temperature to room temperature and increasing the number of equivalents of dilithiated propargyl alcohol but disappointingly only the starting material was recovered again (Table 4.9, entry 3, 4).

Table 4.10: Addition of propargyl alcohol



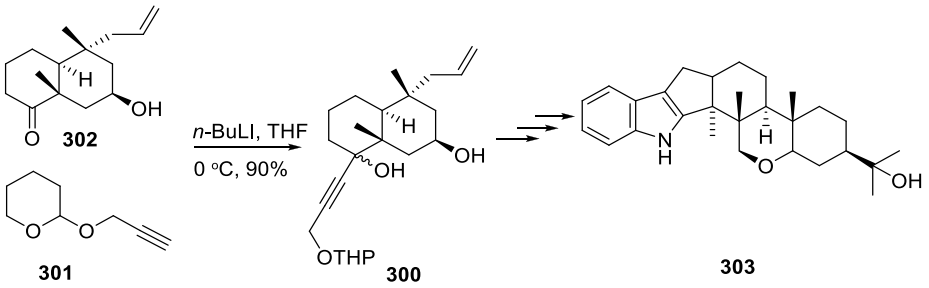
Entry	Conditions	Results
1	Propargyl alcohol, KOH, H_2O, 45- 40 °C	N.R
2	2 equiv. Propargyl alcohol, *n*-BuLi 4 equiv., -84	N.R
3	2 equiv. Propargyl alcohol, *n*-BuLi 4 equiv., -84 – r.t.	N.R
3	2 equiv. Propargyl alcohol, *n*-BuLi 8 equiv., -84 – r.t.	N.R

Since the addition of propargyl alcohol to the ketoacid was problematic, a less basic reagent was used. Compound **299** was prepared by the following the procedure. Propargyl alcohol in anhydrous ether was treated with ethylmagnesium bromide at 0 ˚C and the reaction was allowed to warm to the room temperature for 30 minutes to give magnesium salt of the propargyl alcohol **299**. To this solution was added the ketoacid **217** at 0 ˚C (Scheme 4.44). Unfortunately, the starting material was recovered.



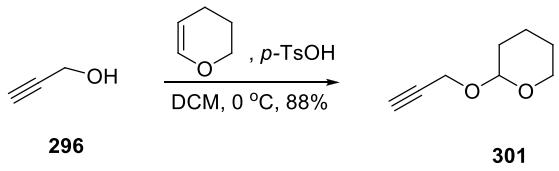
Scheme 4.44: Addition of magnesium bromide salt of propargyl alcohol **299**

Since all attempts of addition of the propargyl alcohol **299** to the ketoacid **217** were unsuccessful, a protected form was used. Mewshaw and co-workers synthesised compound **300** in 90% yield using a THP protected propargyl alcohol **301** to prepare paspaline **303** (Scheme 4.45).[116] The alkyne **301** was treated with *n*-BuLi in anhydrous tetrahydrofuran at -84 ˚C for 1.5 hour then allowed to warm to 0 ˚C. The reaction was cooled again to -84 ˚C, a solution of ketone **302** was added the reaction and the mixture allowed to warm to room temperature to give compound **300** in 90% yield.



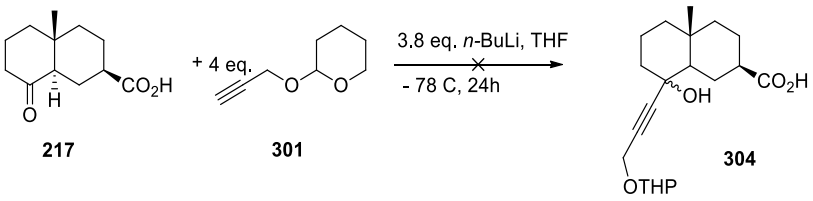
Scheme 4.45

Propargyl alcohol **296** was protected using the tetrahydropyranyl group (THP) following the procedure provided by Hu and co-workers.[117] A solution of propargyl alcohol **296** in dichloromethane was treated with *p*-toluenesulfonic acid and 3,4-dihydro-2*H*-pyran at 0 ˚C for 5 minutes. The reaction was then allowed to warm to room temperature for 3 hours to give the acetal **301** in 88% yield. The ^{1}H NMR spectrum of the compound **301** matched the literature.[118]



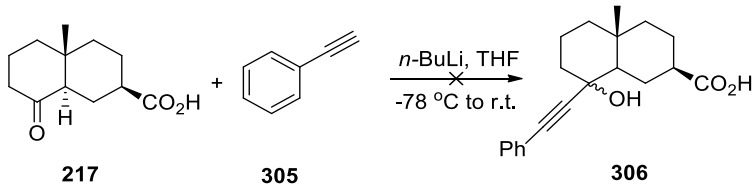
Scheme 4.46

Following the method reported by Mewshaw,[116] a solution of protected propargyl alcohol **301** in anhydrous tetrahydrofuran was treated with *n*-BuLi at -84 ˚C followed by addition of the ketoacid **217** (Scheme 4.47). The ^{1}H NMR spectrum of the crude material showed again only recovered the starting material. Repeating the reaction but using the dianionic salt of propargyl alcohol gave the same result.



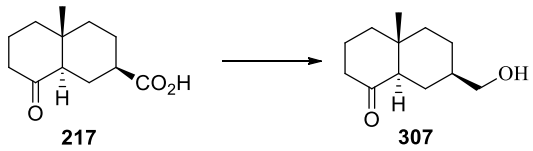
Scheme 4.47: Addition of protected propargyl alcohol **301**

Since addition of protected propargyl alcohol to the ketoacid **217** did not work, the reaction repeated using phenylacetylene **305** as a model (Scheme 4.48). A solution of phenylacetylene **305** in anhydrous ether was treated with 1 equivalent of *n*-BuLi at -84 ˚C, the reaction then allowed to warm to the room temperature. Next the reaction was cooled again to –84 ˚C, and then the ketoacid was added dropwise. The mixture allowed then to warm to the room temperature for 24 hours. The ^{1}H NMR spectrum of the crude showed starting material was recovered.



Scheme 4.48: Alkynylation of ketoacid **217**

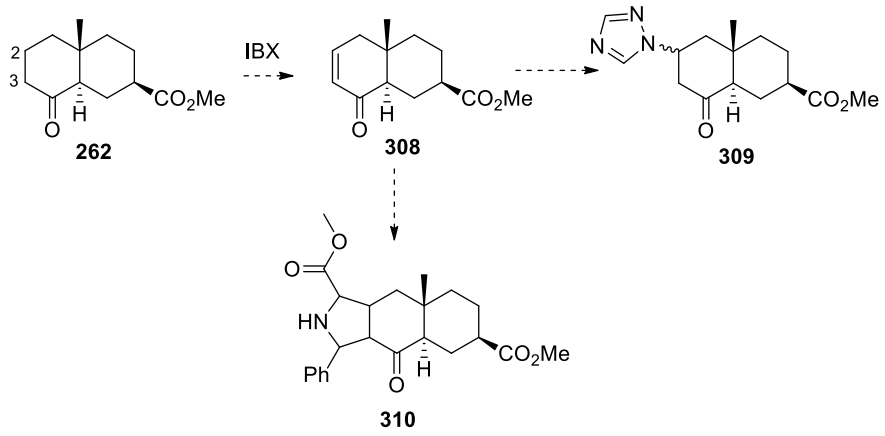
Addition of nucleophiles to the ketoacid was unsuccessful and this unexpected observation could be caused by two factors. The nucleophiles in these reactions may behave as bases and lead to the formation of an enolate (i.e. acid–base reaction). Another potential obstacle is the carboxylate anion may deter the addition of the negatively charged nucleophile by columbic repulsion. To overcome this observation, future endeavours would reduce the ketoacid **217** to ketoalcohol **307** (Scheme 4.49) which should overcome these issues.



Scheme 4.49

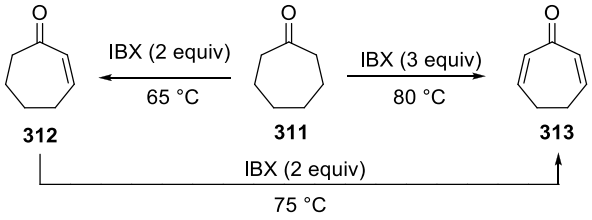
4. 16 **Synthesis of *α,β*-unsaturated carbonyl compound**

Ketoester **262** could be dehydrogenated to *α,β*-unsaturated carbonyl compound **308**. The resulting *α,β*-unsaturated carbonyl compound allows the functionalisation of the C2 and C3 position of the decalin ring by using either a Michael addition or cycloaddition reaction. It would also allow the introduction of nitrogen to the molecule and make it more drug-like (Scheme 4.50)



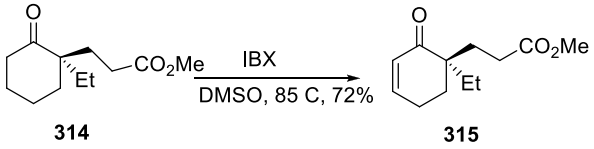
Scheme 4.50: Proposed synthesis

The ketoester **262** could be converted to *α,β*-unsaturated ketoester **308** using 2-iodoxybenzoic acid (IBX). IBX was considered the most appropriate method to dehydrogenate the carbonyl compound to an *α,β*-unsaturated compound. Nicolaou and his co-workers discovered oxidation of alcohols and ketones to enones using IBX.[119] The selectivity of the dehydrogenation using IBX has been explored using a model of cycloheptanone **311** (Scheme 4.51). Manipulation of the equivalents of 2-iodoxybenzoic acid and reaction temperature could be used to form two different compounds, 2-cyclohepten-1-one **312** and cycloheptadienone **313**.



Scheme 4.51: Dehydrogenation of cycloheptanone **311**

Nakajima and co-workers dehydrogenated ketoester **314** to *α,β*-unsaturated carbonyl compound using IBX.[120] A solution of ketoester **314** in DMSO was treated with IBX at 85 ˚C to give compound **315** in 72% yield (Scheme 4.52).



Scheme 4.52: Dehydrogenation of ketoester **314**

Dehydrogenation of ketoester **262** using IBX was investigated following the procedure reported by Nakajima (Scheme 4.53).[120] A solution of ketoester **262** in DMSO was treated with 2 equivalents of IBX. The reaction was heated at 85 ˚C overnight to give an inseparable mixture of unsaturated compound **308** and unreacted starting material in a ratio of 70:30 respectively in 65% yield. The ^{1}H NMR spectrum of the product showed newly vinylic hydrogens at 6.80 and 6.02 ppm.

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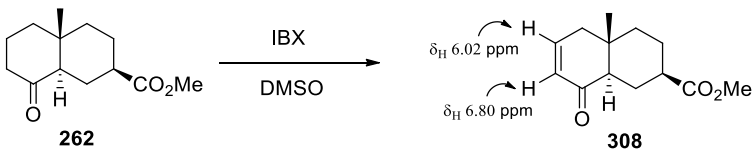
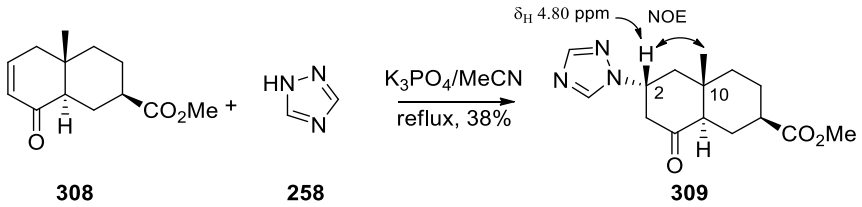


Scheme 4.53: Dehydrogenation of ketoester **262**

4. 17 **Michael addition**

Addition of 1,2,4-triazole **258** to the unsaturated carbonyl compound **308** was performed in the same way as Table 4.5. A solution of compound **308** in acetonitrile

was treated with one equivalent of 1,2,4-triazole **258** and 25 mol% anhydrous potassium phosphate. The reaction was heated under reflux for 24 hours to give compound **309** in 38% yield. The ^{1}H NMR spectrum of the product showed the presence of two new signals at 8.12 and 7.97 ppm indicative of the triazole ring. Also presence a new multiplet at 4.80 ppm in ^{1}H NMR spectrum indicated the hydrogen at C2. The stereochemistry of triazole was established by an NOE interaction between the methyl group hydrogens at C10 and the hydrogen at C2. Thus, the 1,2,4-triazole has added to the least hindered face of the enone **308**.

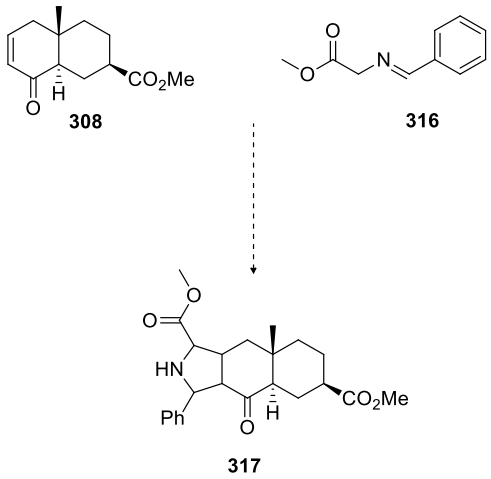


308 **258** **309**

Scheme 4.54: Michael addition

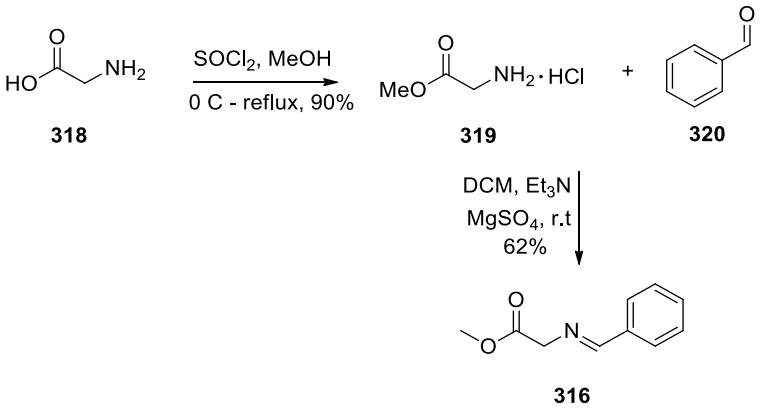
4. 18 **Cycloaddition of azomethine ylides**

Another way to introduce nitrogen atom to the decalin ring **308** using azomethine ylides **316** (Scheme 4.55). Compound **316** could be synthesised in three steps starting from the amino acid glycine. Addition of the compound **316** to the unsaturated compound **308** would give the compound **317**.



Scheme 4.55

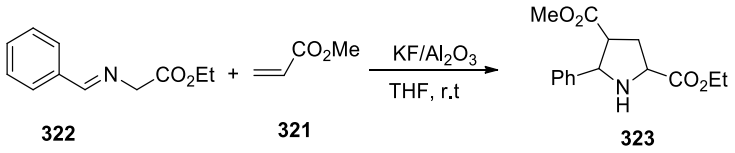
N-benzylidene glycine **316** was made by reported procedures. A solution of glycine **318** in methanol was treated with thionyl chloride under reflux to give glycine methyl ester hydrochloride **319** in 90% yield (Scheme 4.56), then following procedure reported by Cabera and co-workers,[121] a solution of glycine methyl ester hydrochloride **319** in dichloromethane was treated with anhydrous magnesium sulfate, triethyl amine and benzaldehyde **320** at room temperature overnight to give *N*-benzylideneglycine methyl ester **316** in 62% yield (Scheme 4.56).



Scheme 4.56: Synthesis of *N*-benzylidene glycine **316**

The cycloaddition reaction between α,β-unsaturated ketoester **308** and compound **316** was investigated. To a solution of unsaturated ketoester **308** in acetonitrile was added

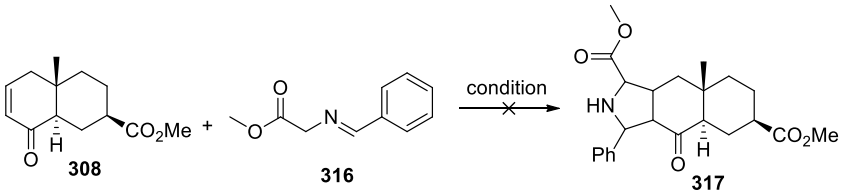
compound **316**, triethylamine, and lithium perchlorate. The reaction mixture was stirred overnight at room temperature (Table 4.11, entry 1). The ^{1}H NMR spectrum of the crude showed only recovered the starting material. The reaction was repeated with adding DBU (Table 4.11, entry 2), but unfortunately no reaction. Boruah and co-workers developed new method to synthesis pyrrolidine using KF/Al_2O_3 (Scheme 4.57).[122] Methyl acrylate **321** was added to the imine ester **322** and treated with KF/Al_2O_3 in THF at room temperature gave compound **323** in 90% yield. Following this procedure,[122] a solution of unsaturated ketoester **308** and compound **316** in anhydrous tetrahydrofuran was treated with KF/Al_2O_3 overnight at room temperature (Table 4.11, entry 3), again only starting material was obtained. An attempt alternative method was investigated following the method repeated reported by Grigg.[123] A solution of the unsaturated ketoester **308** and compound **316** in acetonitrile was treated with silver acetate, DBU at room temperature (Table 4.11, entry 4), but this again gave no result.



322 321 323

Scheme 4.57:

Table 4.11: Cycoaddition of *N*-benzylidene glycine to unsaturated ketoester

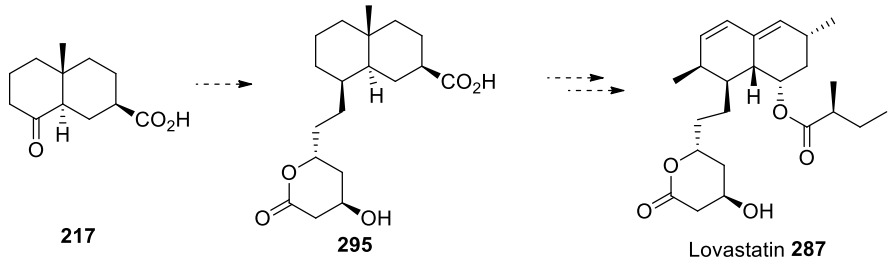


308 316 317

Entry	Conditions	results
1	$LiClO_4$, acetonitrile, room temperature	N.R
2	$LiClO_4$, DBU, acetonitrile, room temperature	N.R
3	KF/Al_2O_3, THF, room temperature	N.R
4	AgOAc, DBU, acetonitrile, room temperature	N.R

4. 19 **Conclusion**

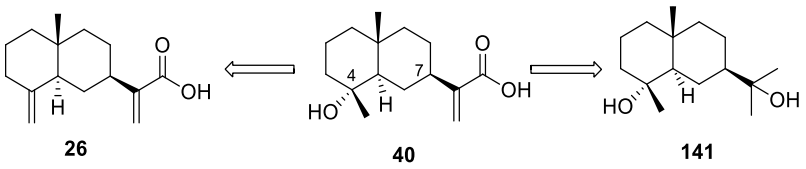
Investigation of reactions into costic acid **26** showed a number of stereoselective reactions could be applied to this compound. The chemistry of costic acid **26** was explored at two different positions, the exocyclic double bond attached to C4 and the acrylic acid attached to C7 or through the both positions at the same time. The epoxidation of costic acid **26** was highly selective. Epoxidation of costic acid using *m*-CPBA gave the epoxide **250** as a single stereoisomer. A simplified decalin framework derived from costic acid may be more useful as starting material for medical chemistry programs as it has a ketone and a carboxylic acid attached to the decalin ring. Oxidation of costic acid **26** with $RuCl.xH_2O$ afforded ketoacid **217**. The ketoacid **217** was successfully transformed to sesquiterpene diol **280** in two steps. The main difficulty discussed in this chapter was the addition of nucleophile to the ketone of the ketoacid **217**, which would have been the first step toward synthesis of the statin-like compounds similar to **287**.



Chapter 5

5. 1 **Chemistry of ilicic acid**

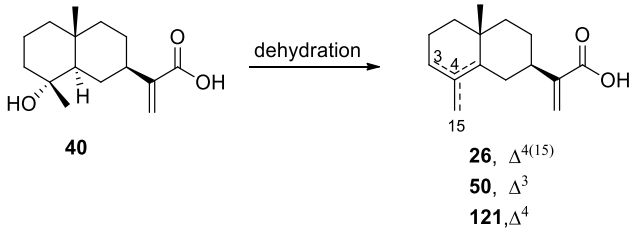
Ilicic acid was one of the sesquiterpene acids from *Dittrichia graveolens* that was also available on a large scale. To determine if this compound is a useful starting material for synthesis, a range of reactions were attempted on this compound (Scheme 5.1). Ilicic acid contains two key functional groups: tertiary hydroxyl group attached to C4 and an acrylic acid attached to C7. Only limited chemistry has been performed on ilicic acid (see Chapter 1).



Scheme 5.1

5. 2 **Transformation of ilicic acid to costic acid**

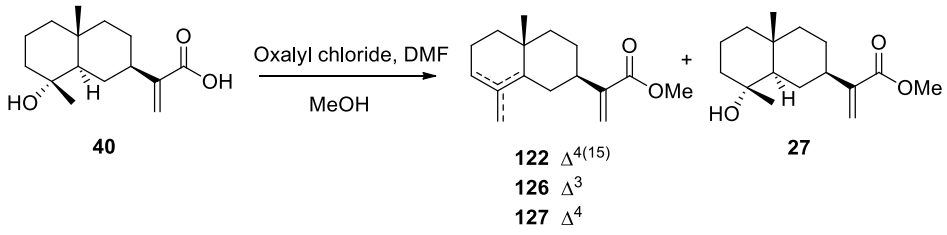
The synthesis of costic acid **26** from ilicic acid **40** was investigated. Dehydration of ilicic acid **40** was performed following the procedure similar to that provided by Kurina, *et al*., and León *et al*.[124,125] A solution of ilicic acid **40** was treated with *p*-TsOH in toluene and the reaction heated under reflux for five minutes. A mixture of inseparable isomers of costic acid as suggested by the ^{1}H NMR spectrum of the crude reaction mixture, therefore this was not a viable method to prepare costic acid.



Scheme 5.2

5. 3 **Esterification of ilicic acid**

Unlike costic acid, esterification of ilicic acid was problematic. Ilicic acid **40** was treated with oxalyl chloride (5 equiv), using a drop of dimethylformamide as a catalyst at 0 °C for one hour. The volatiles were removed from the reaction and the residue was stirred in methanol and triethylamine to produce an inseparable mixture of costic acid methyl ester isomers **122**, **126** & **127** and a small amount of the desired ilicic acid methyl ester **27** (Scheme 5.3). This highlights the acid sensitivity of 3˚ alcohol of the ilicic acid.



40

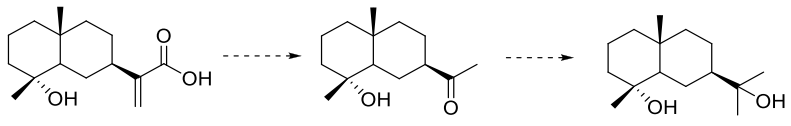
122 $\Delta^{4(15)}$
126 Δ^{3}
127 Δ^{4}

27

Scheme 5.3

5. 4 **Transform ilicic acid to ketone**

The diol **141** was isolated form *Blumea balsamifera* in 0.01% and reported as antispasmodic.[126] Diol **141** could be synthesised from ilicic acid **40** which was isolated in significant quantities. A semisynthetic pathway was proposed as shown in Scheme 5.4. Ilicic acid **40** could be transformed to the ketone **138**. Treatment of the ketone **40** with Grignard reagent could give the diol **141**. The diol **141** is the epimer of the diol **280** (Figure 5.1) which was synthesised in Chapter 4.13



40 **138** **141**

Scheme 5.4: Proposed synthesis of Diol **141**

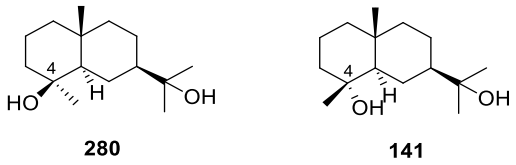
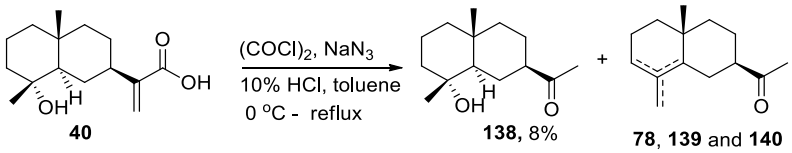


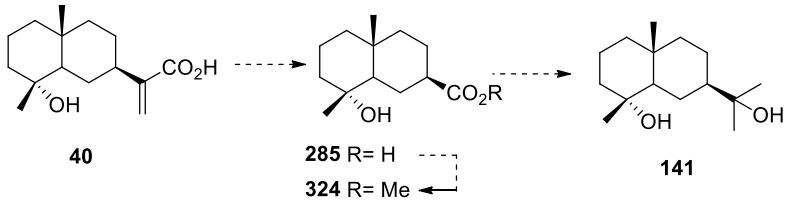
Figure 5.1: Diol epimers

Transformation of ilicic acid **40** to hydroxyketone was investigated following procedure in Scheme 4.2. Ilicic acid **40** was converted to the acid chloride using oxalyl chloride then treated with aqueous solution of sodium azide to give acyl azide. A Curtius rearrangement of the acyl azide would give the ketone **40** upon acid hydrolysis. The ^{1}H NMR spectrum of the crude showed presence of a mixture of products. Chromatography of the mixture gave two products. The first isolated product was a mixture of isomers of dehydrated ketone and the second product was the hydroxyketone **138** in 8% yield (Scheme 5.5). The ^{1}H NMR spectrum of the ketone **138** matched the literature.



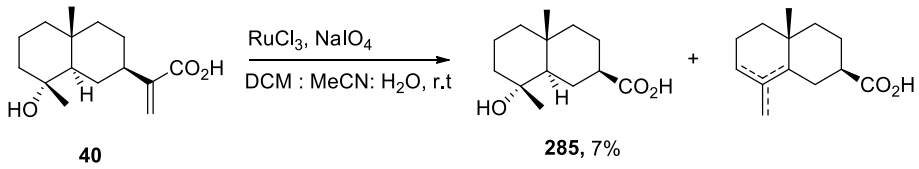
Scheme 5.5: Synthesis of ketone **138**

Since the synthesised of the intermediate ketone **138** was low yielding, an alternative pathway was investigated (Scheme 5.6). Diol **141** could be synthesised from ilicic acid **40** in three steps shown in Scheme 5.6. Oxidative cleavage of ilicic acid **40** may afford the hydroxyacid **285**, which can be esterified using diazomethane to give the hydroxyester **324**. Then treatment of the hydroxyester **324** with methylmagnesium iodide could give the desire diol **142**.



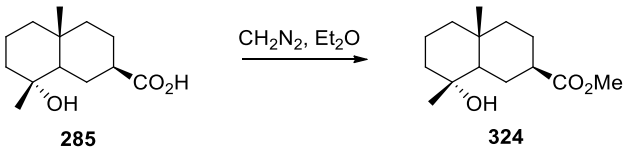
Scheme 5.6: proposed synthesis of diol **141**

The same oxidative cleavage conditions used for costic acid **26** were also used for ilicic acid **40**. When a mixture of ilicic acid **40**, ruthenium chloride hydrate and sodium periodate in water, acetonitrile and dichloromethane was stirred at room temperature overnight (Scheme 5.7), the hydroxyacid **285** was obtained in 7%. Again undesirable products derived from the elimination reaction and starting material was also recovered. The ^{1}H NMR spectrum of the hydroxyacid **285** shows absence of the vinylic hydrogens. In addition, a new signal at 9.88 ppm was present which indicated the carboxylic acid proton (COOH). The compound had 13 carbons in its skeleton by ^{13}C NMR spectrum. Signal 180.1 ppm in ^{13}C NMR indicated the presence of carboxylic acid. The carboxylic group was confirmed by IR spectra where, was a carbonyl stretching frequency at 1705 cm^{-1}, and a broad absorbance at 2500 – 3300 cm^{-1} ascribed to the hydroxyl group. From all spectroscopic data produced compound was the hydroxyacid **285**.



Scheme 5.7: Oxidation of ilicic acid **40**

To avoid the elimination of the hydroxyl group in **285** during the esterification, diazomethane was used (Scheme 5.8). A freshly prepared ethereal solution of diazomethane was added to a solution of the hydroxyacid **285** in ether stirred at 0 °C until the yellow colour remain stable. The ^{1}H NMR spectrum of the crude showed a new signal at 3.67 ppm indicated the methyl ester group. The mass obtained was very low and the hydroxyester **285** could not used in further reactions.



285 **324**

Scheme 5.8: Esterification of hydroxyacid **285**

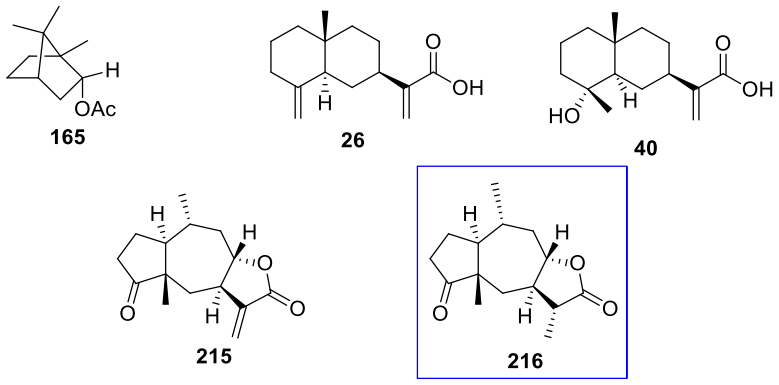
Conclusion

Unlike costic acid, the reaction of ilicic acid was problematic due to the facile dehydration of the 3° alcohol at C4. Although the hydroxyester **324** was made and it is likely to be a useful intermediate, low yields prevented further investigations. The use of ozone for the oxidative cleavage of ilicic acid to the hydroxyacid **285** could be done under neutral or basic condition and may optimise the yield.

Chapter 6

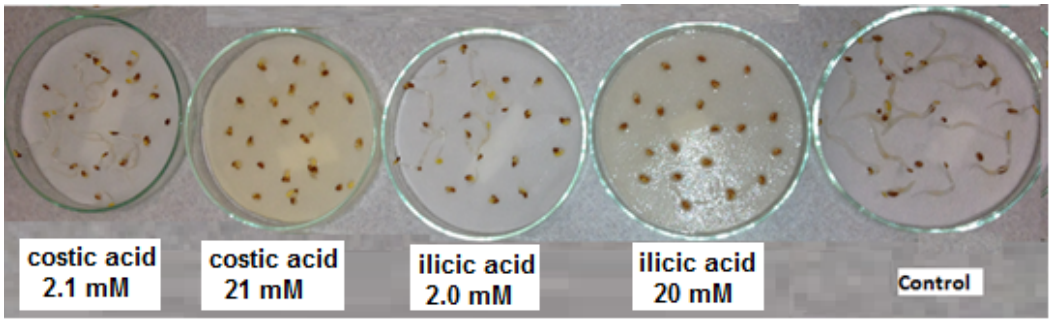
Conclusion

The aim of this study was to find a viable source of sesquiterpenes from *Dittrichia graveolens* growing in south western of Western Australia and investigate their chemistry. The main components of the *Dittrichia graveolens* were isolated using a rapid extraction technique. The resin of *D. graveolens* contained five main compounds (Figure 6.1). Compounds **26**, **40**, **165** and **215** have been previously isolated from this plant. However, the sesquiterpene lactone **216** was isolated and identified as a new compound during this study. The five compounds were isolated in a yield of about 1% w/w of the dry plant. Since costic acid **26** and ilicic acid **40** were available in a good quantity and easy to isolate using aqueous extraction, their biology and chemistry were investigated.



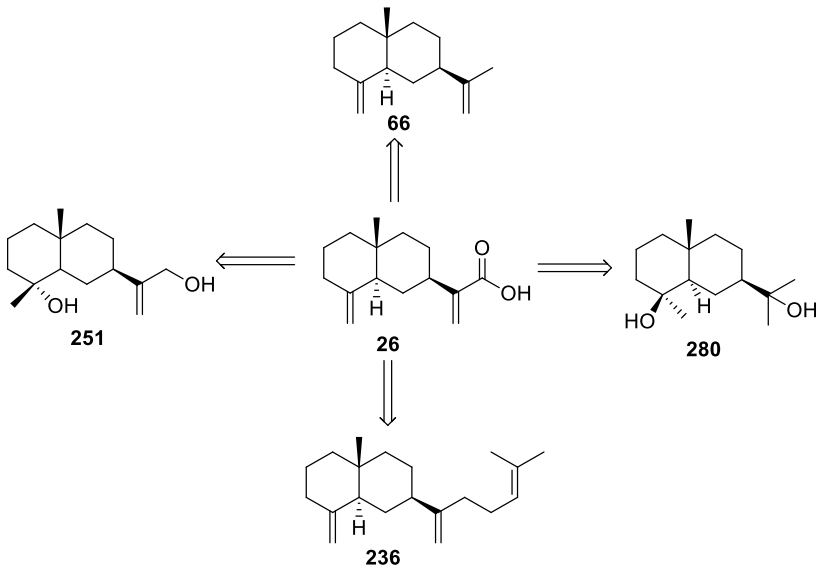
Scheme 6.1:The main components isolated from *D. graveolens*

The effect of the crude extract, costic and ilicic acid were investigated on seed germination. The crude extract showed high inhibition against alfalfa seed germination. In addition, ilicic acid showed 100% seed germination inhibition at 21 mM (Figure 6.2).



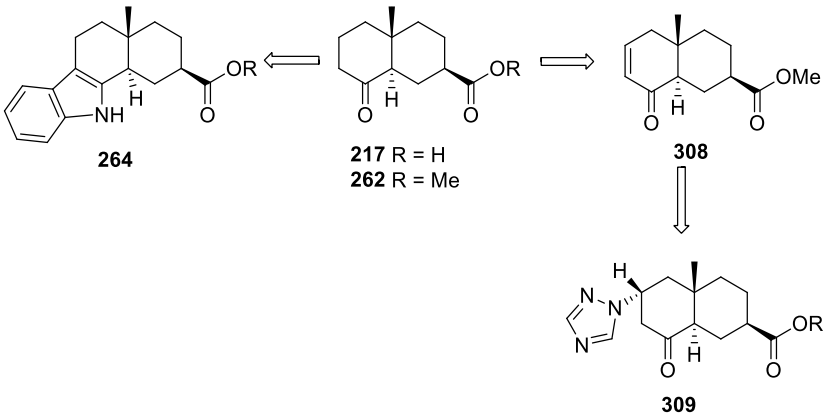
Scheme 6.2: Costic and ilicic acid seed germination bioassay

Costic acid **26** could be used to synthesise many sesquiterpenes. *β*–selinene **67** was synthesised from costic acid in 2 steps. Costic acid **26** was converted to ketone **78** which were then converted to *β*–selinene **66** by a Wittig olefination. Ilicol **280** was isolated from *Fluorensia oolepis* was prepared in two steps. A stereoselective epoxidation of costic acid **26** followed by treatment with $LiAlH_4$ gave ilicol **280**. Diol **280** was also synthesised from costic acid **26** in three steps. Costic acid **26** oxidised to the ketoacid **217** followed by esterification and the treated with methylmagnesium iodide to give the diol **280** as a single isomer. The synthesis of the deoxygenated diterpene **236** was also attempted from costic acid in three steps. Firstly, costic acid **26** was reduced to allylic alcohol **83** and then treated with butyl vinyl ether and through Claisen rearrangement afforded the aldehyde **235**. Unfortunately, olefination of the aldehyde **235** using Wittig reagent did not give the diterpene **236**.

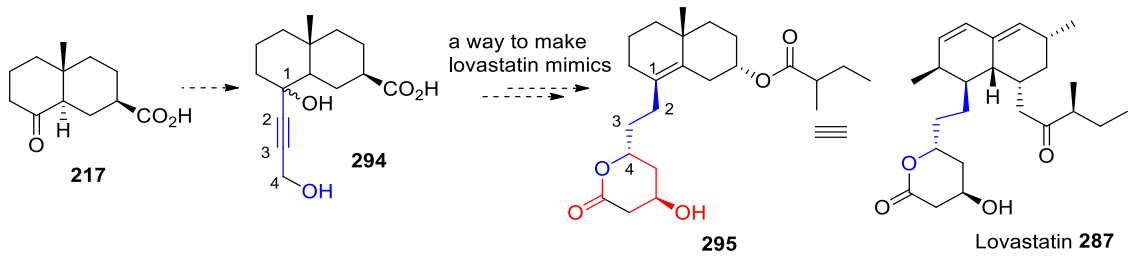


Scheme 6.3: Semisynthesis of sesquiterpenes

Costic acid was not only used for semisynthesis of terpenoids but also used to synthesise drug-like compounds. The ketoacid **217**, made by the oxidation of costic acid using either ruthenium tetroxide or ozone, could be a molecular scaffold for the pharmaceutical industry. Ketoacid **217** was used in the preparation of the indole **264** and triazole **309** (Scheme 6.2). A goal of this research was to prepare molecules resembling the cholesterol drug lovastatin (Scheme 6.3). Unfortunately, all attempts to attach a carbon chain at C4 on the ketoacid **217** were unsuccessful.



Scheme 6.4



Scheme 6.5

Overall, these results showed that useful compounds for synthesis can be obtained from *D. Graveolens* a common weed found in Western Australia.

6. 1 **Experimental**

All reactions were conducted under an atmosphere of nitrogen at room temperature unless otherwise stated. Materials were purchased from commercial sources and used without further purification unless otherwise stated. Dry solvents were prepared according to Armarego and Chai.[127] Melting points were measured using Barnstead Electrothermal 9100 melting point apparatus. IR spectra were recorded using a Perkin Elmer Spectrum 100 FT-IR spectrometer fitted with a universal ATR sampling accessory. NMR spectra were collected using a Bruker AVANCE III 400 MHz spectrometer. All ^{1}H NMR spectra were recorded at a frequency of 400.1 MHz, ^{13}C NMR spectra were recorded at a frequency of 100.6 MHz. NMR spectra were referenced to their respective solvents: chloroform-d ($CDCl_3$, ^{1}H, 7.26 ppm, ^{13}C, 77.16 ppm); acetone d_6 (^{1}H, 2.05 ppm, ^{13}C, 29.84 ppm); dimethylsulfoxide-d_6 (DMSO-d_6 ^{1}H, 2.50 ppm, ^{13}C, 39.5 ppm). Multiplicity was assigned as follows: s = singlet, d = doublet, t = triplet, q = quarter, m = multiplet and br = broad. Optical rotations (α) were obtained from a Rudolph Research Analytical Autopol I polarimeter.

Chromatography was performed using flash chromatography on silica gel (230-400 mesh, SiliaCycle, Canada) with the solvents stated. Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 μm TLC plates with aluminium backing and containing F254 fluorescent indicator. Compounds were visualised on the TLC plates using ultraviolet light followed by staining with potassium permanganate solution or vanillin. Reactions carried out at -84 °C, refer to a liquid nitrogen-ethyl acetate bath.

Plant material

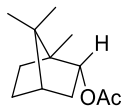
Dittrichia graveolens L. was collected in January to March 2012- 2015 in Western Australia. The leaves were air-dried prior to extraction.

Extraction Methods

Method A - Ethanol extraction

Dried aerial parts of *Dittrichia graveolens* L. (100 g) were soaked in ethanol (1 L) for 30 minutes. The solution was filtered, and the crude extract was concentrated under reduced pressure to afford a brown oil (5.2 g, 5.2%). The oil was subjected to chromatography using silica gel. Gradient elution with ethyl acetate in petrol (5% - 50%) gave bornyl acetate **1,** (1 g, 1%), costic acid **2** (1.02 g, 1.2 %), ilicic acid **3** (1 g, 1%) and mixture of the sesquiterpene lactones **4**, and **5** (2 g, 2%) which were purified by recrystallization.

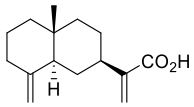
Bornyl acetate **165**



165

$[\alpha]_D^{26}$ –16 (c = 2, MeOH, Lit. [α] -42)[128]; ^{1}H NMR ($CDCl_3$): δ 4.88-4.91 (m, 1H), 2.34-2.38 (m, 1H), 2.05 (s, 3H), 1.92-196 (m, 1H), 1.76 (m, 1H), 1.67-1.69 (m, 1H), 1.21-1.34 (m, 2H), 0.99 (dd, 1H), (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR ($CDCl_3$,): δ 171.6 (C=O), 80.1 (CH), 48.9 (C), 47.9 (C), 45.1 (CH), 36.9 (CH_2), 28.2 (CH_2), 27.3 (CH_2), 21.5 (CH_3), 19.9 (CH_3), 19.0 (CH_3), 13.6 (CH_3). The spectral data for this compound was compatible with that reported.[129]

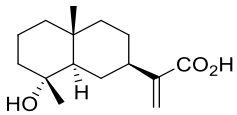
Costic acid **26**



26

M.p 75-80˚C (lit. 87-88 C) [63, 130]; $[\alpha]_D^{23}$ + 22 (c = 1, $CDCl_3$, Lit. + 23.45 , c = 1.3) [63, 130]; ^{1}H NMR ($CDCl_3$): δ 6.33 (s, 1H), 5.69 (s, 1H), 4.71 (s, 1H), 4.41 (s, 1H), 2.53 (t, *J* = 12.0 Hz, 1H), 2.31 (d, *J* = 12.8 Hz, 1H), 1.98 (dd, *J* = 10.8, 10.2 Hz, 1H), 1.90 (d, *J* = 12.0 Hz, 1H), 1.63-1.26 (m, 10H), 0.75 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 173.1 (C=O), 150.6 (C), 145.2 (C), 125.0 (CH_2), 105.5 (CH_2), 49.9 (CH), 41.9 (CH), 41.0 (CH_2), 39.3 (CH_2), 36.8 (CH_2), 36.1 (C) , 30.1 (CH_2), 27.4 (CH_2), 23.5 (CH_2), 16.5 (CH_3) ppm; IR: ν_{max} 3500- 2500, 1690, 1645 1620 cm^{-1}. The spectral data for this compound was identical with that provided in literature.[14, 82]

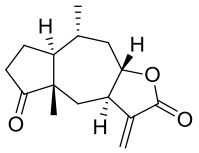
Ilicic acid **40**



40

Mp 165-175 (lit m.p 173-175) [130, 131]; $[\alpha]_D^{23}$ -39 (c = 1, $CDCl_3$. Lit. - 32.8, -35 (c 0.05) [132, 130]; IR: 3440 cm^{-1}, 1690, 1620. ^{1}H NMR ($CDCl_3$): δ 6.29(s, 1H), 5.65 (s, 1H), 2.53 (t, *J* = 12.0 Hz, 1H), 1.98 (d, *J* = 10.8, 10.2 Hz, 1H), 1.85 (d, *J* = 12.0 Hz, 1H), 1.63-1.20 (m, 11H), 1.10 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (CDCl3): δ 171.3 (C=O), 145.1(C), 124.4 (CH), 72.5 (C), 55.0 (CH), 44.5 (CH_2), 43.4 (CH_2), 40.9 (CH_2), 40.1(CH), 34.7 (C), 27.2 (CH_2), 26.7 (CH_2), 22.5 (CH_3), 20.1 (CH_2), 18.7 (CH_3). The spectral data for this compound was identical to that provided in literature.[4, 132, 133]

Lactone **215**

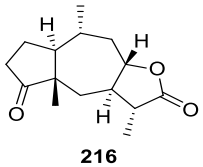


215

IR: 1764, 1730. ^{1}H NMR ($CDCl_3$): δ 6.18 (d, *J* = 3.4 Hz, 1H), 5.50 (d, *J* = 3.4 Hz, 1H), 4.27 (ddd, *J* = 12.4, 9.3, 3.1 Hz, 1H), 2.80 (ddd, *J* = 12.4, 9.5, 6.5, 3.4 Hz, 1H), 2.54 – 2.34 (m, 4H), 2.23 – 2.04 (m, 2H), 1.92 (dq, *J* = 10.8, 5.7 Hz, 2H), 1.69 – 1.32 (m, 2H), 1.16 – 1.00 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 222.66 (C=O), 176.1 (OC=O), 140.38 (C), 120.22 (CH_2), 80.97 (CH), 50.16 (C), 48.85 (CH), 44.91 (CH), 44.21 (CH_2), 35.34 (CH_2), 34.69 (CH_2), 29.75 (CH_2), 24.27 (CH_2), 22.18 (CH_3), 20.13 (CH_3).

Lactone **216**

IR: 1764, 1730. ^{1}H NMR (CDCl3): δ 4.27 (m, 1H), 2.45 (m, 1H), 2.39 (m, 3H), 2.32 (m, 1H), 2.15-1.18 (m, 10H), 1.16 – 1.00 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 222.56 (C=O), 176.10 (OC=O), 80.83 (CH), 50.01 (C), 48.70 (CH), 47.76 (CH), 44.06 (CH_2), 43.35 (CH), 36.10 (CH_2), 35.19 (CH_2), 29.60 (CH), 24.12 (CH_2), 22.03 (CH_3), 19.98 (CH_3), 13.09 (CH_3).



216

Method B – Aqueous extraction

A number of different aqueous solution (10% EtOH, 10% Na_2CO_3, 10% $NaHCO_3$, 5% Na_2CO_3, 1% Na_2CO_3 and 5% $NaHCO_3$, 2% $NaHCO_3$) were tested to optimise the extraction process, and all gave the expected five compounds. The optimal conditions were extraction with the 2-5% of sodium carbonate or sodium bicarbonate for 5 days depending on the amount of the plant material. The general extraction procedure is as follows: The aerial parts of *D. graveolens* were coarsely cut and steeped in 5% sodium

bicarbonate for five days. The extract was filtered and acidified with concentrated hydrochloric acid to pH ≈ 2. The resulting solution was extracted 2 times with dichloromethane. The combined organic extracts were dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. The ^{1}H NMR spectrum of the crude extract showed the presence of the five main components as in the ethanol extraction.

Sodium hydrogen carbonates large scale extraction of fresh

A 10 kg of fresh aerial parts of *D. graveolens* L. were soaked in a 2% $NaHCO_3$ solution (56 L) for 5 days. The solution was filtered, acidified to pH 1 with concentrated HCl solution and extracted with DCM the extract was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford brown oil (40 g) containg the 5 main components.

Non- chromatographic technique

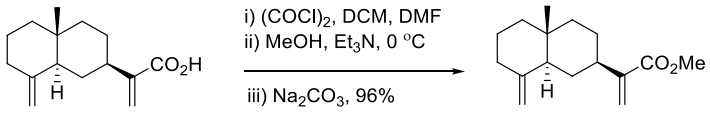
The isolated sesquiterpenes acids were isolated using acid/base extraction. The crude extract was washed with 5% of sodium hydroxide to deprotonate isolated sesquiterpenes acids. The aqueous extract was acidified to pH≈2 using concentrated hydrochloric acid. Thus with the choice of suitable weaker base (5% $NaHCO_3$) could selectively separate ilicic acid over costic acid. This technique was partially successful in isolated ilicic acid from costic acid affording ilicic acid containing 20% costic acid and pure costic acid.

Seed germination bioassay

A 0.125 g of crude extract was dissolved in ethanol (25 mL), serial dilutions of the solution gave 1/10 and 1/100 dilutions. The bioassay was carried out using the procedure provided by Wolf *et al.*[87,88] Two pieces of filter paper (Whatman number 1) were set in 9 cm diameter Petri dishes. First, 4 mL of each test solution (0.125g/25 mL, 1/10, and 1/100) were placed onto each plate, and controls were prepared identically using the pure ethanol. The plates were left overnight to evaporate all the ethanol. Next, the filter papers were moistened with (4 mL) of deionized water.

Finally, Uniform seeds were selected for the test while damaged seeds were discarded. The selected seeds were soaked in deionised water for 3 hours. Then twenty seeds were placed on each plate with three replicates per treatment. The Petri dishes were covered with a lid, and protected from light by aluminium foil and kept in the dark at 25˚C. After three days the Petri dishes were frozen to kill the seedlings and to facilitate measuring the length. The lengths of each seedling for each set were measured (root plus shoot) figure 3.3, and mean values were calculated and converted to the percentage to ease expression this value in bar chart. Germination [SG (%)] was calculated from the equation SG = 100 × T/C, where T and C are the numbers of the germinated seeds on the treated and control filter papers, respectively. Costic acid at (20 mM and 2 mM) and ilicic acid (20 mM and 2.1 mM) was investigated using the same procedure.

Esterification of costic acid 26



26 **123**

A solution of costic acid **26** (100 mg, 0.43 mmol) in dichloromethane (2 mL) was stirred at 0˚C for 10 minutes. Oxalyl chloride (5 equiv, 0.27 g, 2.1 mmol) was added dropwise over 5 min followed by DMF (one drop). The reaction mixture was stirred for 30 min at the same temperature. Then the solvent was evaporated under reduced pressure. Triethylamine (3 eq, 0.13 g, 1.3 mmol) and MeOH (10 mL) were added sequentially to the residue and the mixture stirred at 0˚C for 1 h. The solvent was removed *in vacuo* to give a brown oil (120 mg). The oil was diluted by dichloromethane and washed by 5% sodium carbonate (2 × 20 mL), dried over anhydrous $MgSO_4$, and concentrated under reduce pressure to give a colourless oil (96 mg, 91%) of costic acid methyl ester **123** which did not need to be purified further. The ^1H NMR spectrum was identical with the reported ester.[100,134]

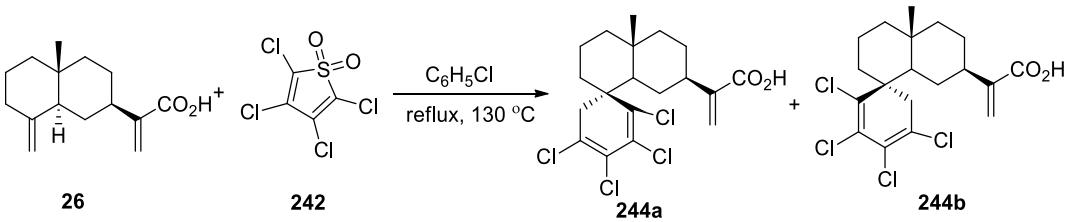
^{1}H NMR ($CDCl_3$): δ 6.14(d, 1H), 5.56 (t, 1H), 4.70 (q, 1H), 4.40 (q, 1H), 3.76 (s, 3H), 2.53 (t, *J* = 12.4 Hz, 1H), 2.31 (d, *J* = 12.4 Hz, 1H), 1.98 (dd, *J* = 10.8, 10.2 Hz, 1H), 1.90 (d, *J* = 12.0 Hz, 1H), 1.63-1.26 (m, 10H), 0.74 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.00 (C=O), 150.84 (C), 146.04 (CH_2), 122.58 (C), 105.52 (CH_2), 51.89 (OCH_3), 49.97 (CH), 41.95 (CH_2), 41.18 (CH_2), 39.78 (CH), 36.80 (CH_2), 36.05 (C), 30.08 (CH_2), 27.43 (CH_2), 23.58 (CH_2), 16.49 (CH_3).

Synthesis of 2,3,4,5-tetrachlorothiophene-1,1-dioxide

247 **242**

A solution of anhydrous *m*-chloroperoxybenzoic acid (water reduced under high vacuum for 1 h) (22.7 g, 0.132 mol) in 1,2-dichloroethane (173 mL) was stirred and warmed to form a solution and then 2,3,4,5-tetrachlorothiophene **247** (10 g, 0.045 mol) was added to the solution. The mixture was heated under reflux under nitrogen for 48 h. The mixture was allowed to cool to room temperature, filtered and the filtrated was washed with solution of 10% Na_2CO_3, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give a solid. The solid was recrystallised from a minimal amount of DCM to afford the thiophene dioxide **242** as colourless crystals (5.25 g, 46%). ^{13}C NMR (100 MHz, $CDCl_3$): δ 131.34 (C), 127.57 (C).

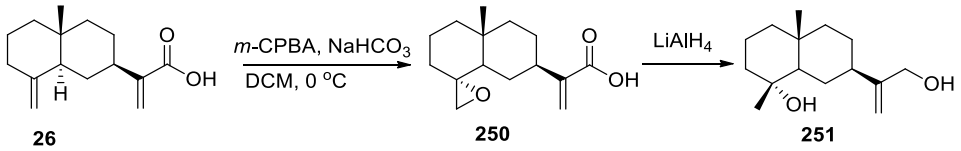
Diels-Alder reaction



26 **242** **244a** **244b**

A solution of costic acid **26** (500 mg, 2.13 mmol) in chlorobenzene (5 mL) and 2,3,4,5-tetrachlorothiophene-1,1-dioxide **242** (542 mg, 2.13 mmol) was heated under reflux for 24 h. under nitrogen. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure to give an oil (820 mg). The oil was subjected to flash chromatography. Elution with 10% - 25% ethyl acetate and petroleum spirit gave a mixture of stereoisomers **244a** and **244b** in 9 : 1 ratio (0.66 g, 67%). ^{1}H NMR ($CDCl_3$): δ 6.32 (dd, *J* = 8.4, 1.1 Hz, 1H), 5.68 (dt, *J* = 7.4, 1.3 Hz, 1H d, 1H), 2.66 (ddd, *J* = 13.6, 3.4, 2.2 Hz, 1H), 2.52 (m, 1H), 2.10-1.20 (m, 14H), 1.06 (s, 3H).

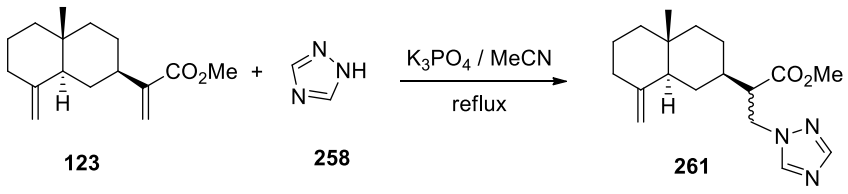
Epoxidation of costic acid



26 **250** **251**

A solution of costic acid **26** (46 mg, 0.19 mmol) in anhydrous DCM (5 mL) was added to a mixture of *m*-CPBA (90 mg, 0.52 mmol) and sodium hydrogen carbonate (90 mg) in DCM (2 mL), and the resulting mixture was stirred at 0 ˚C for 1 h and then allowed to warm to room temperature for 16 h. The resulting mixture was washed with deionised water (50 mL), dried over anhydrous $MgSO_4$ and concentrated under vacuum to give an oil (52 mg). The oil was diluted with anhydrous diethyl ether. Lithium aluminium hydride (50 mg) was added and the mixture reaction was stirred for 3 h. The resulting oil (30 mg) was subjected to flash chromatography. Elution with ethyl acetate in petrol 1:1 gave the allylic alcohol **251** as a colourless oil (20 mg, 44%). The ^{1}H NMR spectrum of the resulting alcohol was identical the literature.[101] ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.04 (d, *J* = 1.5 Hz, 1H), 4.94 (t, *J* = 1.2 Hz, 1H), 4.10 (d, *J* = 5.8 Hz, 2H), 2.15-1.14 (m, 13H) 1.13 (s, 3H), 1.0- 0.93 (m, 1H), 0.90 (s, 3H) ppm.

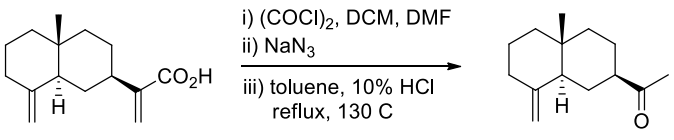
Michael addition



123 258 261

A solution of costic acid methyl ester **123** (50 mg, 0.20 mmol) in acetonitrile (2 mL) was stirred at room temperature under nitrogen. Potassium phosphate tribasic (30 mg, 0.047 mmol) and 1,2,4-1*H*-triazole **258** (16 mg, 0.24 mmol) were added successively, and the reaction mixture was heated under reflux overnight. The reaction mixture was allowed to cool to room temperature, filtered and the solvent evaporated under reduced pressure to afford an oil (73 mg). The crude oil was subjected to flash chromatography. Elution with 2% methanol in dichloromethane gave a mixture of triazoles **261** as yellow oil (30 mg 45%). ^{1}H NMR of stereoisomers (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.92 (s, 1H), 4.72 (dt, *J* = 6.1, 1.7 Hz, 1H), 4.59 – 4.44 (m, 1H), 4.41 (dd, *J* = 6.0, 1.6 Hz, 1H), 4.32 (ddd, *J* = 13.6, 12.1, 4.1 Hz, 1H), 3.68 – 3.55 (m, 4H), 3.00 (ddd, *J* = 10.5, 6.4, 4.1, Hz, 1H), 2.45 – 2.12 (m, 1H), 2.10 – 0.94 (m, 12H), 0.73 (s, 3H) ^{13}C NMR (100 MHz, $CDCl_3$): δ173.57, 173.48, 152.17, 150.37, 150.30, 143.93, 105.86, 52.03, 51.88, 51.78, 49.63, 49.57, 49.17, 41.79, 40.76, 40.67, 39.33, 39.31, 36.84, 36.04, 29.83, 28.27, 28.03, 25.76, 25.62, 23.46, 16.28. The ^{1}H and ^{13}C NMR spectra contained duplicate signals which are suggested the compound formed in isomers, and they are inseparable. HRMS (ESI) m/z $C_{18}H_{27}O_3N_3$ $[M + H]^+$ requires 317.2103, found 318.2175.

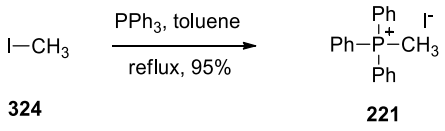
Conversion of costic acid to ketone



26 78

To a stirred suspension of costic acid **26** (100 mg, 0.42 mmol) in dry DCM (5 mL) was stirred at 0 ˚C, was added a solution of oxalyl chloride (5 equiv., 271 mg, 2.14 mmol) and DMF (1 drop). The reaction mixture was stirred at the same temperature for 3 h. A solution of NaN_3 (83 mg, 1.28 mmol) in H_2O (35 mL) was added, and the reaction mixture stirred at 0 ˚C for 1.5 h. Then the reaction mixture was extracted with DCM (3 × 20 mL) and the combined organic extracts were concentrated under reduced pressure. The residue was diluted with toluene (30 mL) and gently heated under reflux overnight. The mixture was allowed to cool to room temperature and acidified with 10% HCl (30 mL) and heated under reflux again for an extra 2 h. The organic layer was decanted and washed with 5% Na_2CO_3 (30 mL), brine (30 mL), and then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to give an oil (76 mg). The crude oil was subjected in flash chromatography. Elution with 2% of ethyl acetate in petrol gave the ketone **9** (0.013 mg, 13%). The presence of the ketone **78** was confirmed by ^{1}H and ^{13}C NMR spectroscopy which, was identical to the spectra provided by Bohlmann.[90] ^{1}H NMR ($CDCl_3$) δ 4.73 (q, *J* = 1.7 Hz, 1H), 4.45 (q, *J* = 1.7 Hz, 1H), 2.40 (tt, *J* = 12.4, 3.6 Hz, 1H), 2.31 (dtd, *J* = 13.0, 3.5, 1.7 Hz, 1H), 2.16 (s, 3H), 2.06 – 1.89 (m, 1H), 1.80 (dd, *J* = 12.3, 3.6, 1.8 Hz, 1H), 1.80 – 1.66 (m, 2H), 1.69 – 1.15 (m, 8H), 0.72 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 212.14 (C=O), 150.29(C), 105.98 (CH_2), 52.10 (CH), 49.32 (CH), 41.92 (CH_2), 40.48 (CH_2), 36.97 (CH_2), 36.02 (C), 28.24 (CH_3), 26.28 (CH_2), 23.75 (CH_2), 23.48 (CH_3), 16.27 (CH_3) ppm.

Preparation of phosphonium salt

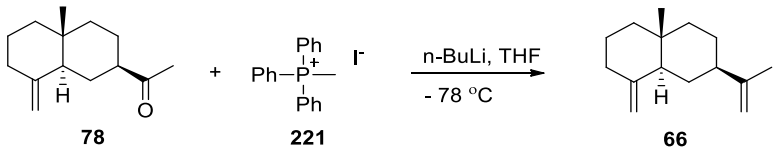


324 **221**

This procedure was performed followed that published by Li *et al.*[76] Methyl iodide **324** (2.9 g, 20 mmol) was added dropwise to a solution of triphenylphosphine (5 g, 19 mmol) in anhydrous THF (10 mL) was stirred at room temperature for 12 h. The white precipitate was collected by filtration the washed with THF to give yellow crystalline

solid triphenylmethylphosphonium iodide **221** (6.35, 82%). The spectral data for this compound was identical to that provided in literature.[76] ^{1}H NMR (400 MHz, CDCl3) δ= 7.79- 7.72 (15H, m), 3.24 (3H, d) ppm.

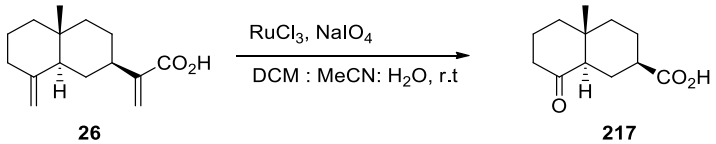
Synthesis of *β*-selinene



78 221 66

n-BuLi (1.6 M in hexanes, 240 µl, 0.38 mmol, 1.7 eq.) was added dropwise to a solution of triphenylmethylphosphonium iodide **221** (150 mg, 0.37 mmol, 1.7 eq) in anhydrous tetrahydrofuran (2 mL) at 0 ˚C. The reaction mixture was stirred for 30 minutes. The mixture was allowed to warm to room temperature for a further 30 minutes. The mixture was then cooled to 0 ˚C and a solution of the ketone 7**8** (45 mg, 0.22 mmol) in anhydrous tetrahydrofuran (2 mL) was added dropwise over 2 minutes. The mixture was then allowed to warm to room temperature for 12 h. A few drops of MeOH was added to the reaction and then concentrated under reduced pressure to afford an oil (30 mg, 67%). The ^{1}H NMR spectrum of the oil contained **66**.[135, 136] and an unknown compound. Purification of the oil by flash chromatography was unsuccessful due to the volatility of the product.

Transformation of costic acid to ketoacid

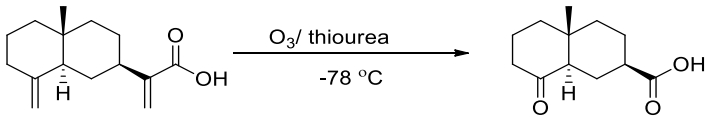


26 217

Sodium periodate (316 mg, 1.6 mmol) was added to a stirred solution of costic acid **26** (100 mg, 0.43 mmol) in dichloromethane (1 mL), acetonitrile (1 mL) and H_2O (1.5 mL) under nitrogen at room temperature. The reaction mixture was stirred vigorously until the sodium periodate was completely dissolved then, ruthenium chloride.xH_2O

(30 mg, 0.14 mmol) was added. The resulting black reaction mixture was stirred at room temperature and monitored with TLC. When the starting material was consumed the reaction mixture was extracted with dichloromethane (10 mL). The aqueous layer was washed with DCM (2 × 20 mL). The combined organic extracts were filtered, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give a solid (115 mg). The residue was purified by flash chromatography (20% ethyl acetate: petroleum spirit) to give the ketoacid **217** (50 mg, 56%). A sample from this compound was recrystallized from 20% dichloromethane – petroleum spirit which afforded the ketone **217** as white needles crystals: mp 128 – 133 ˚C; $[\alpha]_D^{23}$ -10.71 (c = 1, $CDCl_3$); IR 2600-3200,16902 cm^{-1}.; ^1H NMR ($CDCl_3$) δ 9.9 (s, COOH), 2.41 – 2.19 (m, 4H), 2.04 – 1.87 (m, 3H), 1.90 – 1.78 (m, 1H), 1.75 – 1.52 (m, 5H), 1.41 (td, *J* = 13.5, 4.3 Hz, 1H) 0.82 (s, 3H). ^{13}C NMR, ($CDCl_3$) δ 211.58 (C=O), 180.79 (COOH), 56.48 (CH), 42.61 (CH), 41.30 (CH_2), 40.36 (CH_2), 39.88 (CH_2), 39.22 (CH_2), 23.68 (C), 23.21 (CH_2), 22.62 (CH_2), 16.95 (CH_3). Elemental Analysis: Found: C, 68.54%; H, 8.63%. $C_{12}H_{18}O_3$ calculated C, 68.49%; H, 8.98%). HRMS (ESI) m/z $C_{12}H_{12}O_3$ [M + H]$^+$ requires 210.1256, found 211.1149.

Ozonolysis of costic acid

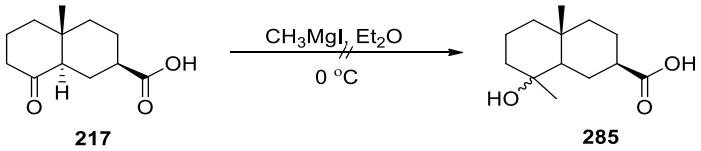


26 **217**

A solution of costic acid (103 mg, 0.44 mmol) in dichloromethane (80 mL) and methanol (20 mL) was stirred at -84 ˚C. Then ozonized oxygen was bubbled through the solution until the reaction was completed as indicated by the blue colour of excess ozone. The reaction was purged with oxygen until the blue colour disappeared, reaction become colourless. A solution of thiourea (66 mg, 0.88 mmol) in methanol (1 mL) was added to the reaction and stirred at 0 ˚C for 1.5 h. The mixture was washed with deionised water (50 mL), extracted with dichloromethane (2 × 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give a solid (100 mg). The solid was subjected to flash chromatography. Elution with 50% ethyl acetate/light petroleum afforded ketoacid **217** as a colourless solid (65 mg, 70%). ^1H NMR ($CDCl_3$) δ 9.9 (s,

COOH), 2.41 – 2.19 (m, 4H), 2.04 – 1.87 (m, 3H), 1.90 – 1.78 (m, 1H), 1.75 – 1.52 (m, 5H), 1.41 (td, *J* = 13.5, 4.3 Hz, 1H) 0.78 (s, 3H). The ^{1}H NMR spectrum was identical with the transformation of costic acid to ketoacid page 129

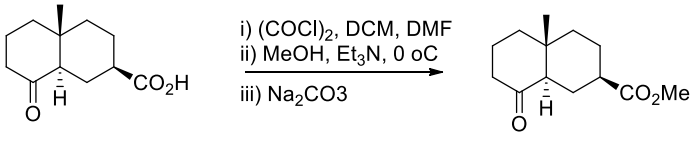
Reaction of Ketoacid with methylmagnesium iodide



217 **285**

A solution of ketoacid **217** (50 mg, 0.24 mmol) in Et_2O (2 mL) was stirred 0 ˚C for 20 minutes. A solution of methylmagnesium iodide (3 M, 250 µl, 0.75 mmol, 3 equiv) was added dropwise. The reaction mixture was stirred for 30 minutes at 0 °C and allowed to heat up to the room temperature. The reaction was quenched with 1 M hydrochloric acid and extracted with DCM. The solvent was removed under reduced pressure and ^{1}H NMR spectrum showed only the starting material was recovered.

Esterification of ketoacid

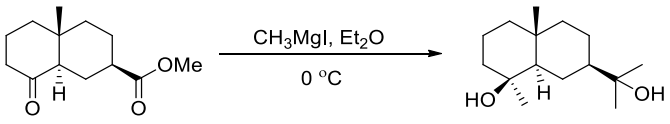


217 **262**

A solution of ketoacid **217** (100 mg, 0.47 mmol) in DCM (2 mL) was stirred at 0˚C for 10 minutes. Oxalyl chloride (30 mg, 2.4 mmol, 5eq) was added dropwise over 5 minutes followed by adding DMF (2 drops, 0.001eq). The reaction mixture stirred for 30 min at the same temperature. Then the solvents were evaporated under reduced pressure. Triethylamine (5 eq, 240 mg, 330 µL) and MeOH (10 mL) were added successively to the residue and the reaction mixture was stirred at 0˚C for 1 h. Once the reaction was complete the solvent was removed *in vacuo* to leave a brown oil (120 mg). The oil was diluted with DCM and washed with 5% sodium carbonate (2 × 50 mL). The organic layer dried over anhydrous magnesium sulfate, and concentrated

under reduced pressure to give the pure ester **262** (96 mg, 87%). The ^{1}H-NMR spectrum of the resulting ester was identical to the literature.[105] ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.66 (s, 3H), 2.28 (m, 4H), 1.95 (m, 1H), 1.91 (m, 1H), 1.91 – 1.78 (m, 6H), 1.38 (dd, *J* = 13.5, 4.4 Hz, 1H), 1.16 (m, 1H), 0.81 (s, 3H).

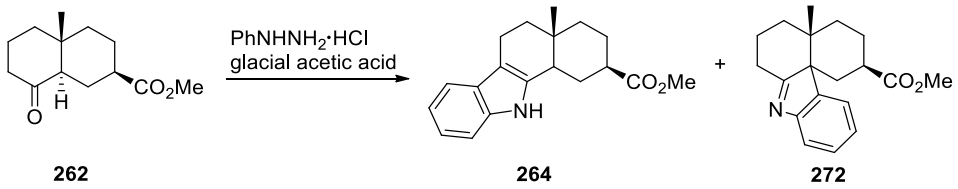
Synthesis Eudesmane- 4β, 11- diol



262 **280**

A solution of ketoester **262** (100 mg, 0.48 mmol, 1 equiv) in Et_2O (5 mL) was stirred under nitrogen at 0˚C. Methylmagnesium iodide (3 M, 7 equiv, 1.12 mL, 3.36 mmol) was added dropwise and the mixture reaction was stirred for 2 h, and the reaction then allowed to warm to room temperature over 30 minutes and monitored by TLC. Then the reaction mixture was quenched with saturated NH_4Cl and extracted with DCM (3 × 30 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under vacuum to give crude (88 mg). The crude was subjected to flash chromatography. Elution with 50% ethyl acetate in petrol gave diol **280** (75 mg, 70%) The ^{1}H NMR spectrum of the product compound matched the spectroscopic data of compound **10** reported by Ando.[113] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 – 1.76 (m, 2H), 1.73 – 1.62 (m, 1H), 1.58 (ddd, *J* = 5.4, 4.1, 2.0 Hz, 3H), , 1.48 – 1.31 (m, 5H), 1.31 – 1.23 (m, 2H), 1.21 (d, *J* = 1.8 Hz, 6H), 1.17 (s, 3H), 1.15 – 1.04 (m, 2H), 0.95 (m, 3H); ^{13}C NMR ($CDCl_3$): 73.04 (C), 72.10 (C), 51.66 (CH), 49.95 (CH), 43.81 (CH_2), 41.53 (CH_2), 41.39 (CH_2), 33.63 (C), 30.29 (CH_3), 27.48 (CH_3), 26.81 (CH_3), 22.42 (CH_2), 21.36 (CH_2), 18.66 (CH_3), 18.09 (CH_2).

Synthesis of indole

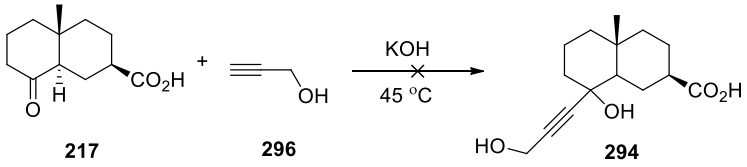


262 **264** **272**

A solution of ketoester **262** (100 mg, 0.452 mmol) and phenyl hydrazine hydrochloric acid (70 mg, 0.48 mmol) in glacial acetic acid (2 mL) was heated under reflux for 2 h. The mixture was allowed to cool to room temperature, washed with H_2O (50 mL) and extracted with DCM (3 × 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to afford a yellow solid (128 mg). The solid was subjected to flash chromatography. Elution with 20% to 50% ethyl acetate in petrol gave **264** (55 mg, 41%) and **272** (35 mg, 26%). The ^{1}H NMR spectrum for the indole **264**, ^{1}H NMR ($CDCl_3$): δ 7.78(d, 1H), 7.44 (d, 1H), 7.30 (m, 1H), 7.08 (m, 2H), 3.69 (s, 3H), 2.82 (s, 1H), 2.70 (ddd, 1H), 2.5-1.5 (m, 10H), 1.14 (s, 3H). HRMS (ESI) m/z $C_{19}H_{23}O_2N$ $[M + H]^+$ requires 297.1729, found 298.1802.

Addition of the propargyl alcohol

Attempt 1

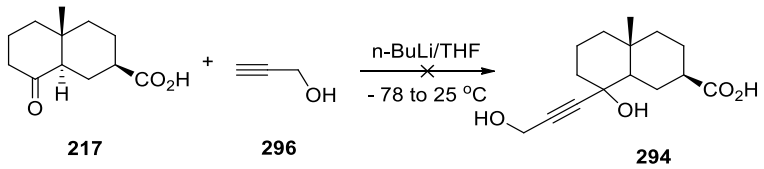


217 **296** **294**

A solution of propargyl alcohol **296** (21 µl, 1.5 eq) and deionised water (3 mL) was stirred at room temperature under nitrogen. Potassium hydroxide (67 mg, 1.2 mmol, 5 eq) was added to the mixture and heated at 45 ˚C for 30 minutes. Ketoacid **217** (50 mg, 0.24 mmol) was added and the reaction mixture stirred at the same temperature

for 24 h. The ^{1}H NMR spectrum of the crude showed only the starting material was recovered.

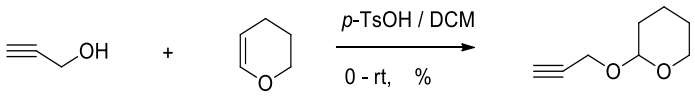
Attempt 2



217 **296** **294**

n-BuLi (1.6 M in hexanes, 1 mL, 1.6 mmol) was added dropwise to a solution of propargyl alcohol **296** (55 µL , 0.48 mmol, 2 equiv) in anhydrous tetrahydrofuran (1 mL) and the mixture was stirred for 30 minutes at −78 ˚C. A solution of ketoacid **217** (50 mg, 0.24 mmol) in anhydrous tetrahydrofuran (2 mL) was added dropwise to the mixture and the reaction stirred for another 30 minutes then allowed to warm to room temperature overnight. The reaction mixture was quenched with 1 M hydrochloric acid, extracted with dichloromethane (2 × 50 mL), washed with deionised water, dried over anhydrous magnesium sulfate. The combined organic extracts were concentrated under reduced pressure. Only the starting material was recovered.

Protection of propargyl alcohol

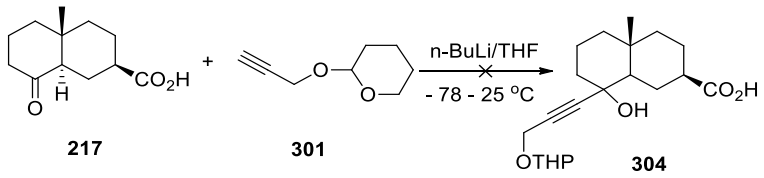


296 **325** **301**

Following the procedure by Hu *et al.*[117] *p*-TsOH (0.13 g, 0.75 mmol) 3,4-dihydro-2*H*-pyran **325** (7.50 mL, 82 mmol) were added successively to a cooled (0 °C) solution of propargyl alcohol **296** (4.43 mL, 75 mmol) in dichloromethane (50 mL). The reaction mixture was stirred at 0 °C for 5 minutes then allowed to warm to room temperature for 3 h, then the reaction was quenched with saturated sodium hydrogen carbonate (5 mL), extracted with DCM (2 × 50 mL) and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give an oil of 2-(prop-2-yn-1-yloxy) tetrahydro-2*H*-pyran **301** (9.4 g, 90.5%). The

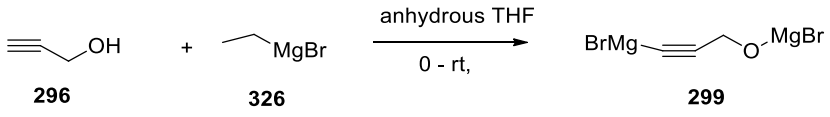
spectroscopic spectrum identical to that provided by Higgins and co-workers.[137] ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.79 (t, *J* = 3.3 Hz, 1H), 4.35 – 4.09 (m, 2H), 3.82 (m, 1H), 3.51 (m, 1H), 2.39 (td, *J* = 2.4, 0.6 Hz, 1H), 1.96 – 1.27 (m, 6H).

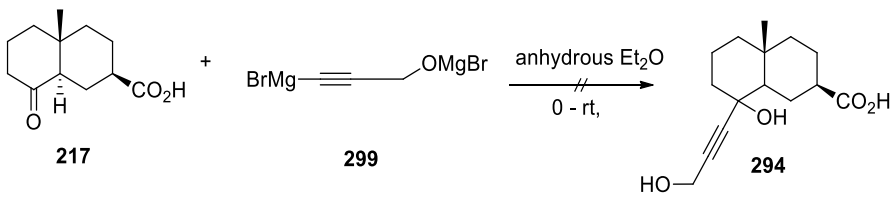
Attempt 3



To a solution of 2-(prop-2-yn-1-yloxy) tetrahydro-2*H*-pyran **301** (133 mg, 0.95 mmol) in anhydrous tetrahydrofuran (5 mL) stirred at -84 ˚C was added dropwise *n*-BuLi (1.6 M in hexanes, 340 µL, 0.54 mmol, 2.3 equiv.). The reaction mixture was stirred for 30 minutes then allowed to warm to room temperature over 2 h. A solution of ketoacid **217** (50 mg, 0.24 mmol) in anhydrous tetrahydrofuran (2 mL) was added dropwise at -84 ˚C, then the reaction mixture allowed to warm to room temperature and stirred overnight. The reaction was quenched with 1 M hydrochloric acid, extracted with dichloromethane (2 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the combined organic extract concentrated under reduced pressure. Only the starting material was recovered.

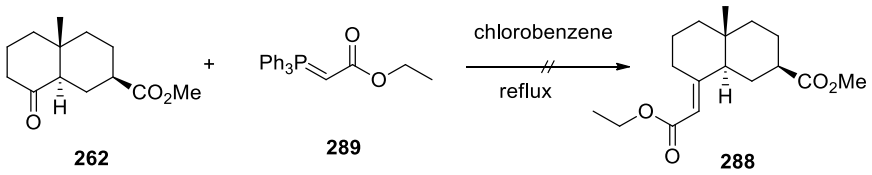
Attempt 4





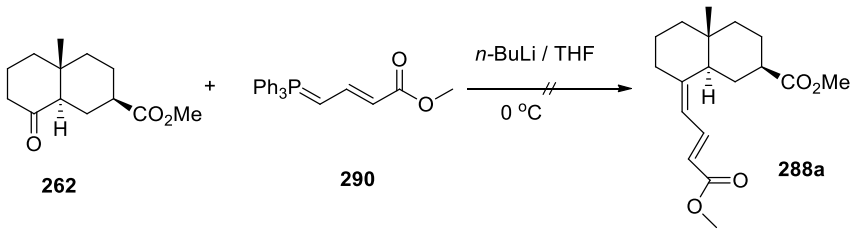
A solution of propargyl alcohol **299** (53 mg, 0.95 mmol, 4 eq) in anhydrous diethyl ether (2 mL) was stirred at 0˚C. Ethylmagnesium bromide (3 M, 650 µL, 1.95 mmol, 8 equiv) was added dropwise and the solution stirred for 30 minutes at the same temperature. The solution was allowed to warm to room temperature. The reaction mixture was cooled to 0˚C. A solution of ketoacid **217** (50 mg, 0.24 mmol, 1 equiv) in anhydrous diethyl ether (2 mL) was added dropwise, and the reaction allowed to warm to room temperature for further 1 h. The reaction was quenched with 1 M hydrochloric acid and extracted with dichloromethane (2 × 50 mL), dried over anhydrous magnesium sulfate, filtered and the combined organic extract was concentrated under reduced pressure. Only starting material was recovered.

Addition of phosphonium salt 289 to ketoester 262



262 **289** **288**

A solution of phosphonium salt **289** (2 equiv., 174 mg, 0.50 mmol) in chlorobenzene (2 mL) was stirred at room temperature. The ketoester **262** (50 mg, 0.22 mmol) was added then the reaction mixture heated under reflux overnight. The solvent was removed under reduced pressure. The ^{1}H NMR of the crude showed only recovered starting material.



262 **290** **288a**

An attempt to olefination the ketone in the ketoester **262** using less stable more reactive phosphonium salt was performed. A solution of phosphonium salt **290** (80 mg, 0.22 mmol, 1 equiv.) in anhydrous tetrahydrofuran (2 mL) was stirred at 0 ˚C. *n*-BuLi (1.6

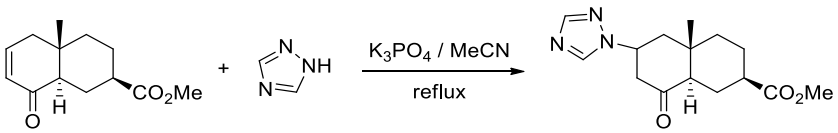
M in hexanes, 260 μL, 0.42 mmol, 1.91 equiv.) was added dropwise. The mixture was allowed to warm to room temperature over 30 minutes. A solution of ketoester **262** (50 mg, 0.22 mmol, 1 equiv.) in anhydrous tetrahydrofuran (2 mL) was added at 0 ˚C. The reaction mixture was stirred for 30 minutes the allowed to warm to room temperature over 12 h. The reaction was extracted with dichloromethane (2 × 50 mL) and concentrated under reduced pressure. Only starting material was recovered.

Dehydrogenation of Ketoester 262

262 **308**

A mixture of ketoester **262** (337 mg, 1.50 mmol) and IBX (1.26 g, 4.50 mmol) in DMSO (2 mL) was heated at 85 ˚C in oil bath overnight. The reaction mixture poured into water (100 mL) and extracted with DCM (3 × 50 mL). The combined organic extracts were washed with 5% sodium carbonate (4 × 50 mL), dried over anhydrous magnesium sulfate and the solvent evaporated under reduced pressure to afford a mixture (225 mg) of starting material and *α,β*–unsaturated ketoester **308** in a 35:65 ratio (nmr). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.80 (m, 1H), 6.00 (m, 1H), 3.67 (m, 3H), 2.98 (s, 1H), 2.88 – 2.67 (m, 1H), 2.69 – 2.52 (m, 1H), 2.29 (s, 5H), 2.45 – 2.12 (m, 7H), 2.16 – 2.00 (m, 1H), 2.02 – 1.83 (m, 4H), 1.88 – 1.29 (m, 12H), 1.33 – 1.25 (m, 1H), 1.25 (s, 1H), 1.27 – 1.08 (m, 5H), 0.80 (m, 3H).

Michael addition of 1,2,4-1H-triazole



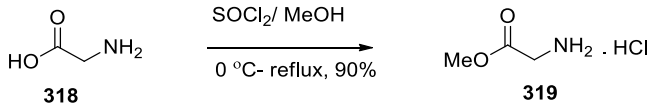
308 **258** **309**

A solution of the mixture of the *α,β*–unsaturated ketoester **308** (ca. 65%, 52 mg, 0.24 mmol) in acetonitrile (2 mL) was stirred at room temperature under nitrogen.

Potassium phosphate tribasic (10 mg, 0.005 mmol) was added followed by adding 1,2,4-1H-triazole (16 mg, 0.24 mmol), and the mixture heated under reflux overnight, the reaction was monitored by (TLC), when the starting material was consumed the mixture filtered and concentrated under reduced pressure to afford a yellow oil (73 mg). The oil was subjected to flash chromatography. Elution with 2% methanol in dichloromethane afforded a yellow oil from triazole compound **309 (**26 mg, 38%, 59% based on **308**).

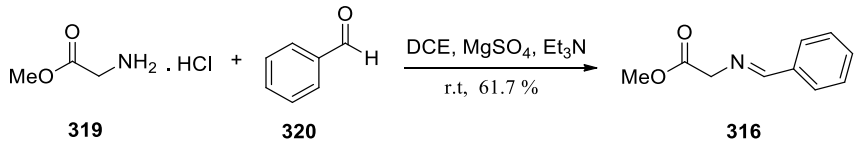
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (m, 1H), 7.97 (s, 1H), 4.80 (tt, *J* = 12.3, 5.1 Hz, 1H), 3.69 (s, 3H), 3.03 (ddd, *J* = 13.5, 12.0, 1.2 Hz, 1H), 2.83 (ddd, *J* = 13.7, 5.5, 2.0 Hz, 1H), 2.50 – 2.18 (m, 3H), 2.13 – 1.21 (m, 7H), 1.30 – 1.17 (m, 2H), 0.93 (s, 3H); ^{13}C NMR ($CDCl_3$): 206.23 (C=O), 175.47 (CO_2Me), 152.37 (CH), 141.85 (CH), 58.60 (CH), 55.73(CH), 55.22 (CH), 51.92 (CH_2), 47.37 (CH_2), 46.65 (CH_2), 42.54 (CH_2), 39.28 (CH_2), 36.21 (C), 23.55 (CH_3), 17.59 (CH_3). HRMS (ESI) m/z $C_{15}H_{21}O_3N_3$ [M + H]$^{+}$ requires 291.1583, found 292.1654.

Synthesis of glycine methyl ester hydrochloride 319



Thionyl chloride (6.33 g, 53.3 mmol) was added dropwise over 5 minutes to a solution of glycine **318** (2 g, 26.6 mmol) in methanol (30 mL) at 0 °C. The reaction mixture was heated at reflux overnight. The solvent was removed under reduced pressure to give a white solid (3.0 g, 90%) of the ester **319**. The NMR spectrum was matched with that in literature. ^{1}H NMR (DMSO): δ 8.59 (s, NH_3^{+}), 3.76 (s, CH_2), 3.72 (s, OCH_3) ppm; ^{13}C NMR (DMSO): δ 167.9 (CO), 52.4(OCH_3), 39.41 (CH_2) ppm.

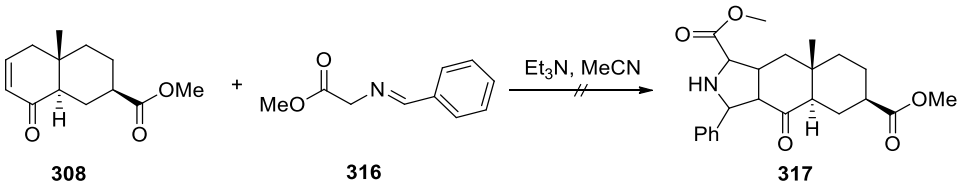
Synthesis of methyl-N-benzylidene glycinate 316



319 320 316

Triethyl amine (2.22 mL, 16 mmol) was added a solution of methyl glycinate hydrochloride **319** (2 g, 16 mmol) and magnesium sulphate (1.92 g, 16 mmol) in anhydrous 1,2-dichloroethane (40 mL), and the mixture was stirred at room temperature for 1 h. Benzaldehyde **320** (1.35 mL, 13 mmol) was added to the reaction mixture continuous stirred overnight. The resulting mixture was filtered, washed with brine (10 mL), extracted with dichloromethane (3 ×20 mL), and combined organic extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give a yellow oil from methyl-*N*-benzylidene glycinate **316** (1.73 g, 61.7%). The spectroscopic data was identical to the literature.[138] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (t, *J* = 1.4 Hz, 1H), 7.83 – 7.70 (m, 2H), 7.57 – 7.32 (m, 2H), 4.41 (t, *J* = 1.3 Hz, 2H), 3.77 (s, 3H) ppm.

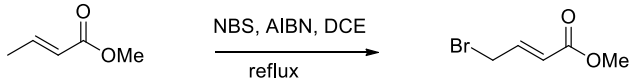
Addition reaction



308 316 317

A mixture of *α,β*-unsaturated carbonyl compound **308** (102 mg, 1.06 mmol), methyl-*N*- benzylidene glycinate **316** (188 mg, 1.06 mmol) and triethyl amine (215 mg, 2.12 mmol) in acetonitrile (4 mL) were stirred at room temperature overnight. The reaction mixture was concentrate under reduced pressure, diluted with DCM, washed with water (2 × 50 mL) and washed with brine (10 mL). The organic layer was concentrated under reduced pressure to yield the starting material.

Bromination of methyl crotonate

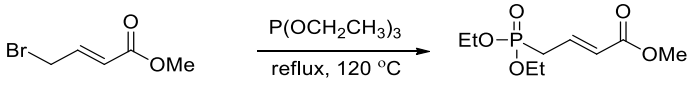


292 **293**

The procedure described by Baeckström and co-workers was following.[139] A mixture of methyl crotonate **292** (10.0 g, 100 mmol, 1.0 equiv) and *N*-bromosuccinimide (17.8 g, 100 mmol, 1.0 equiv.) in 1,2-dichloroethane (36 mL), was heated under reflux for 15 min. AIBN (0.5 g, 3 mmol) was added and the reaction heated under reflux for a further 5 h. The reaction mixture was allowed to cool to room temperature, filtered and washed with 1,2-dichloroethane (10 mL). The yellow liquid was concentrated under reduced pressure and subjected to flash chromatography. Elution with 5% ethyl acetate in petrol afforded **293** as a colourless solid (9.433 g, 53%). The ^{1}H NMR spectrum of the resulting compound **279** was identical to the literature.[139, 140, 141]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01-6.93 (1H, m), 5.99 (1 H, d), 3.97 (2 H, d), 3.71 (3H, s) ppm.

Preparation of the phosphonate ester 291

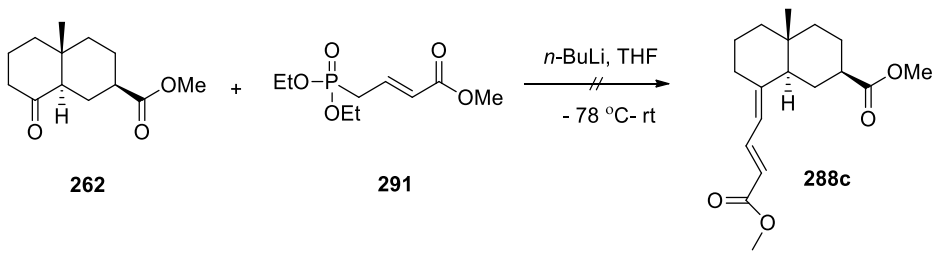


293 **291**

The procedure described by Baeckström and co-workers was following.[139] Methyl-4-bromo-2-butenoate **293** (9.4 g, 53 mmol) and triethylphosphite (9.8 g, 59 mmol) was stirred under nitrogen and heated under reflux for 4 h. The reaction monitored with TLC, after the starting material was consumed the reaction mixture allowed to cool to room temperature. The solvent was evaporated and the residue was subjected in flash chromatography. Elution with 10% ethyl acetate in petrol over to 50% and with MeOH afforded a colour solution from phosphonate ester **291** (11.2 g, 90%) ^{1}H NMR spectrum of the resulting compound **291** identical with the literature.[139] ^{1}H NMR (400

MHz, CDCl3): δ 6.66 (m, *J* = 15.2, 7.4 Hz, 1H), 5.77 (dd, *J* = 15.6, 5.0 Hz, 1H), 4.10 (m, 4H), 3.72 (s, 3H), 2.73 (dd, *J* = 23.0, 1.4 Hz, 2H), 1.31 (t, 6H) ppm.

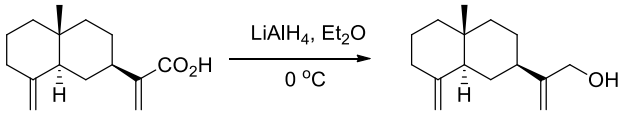
Addition of phosphonate ester 291 to the ketoester 262



262 **291** **288c**

A solution of phosphonate ester **291** (60 mg, 0.25 mmol) in anhydrous tetrahydrofuran (2 mL) was stirred at -84 ˚C. *n*-BuLi (1.6 M in hexanes, 0.5 mL, 0.8 mmol, 3.6 equiv.) was added dropwise. The reaction was allowed to warm to room temperature over 30 minutes. A solution of ketoacid methyl ester **262** (50 mg, 0.22 mmol, 1 equiv.) in anhydrous tetrahydrofuran (2 mL) was added at -84 ˚C. The reaction mixture was stirred for 30 minutes then allowed to warm up to room temperature over 12 h. The reaction was extracted with dichloromethane (2 × 50 mL) and concentrated under reduced pressure, but only the starting material was recovered.

Synthesis of allylic alcohol 83

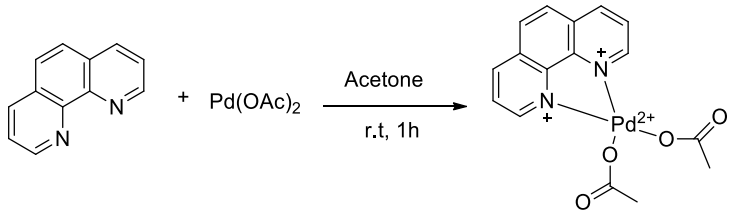


26 **83**

Lithium aluminiumhydride (90 mg, 2.38 mmol, 3 equiv.) was added to stirred solution of costic acid **26** (213 mg, 0.91 mmol) in dry ether (5 mL) at 0°C. The mixture was allowed to warm to room temperature overnight. The reaction was quenched with saturated solution of NH_4Cl and extracted with DCM (3 x 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentration *in*

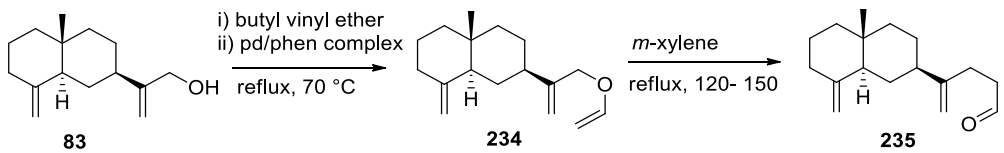
vacuo to give a colourless oil (96 mg). The oil was subjected to flash chromatography. Elution with 5-20% ethyl acetate in petrol afforded allylic alcohol **83** as a colourless oil (139 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 5.04 (q, *J* = 1.5 Hz, 1H), 4.94 (p, *J* = 1.1 Hz, 1H), 4.70 (p, *J* = 1.8 Hz, 1H), 4.42 (q, *J* = 1.8 Hz, 1H), 4.14 (d, *J* = 4.9 Hz, 2H), 2.30 (dtd, *J* = 13.1, 3.6, 1.7 Hz, 1H), 2.02 (m, 2H), 1.83 (m, 1H), 1.62- 0.85 (10H, m), 0.73 (s, 3H). ^{13}C NMR (CDCl3): δ 154.3 (C), 150.9 (C), 107.9 (CH_2), 105.8 (CH_2), 65.4 (C-O), 50.1 (CH), 42.03 (CH_2), 41.7 (CH_2), 41.3 (CH_2), 36.98 (CH_2), 36.12 (CH_2), 30.12 (CH_2), 27.45 (CH_2), 23.58 (CH_2), 16.48 (CH_3). The spectral data for this compound was compatible with that provided in literature.[63]

Synthesis of Pd/phen complex



The procedure was adapted from woolley and co-workers.[142] A solution of $Pd(OAc)_2$ (200 mg, 0.90 mmol) and 1,10-phenanthroline (162 mg, 0.90 mmol) in acetone (20 mL) was stirred at room temperature for 1 h. the yellow powder was collected with filtration and washed with cold acetone to give $Pd(OAc)_2$ Phen. (269 mg, 74%).
^{1}H NMR: ($CDCl_3$): δ 8.68 (m, 1H), 8.56 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.98 (s, 1H), 7.8 (s, 1H), 2.2 (s, 3H).

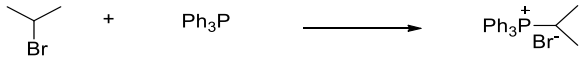
Synthesis of aldehyde 234



Butyl vinyl ether (2 mL), allylic alcohol **83** (70 mg, 0.32 mmol) and $Pd(OAc)_2$ Phen. (0.23 mg, 0.0055 mmol) was stirred at 70 ˚C overnight. The reaction monitored by NMR. When the start material was consumed the solvent evaporated under reduced

pressure. The obtained mixture was diluted with m-xylene and heated under reflux overnight. The m-xylene was removed under nitrogen stream to afford a yellow oil (40 mg). The crude oil was subjected to flash chromatography. Elution with 2% ethyl acetate in petrol afforded colourless oil of the aldehyde **235** (21 mg, 27%). ^{1}H NMR (400 MHz, CDCl3): δ 9.79 (s, 1H), 4.89 (s, 1H), 4.43 (s, 1H), 4.43 (s, 1H), 4.42 (s, 1H), 2.6 (m, 2H), 2.4 (m, 2H), 2.1-1.2 (m, 14H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, CDCl3): δ 202.36 (HC=O), 152.83 (C), 150.83 (C), 107.90 (CH_2), 105.45 (CH_2), 49.97 (CH), 44.88 (CH), 42.22 (CH_2), 41.89 (CH_2), 41.19 (CH_2), 36.85 (CH_2), 36.01 (CH_2), 29.87 (CH_2), 27.25 (CH_2), 26.90 (CH_2), 23.44 (CH_2), 16.37 (CH_3) ppm. HRMS (EI) *m/z* $C_{17}H_{26}O$ $[M + H]^+$ requires 246.1984, found 247.2055.

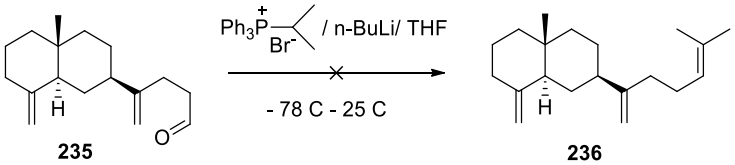
Synthesis of phosphonium salt 241



241

The procedure was adapting from Vassilikogiannakis and co-workers,[143] a solution of 2-bromopropane (11.72 g, 0.095 mol) and triphenylphosphine (20 g, 0.076 mol) in toluene (15 mL) was stirred and heated in sealed tube at 110 ˚C for 48 h. The reaction was allowed to cool to room temperature and the white solid powder filtrated. The solid was washed with hot toluene (10 mL) and dried under vacuum to give phosphonium salt **241** (3.24 g). The ^{1}H NMR spectrum identical to the literature.[143]
1H NMR (400 MHz, $CDCl_3$): δ 8.01 (m, 6H), 7.75 (m, 3H), 7.69 (m, 6H), 5.70 (m, 1H), 1.34 (dd, *J* = 19.1, 6.8 Hz, 6H).

Preparation of diterpene 236

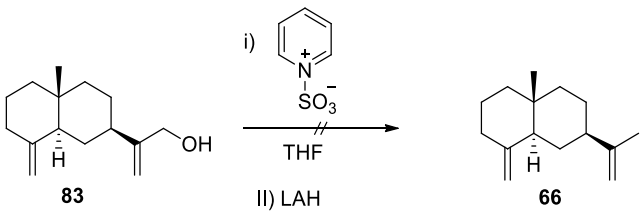


235 **236**

A solution of isopropyl triphenyl phosphonium bromide (30 mg, 0.081 mmol) in THF (3 mL) was stirred at 0 °C. Excess a solution of *n*-BuLi (1.6 M in hexane, 100 µL, 0.16

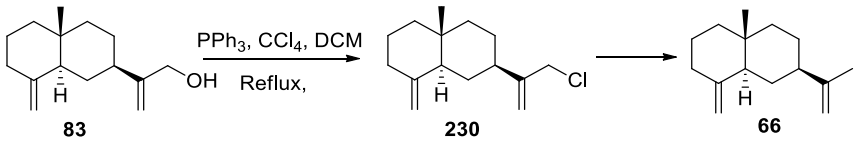
mmol) was added dropwise, and stirred for 1 h. The colour to the orange as mention in the literature. When a solution of the aldehyde **235** (20 mg, 0.08 mmol) was added, the result was a complex mixture.

Synthesis of beta-selinene



This procedure was performed followed that published by Corey and Achiwa.[93]
A solution of allylic alcohol **83** (40 mg, 0.18 mmol) in anhydrous tetrahydrofuran (5 mL) was stirred at 0 ˚C. Pyridine trioxide complex (43 mg, 0.27 mmol) was added. The reaction mixture was stirred for 3 h. Then the mixture allowed to warm to room temperature overnight. The mixture cool to 0 ˚C again and a solution of lithium aluminium hydride (30 mg, 0.8 mmol) was added then the reaction was allowed to warm to room temperature overnight. Only the starting material was recovered.

Transformation of allylic alcohol 83 to allyl chloride

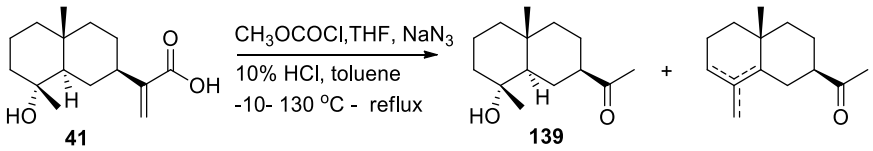


A solution of triphenylphosphine (562 mg, 2.14 mmol) in dichloromethane (3 mL) was stirred at room temperature. Allylic alcohol **83** (296 mg, 1.34 mmol) in carbon tetrachloride (3 mL) was added. The reaction mixture was refluxed 3 h. Then the mixture allowed to cool to room temperature. The solvent was removed under reduced pressure, and the crude was dissolved in hexane 3×50 mL and the suspended triphenylphosphine oxide was filtrated. The filtrate solution was concentrated under vacuo to afford an oil (256 mg). The oil was subjected to flash chromatography. Elution with 2% oh ethyl acetate in petrol gave the allyl chloride **230** (160 mg).

1H NMR (400 MHz, $CDCl_3$): δ 5.16 (t, *J* = 1.1 Hz, 1H), 5.04 (t, *J* = 1.1 Hz, 1H), 4.72 (p, *J* = 2.0 Hz, 1H), 4.43 (dq, *J* = 3.9, 1.8 Hz, 1H), 4.10 (d, *J* = 1.0 Hz, 2H), 2.37 – 2.27 (m, 2H), 2.08-1.94 (m, 1H), 1.90-0.94 (m, 11H), 0.72 (s, 3H).

Continues, a solution of allyl chloride **230** (160 mg, 0.67 mmol) in anhydrous ether (2 mL) was added to a suspension of lithium aluminiumhydride (80 mg, 2.1 mmol, 3 equiv.) and stirred at 0 ˚C. The mixture was allowed to warm to room temperature overnight. The reaction was quenched with saturated solution of NH_4Cl and extracted with ether (3 x 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentration *in vacuo* to give a colourless oil (115 mg). The oil was subjected to flash chromatography. Elution with 2% ethyl acetate in petrol afforded an oil (80 mg). 1H NMR spectrum contained **66** and unknown compound.

Transformation of ilicic acid to ketone



Ilicic acid **41** converted to ketone followed the procedure reported by Tebbaa *et al*.[67] A solution of ilicic acid (400 mg, 1.6 mmol) and triethylamine (1.7 eq, 273 mg, 2.7 mmol) in anhydrous tetrahydrofuran (50 mL) was stirred at -10 ˚C. Methyl chloroformate (1.8 eq, 270 mg, 2.8 mmol) was added and the mixture was stirred for 2 h. Then the solvent was evaporated under reduced pressure. The crude mixture was diluted with anhydrous tetrahydrofuran (10 mL) and a solution of sodium azide (1.5eq, 155 mg, 2.4 mmol) in water was added, and then the mixture stirred at 0 ˚C for 2 h. The solvent was removed, the residue was diluted with toluene (20 mL) and heated under reflux overnight. The mixture was allowed to cool to room temperature. The mixture was allowed to cool to room temperature and acidified with 10% HCl (30 mL) and heated under reflux again for extra 2 h. The organic layer was decanted and washed with 5% Na_2CO_3 (20 mL), brine (20 mL), then dried over anhydrous $MgSO_4$ and filtered and the solvent was removed *in vacuo* to give an oil (285 mg). The oil was subjected to flash chromatography. Elution with 5% ethyl acetate/spirit petroleum up to 50% afforded ketone **139** (22 mg, 6%) and by product mixture from ketone isomers.

^{1}H NMR (400 MHz, CDCl3): δ 2.39 (m, 1H), 2.15 (s, 3H), 2.08-1.98 (m, 1H), 1.79 (dtd, *J* = 12.4, 3.2, 1.6 Hz, 1H), 1.70–1.10 (m, 11H), 1.14 (s, 3H), 0.88 (s, 3Hs) ppm. The spectral data for this ketone **139** was identical to the literature.[67]

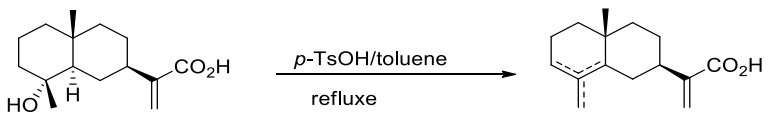
Esterification of ilicic acid

41

123 $\Delta^{4(15)}$
127 Δ^3
128 Δ^4

A solution of ilicic acid **3** (50 mg, 0.20 mmol) in DCM (2 mL) was stirred at 0˚C for 10 minutes. Oxalyl chloride (1.5 equiv., 28 mg, 0.22 mmol) was added dropwise over 5 min flowed by adding DMF (one drop). The reaction mixture stirred for 30 min at 0 ˚C. Then the solvents were evaporated under reduced pressure. To the residue was added Et_3N (3 eq), followed by adding MeOH (10 mL), and the reaction mixture stirred at 0˚C for 1 h. finely, the solvent was removed under reduced pressure to give a brown oil (52 mg). The oil was diluted by DCM and washed 5% sodium carbonate (2 × 20 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered and concentrated under reduce pressure to give inseparable mixture products from costic acid methyl ester isomers.

Dehydration of ilicic acid 41

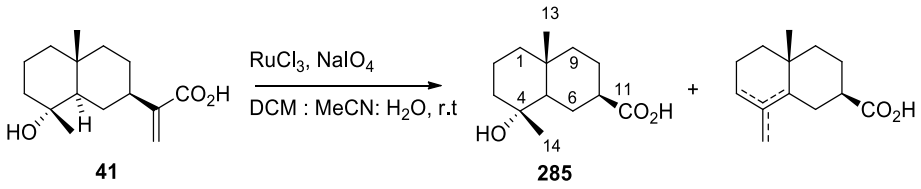


41 **26**, **51** and **122**

A solution of ilicic acid (50 mg, 0.29 mmol) in dry toluene (4 mL) was stirred under nitrogen. *p*-TsOH (50 mg, 0.20 mmol) was added and the reaction mixture was heated under reflux for 1 h. The the mixture reaction allowed to cool to room temperature and the solvent was removed under reduced pressure. The resulting crude was diluted with

ether (20 mL), washed with H_2O (20 mL) and brine (20 mL). The organic extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure afforded a brown oil (25 mg). The ^1H NMR spectrum of the crude showed inseparable a mixture product from costic acid isomers.

Oxidation of ilicic acid



Solution of sodium periodate (1.9 g, 9 mmol) was added to a stirred solution of ilicic acid (1 g, 4 mmol) in dichloromethane (1.5 mL), acetonitrile (1.5) and deionised water (3 mL) under nitrogen at room temperature. The mixture was stirred vigorously until sodium periodate was dissolved then, ruthenium chloride hydrate (200 mg, 0.96 mmol) was added. The resulting black mixture was stirred at room temperature for 24 h. The resulting black mixture was stirred at room temperature and monitored with TLC. The crude mixture was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were filtered, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a solid (1.15 g). The solid was subjected to flash chromatography. Elution with 50% ethyl acetate in petrol gave the mixture of ketoacid isomers, starting material and hydroxyacid **285** (73 mg, 7%).

IR (film, cm^{-1}): 3301.2, 3300-2500, and 1693.5; ^1H NMR (400 MHz, $CDCl_3$): δ 2.38 (m, 1H), 2.13 (ddt, J = 12.7, 3.9, 1.9 Hz, 1H), 1.80 (m, 2H), 1.67- 1.05 (m, 10H), 1.13 (s, 3H), 0.92 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 180.0 (C=O), 72.30 (C), 66.09 (CH), 44.03 (CH_2), 43.79 (CH_2), 43.43 (CH_2), 41.00 (CH), 34.55 (C), 24.35 (CH_2), 23.58 (CH_2), 22.84 (CH_3), 20.17 (CH_2), 18.64 (CH_3) ppm.

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Appendix A

Crystallography Data

Crystallography

The crystal data for **217** and **216** are summarized in Table 1, 2 and 3 with the structure depicted in Figs. A1 and A2 where ellipsoids have been drawn at the 50% probability level. Crystallographic data for the structure were collected at 100-150(2) K on an Oxford Diffraction Gemini diffractometer fitted with Mo Kα radiation. Following multi-scan absorption corrections and solution by direct methods, the structure was refined against F^2 with full-matrix least-squares using the program SHELXL-97 [ref: Sheldrick, G.M. (2008). *Acta Cryst*. A64, 112-122]. All hydrogen atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atoms. Anisotropic displacement parameters were employed throughout for the non-hydrogen atoms.

Structural Description

The formula is depicted in the scheme. The two independent molecules in the asymmetric unit are similar. They form a hydrogen bonded dimer through the carboxylic acid groups. The hydrogen bonding details are listed in Table 2.

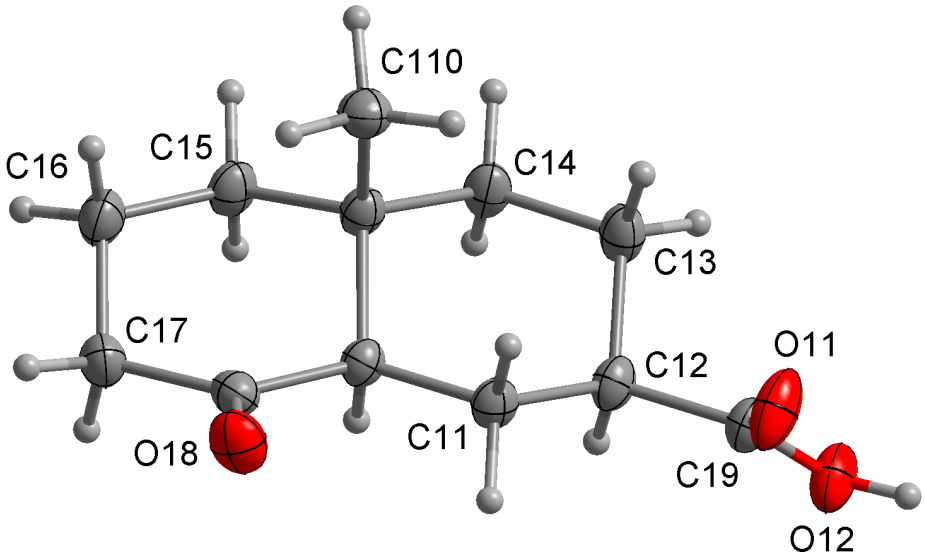


Figure A 1: X-ray structure of **217**

Table 1. Crystal data and structure refinement for (**217**)

Identification code	br25
Empirical formula	$C_{12}H_{18}O_3$
Formula weight	210.26
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 7.1821(2) Å
	b = 10.8305(2) Å
	c = 14.6280(4) Å
	β= 102.344(3)°
Volume	1111.54(5) $Å^3$
Z	4
Density (calculated)	1.256 Mg/m^3
μ	0.089 mm^{-1}
Crystal size	0.34 x 0.27 x 0.21 mm^3
θ range for data collection	3.50 to 32.66°.
Index ranges	-10<=h<=10, -15<=k<=15, -21<=l<=21
Reflections collected	13743
Independent reflections	3938 [R(int) = 0.0330]
Completeness to θ = 31.00°	99.4%
Absorption correction	Semi-empirical from equivalents
Max./min. transmission	0.9816/0.9704
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3938 / 1 / 275
Goodness-of-fit on F^2	1.034
Final R indices [I>2σ(I)]	R1 = 0.0451, wR2 = 0.1001
R indices (all data)	R1 = 0.0601, wR2 = 0.1105
Largest diff. peak and hole	0.277 and -0.192 $e.Å^{-3}$

Table 2. Hydrogen bonds for **217** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(12)-H(12A)...O(21)	0.84	1.85	2.691(2)	173.2
O(22)-H(22A)...O(11)	0.84	1.78	2.620(2)	173.8

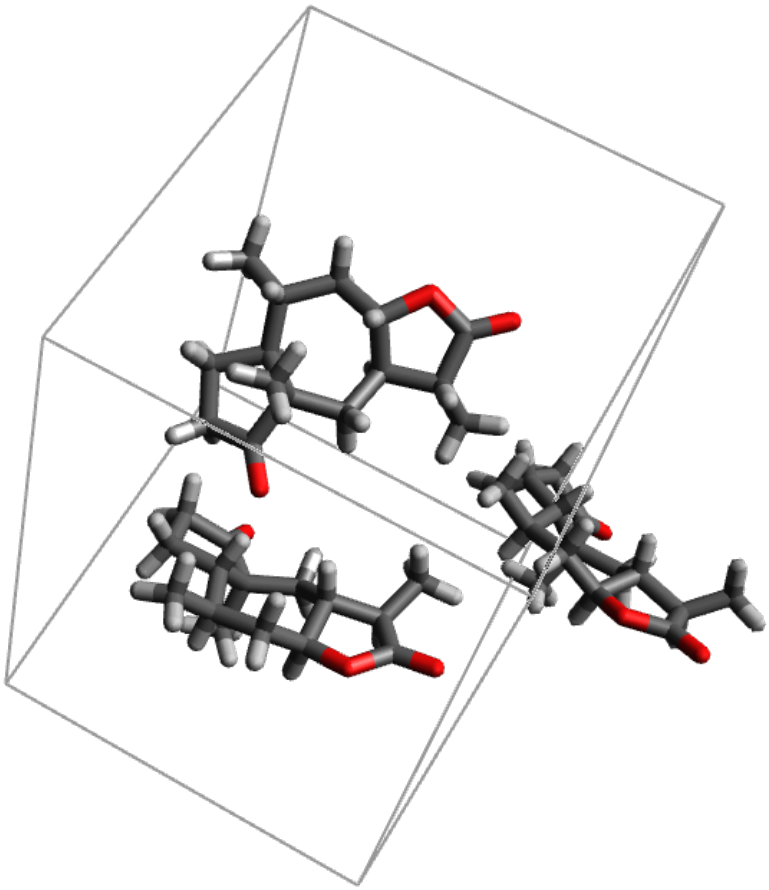


Figure A 2: X-ray structure of **216**

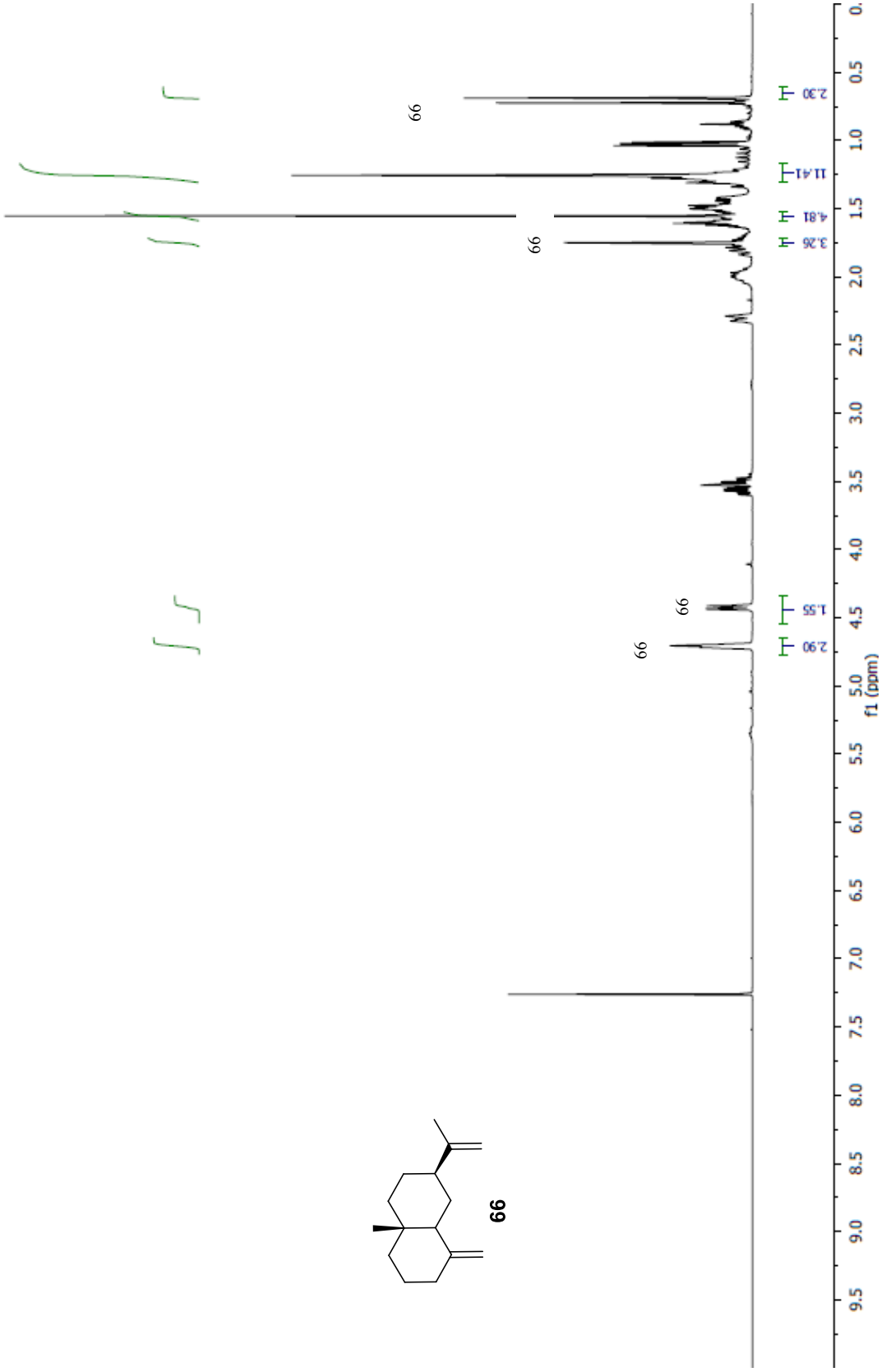
Table3. Crystal data and structure refinement for (**216**)

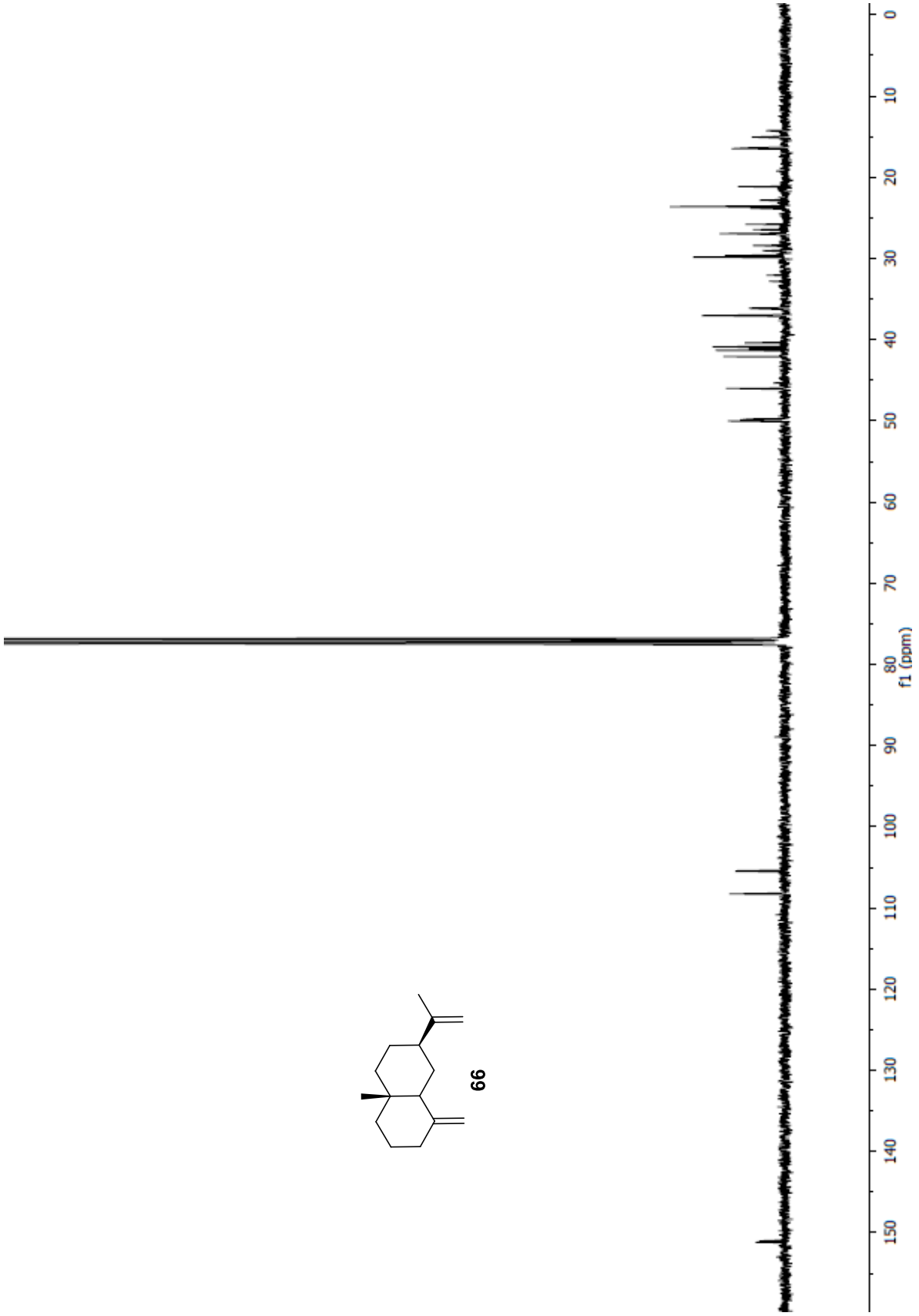
Identification code	ap02
Empirical formula	$C_{15}H_{22}O_3$
Formula weight	250.33
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 12.157(2) Å
	b = 10.5495(14) Å
	c = 15.762(2) Å
	β = 93.343(3)°
Volume	2017.9(5) $Å^3$
Z	6
Density (calculated)	1.236 Mg/m^3
μ	0.084 mm^{-1}
Crystal size	0.42 x 0.07 x 0.045 mm^3
θ range for data collection	2.82 to 26.5°.
Index ranges	-14<=h<=15, -12<=k<=13, -19<=l<=19
Reflections collected	14705
Independent reflections	4413 [R(int) = 0.1024]
Completeness to θ = 26.50°	99.8%
Absorption correction	Semi-empirical from equivalents
Max./min. transmission	0.9816/0.9704
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	4413 / 13 / 496
Goodness-of-fit on F2	0.895
Final R indices [I>2σ(I)]	R1 = 0.0643, wR2 = 0.0708
R indices (all data)	R1 = 0.1777, wR2 = 0.092
Largest diff. peak and hole	0.161 and -0.159 $e.Å^{-3}$

Appendix B

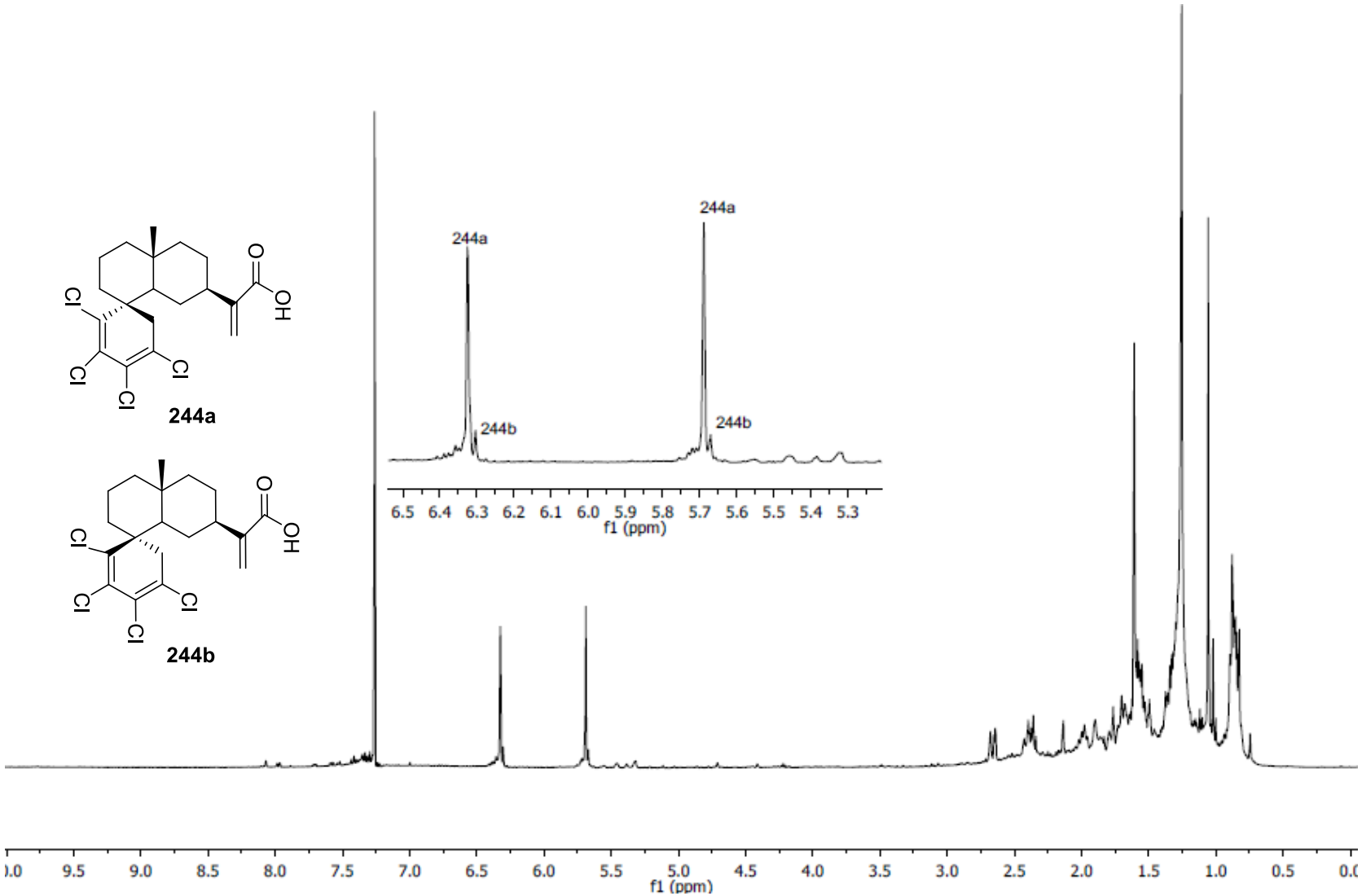
NMR Spectra

β-selinene **66** + Unknown compound

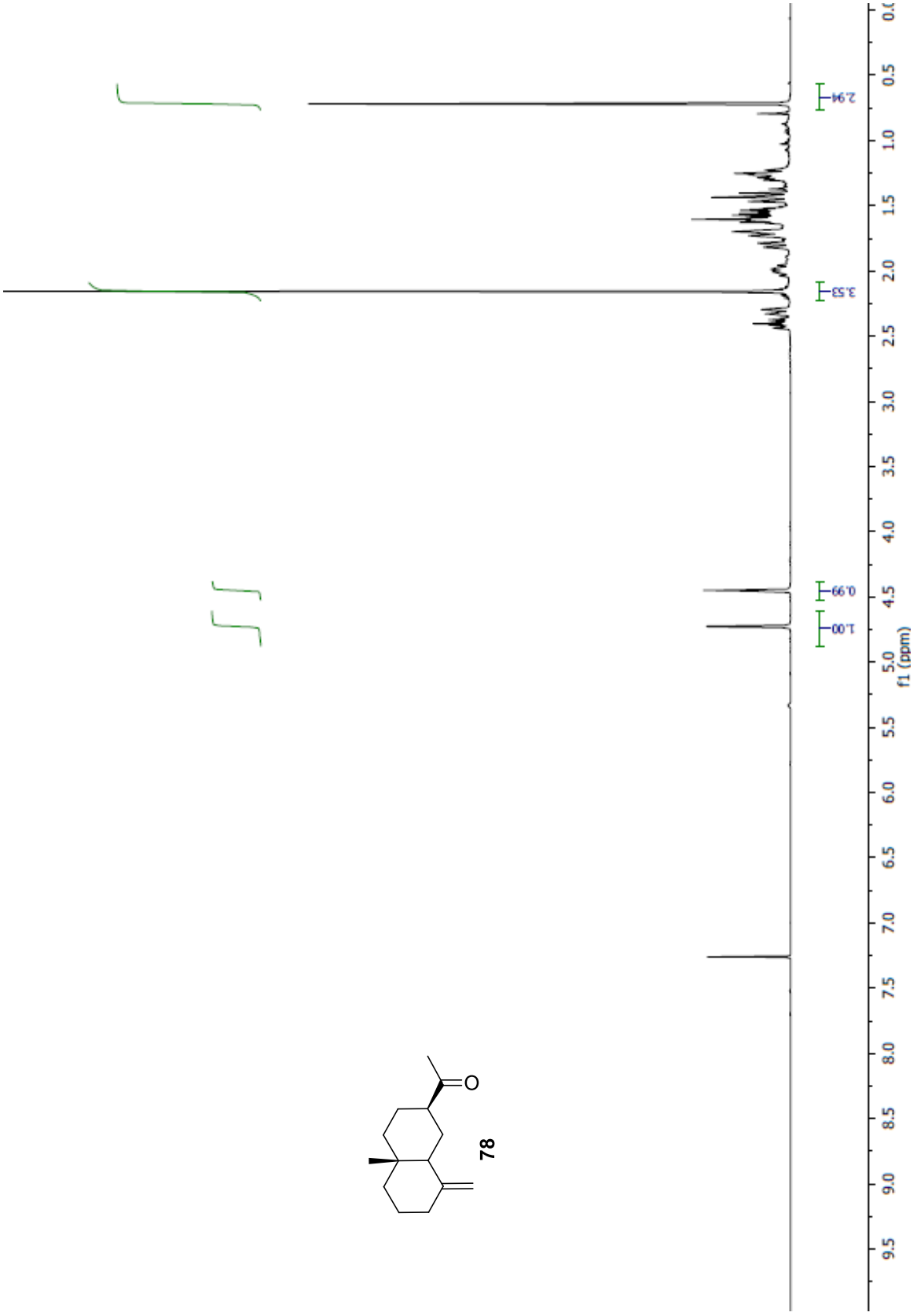


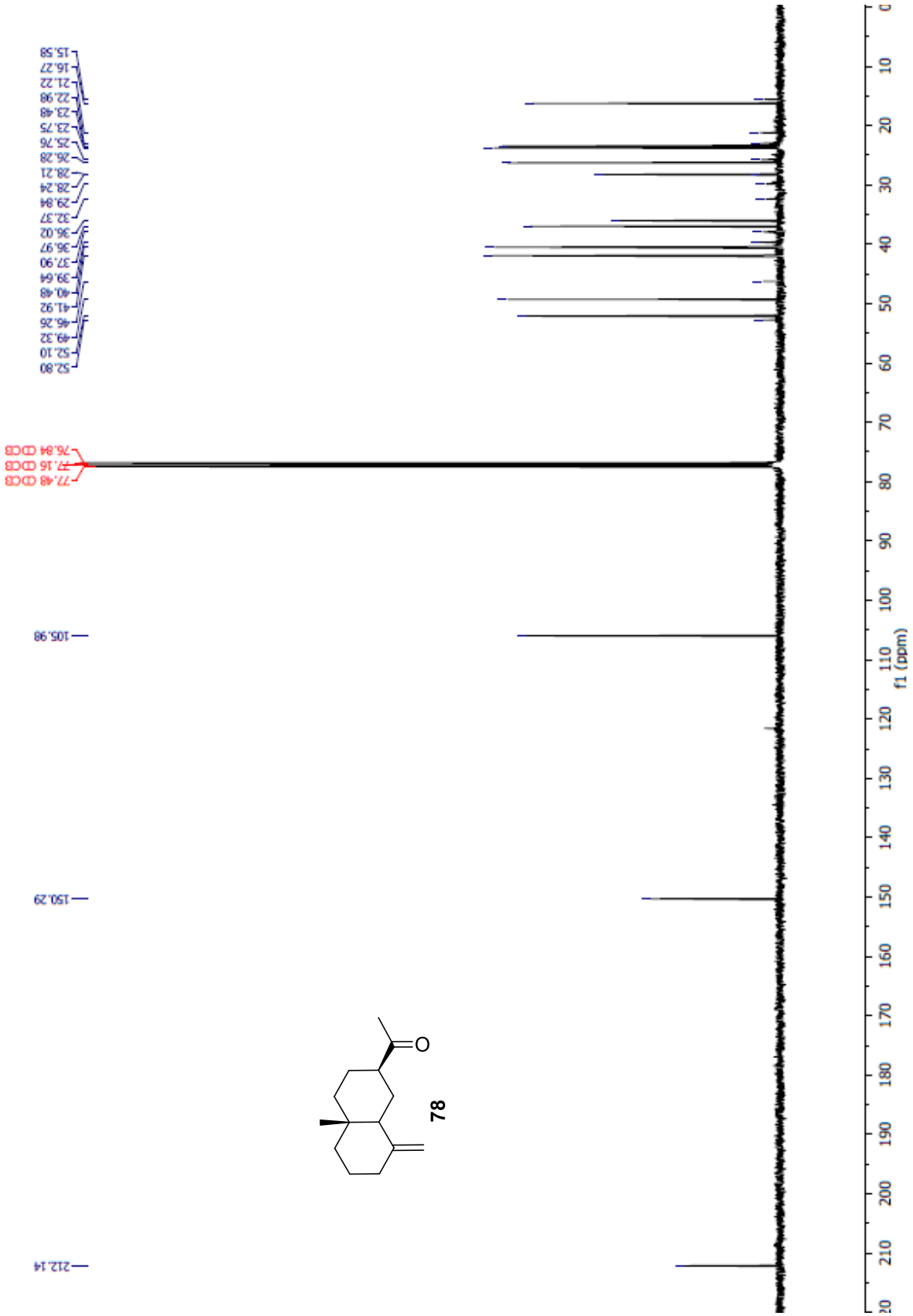


99


Cl
Cl
Cl
Cl
O
OH
244a
Cl
Cl
Cl
Cl
O
OH
244b
244a
244b
244a
244b
6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3
f1 (ppm)
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

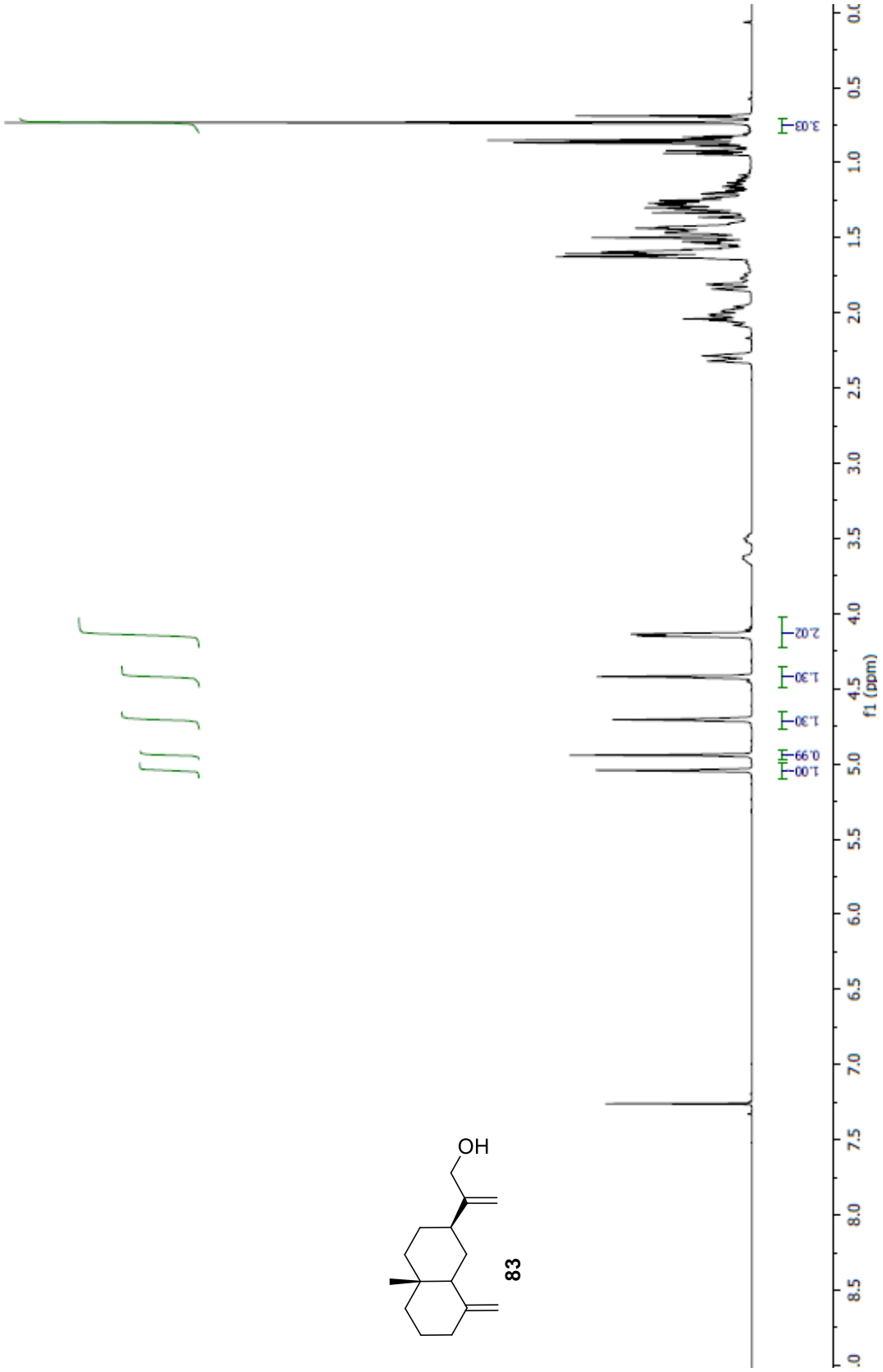
Ketone **78**

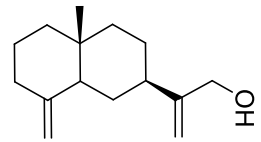



212.14
150.29
105.98
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
52.80
49.32
46.25
41.92
40.48
39.64
37.90
36.97
36.02
32.37
29.84
28.24
28.21
26.28
25.76
23.75
23.48
22.98
21.22
16.27
15.58
f1 (ppm)

78

Allyl alcohol **83**

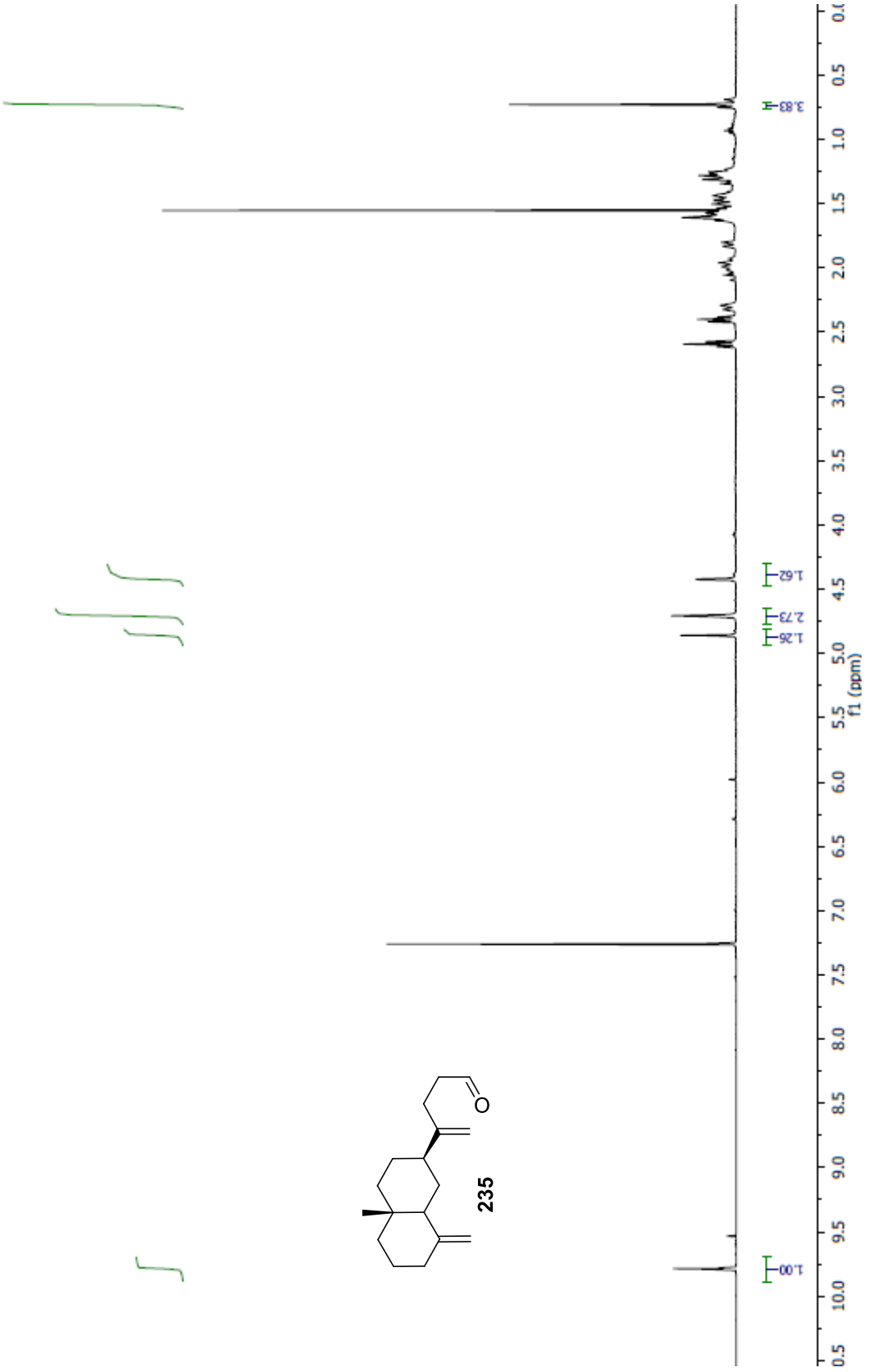



HO

83

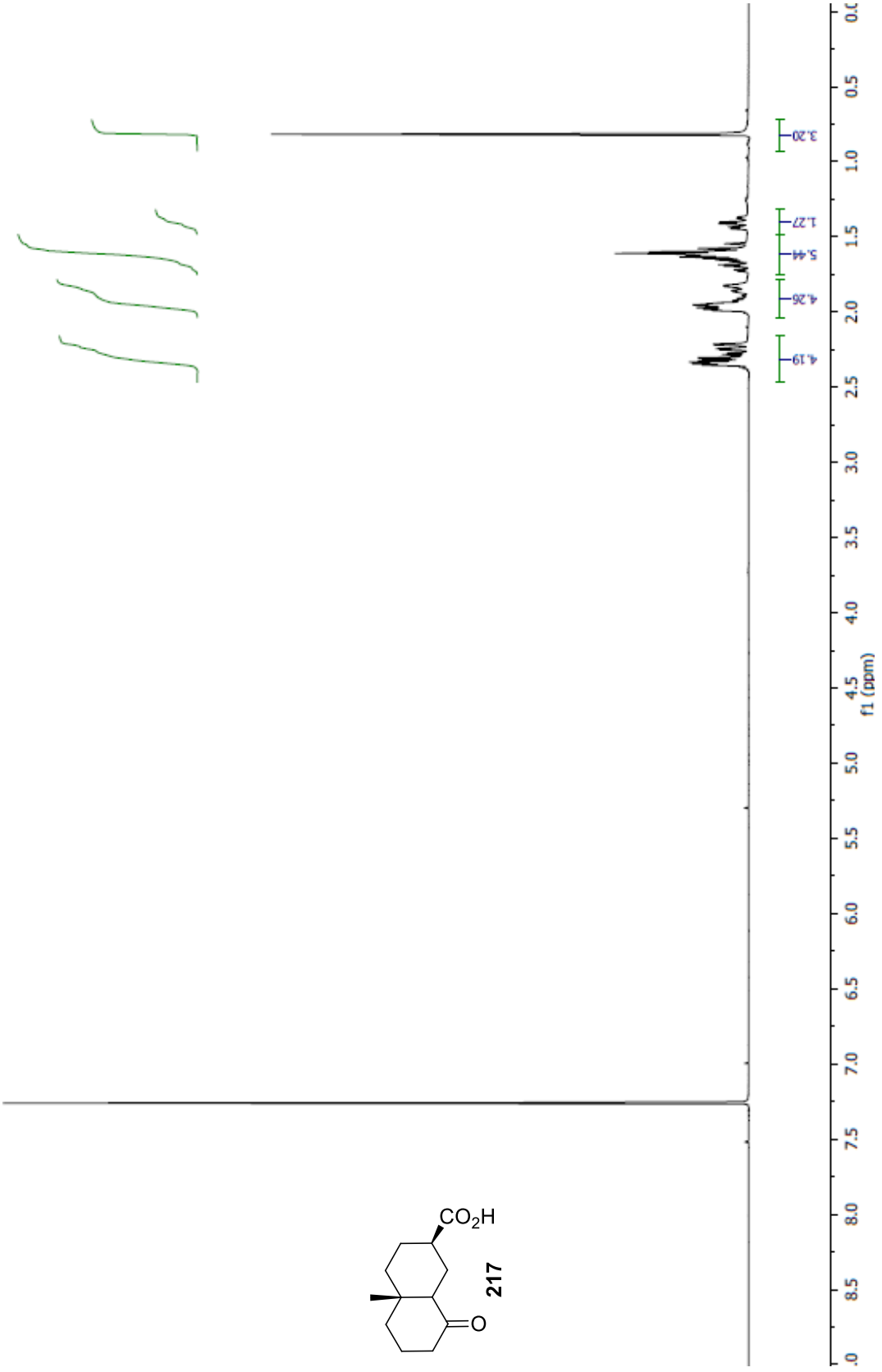
154.30
151.47
151.37
150.96
107.93
105.57
105.35
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
66.55
66.51
65.42
50.10
50.09
49.94
45.67
42.11
42.08
42.03
41.73
41.49
41.41
41.33
41.28
41.03
41.02
39.78
39.76
37.05
37.02
36.98
36.28
36.21
36.12
30.12
29.84
29.20
28.64
27.81
27.45
26.39
26.05
23.77
23.64
23.58
22.76
20.58
19.57
18.90
16.48
16.42
14.46
14.26
13.71
13.66
11.57
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

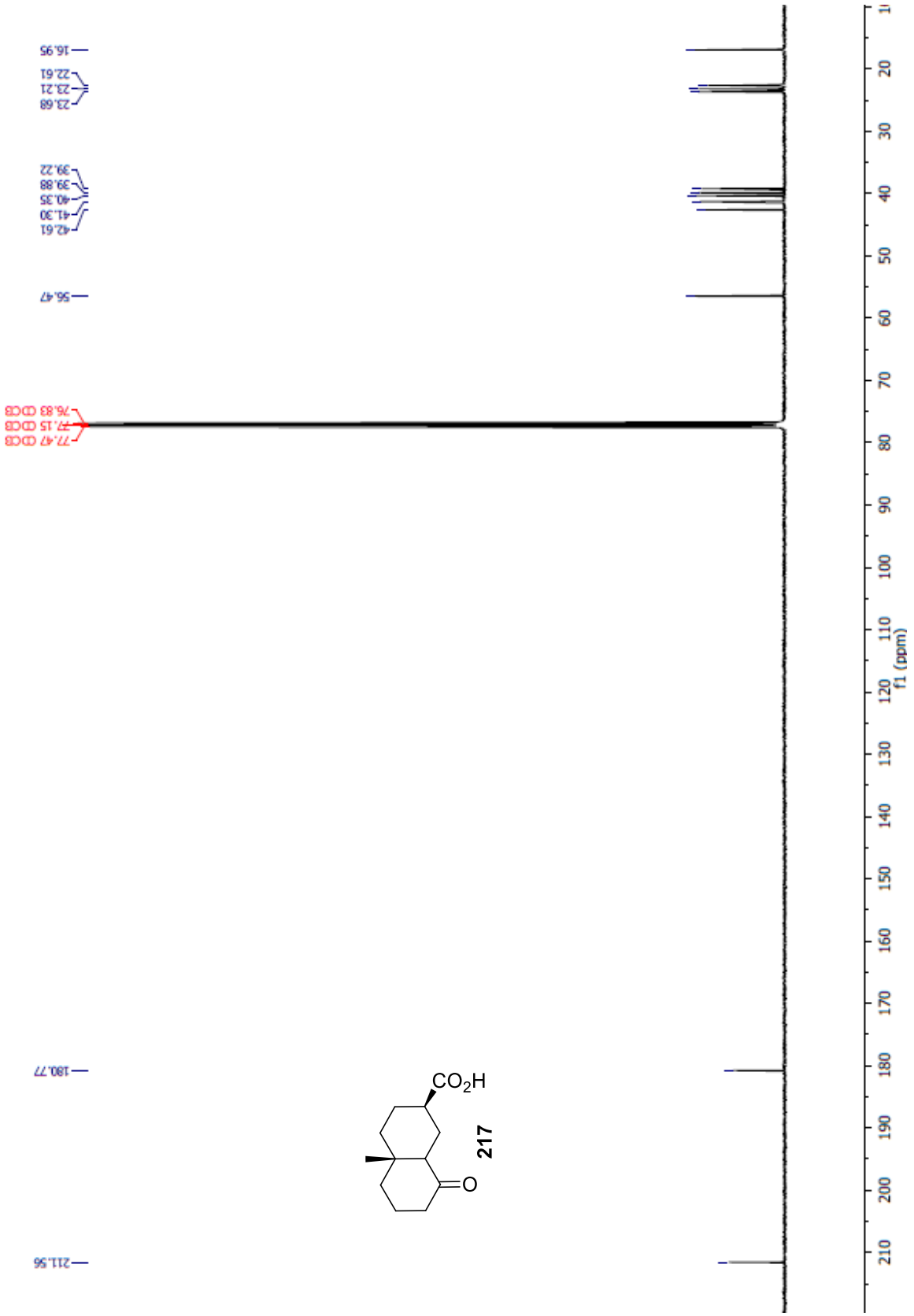
Aldehyde **235**



O
235
3.83
1.62
2.73
1.26
1.00
f1 (ppm)

Ketoacid **217**




16.95
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23.21
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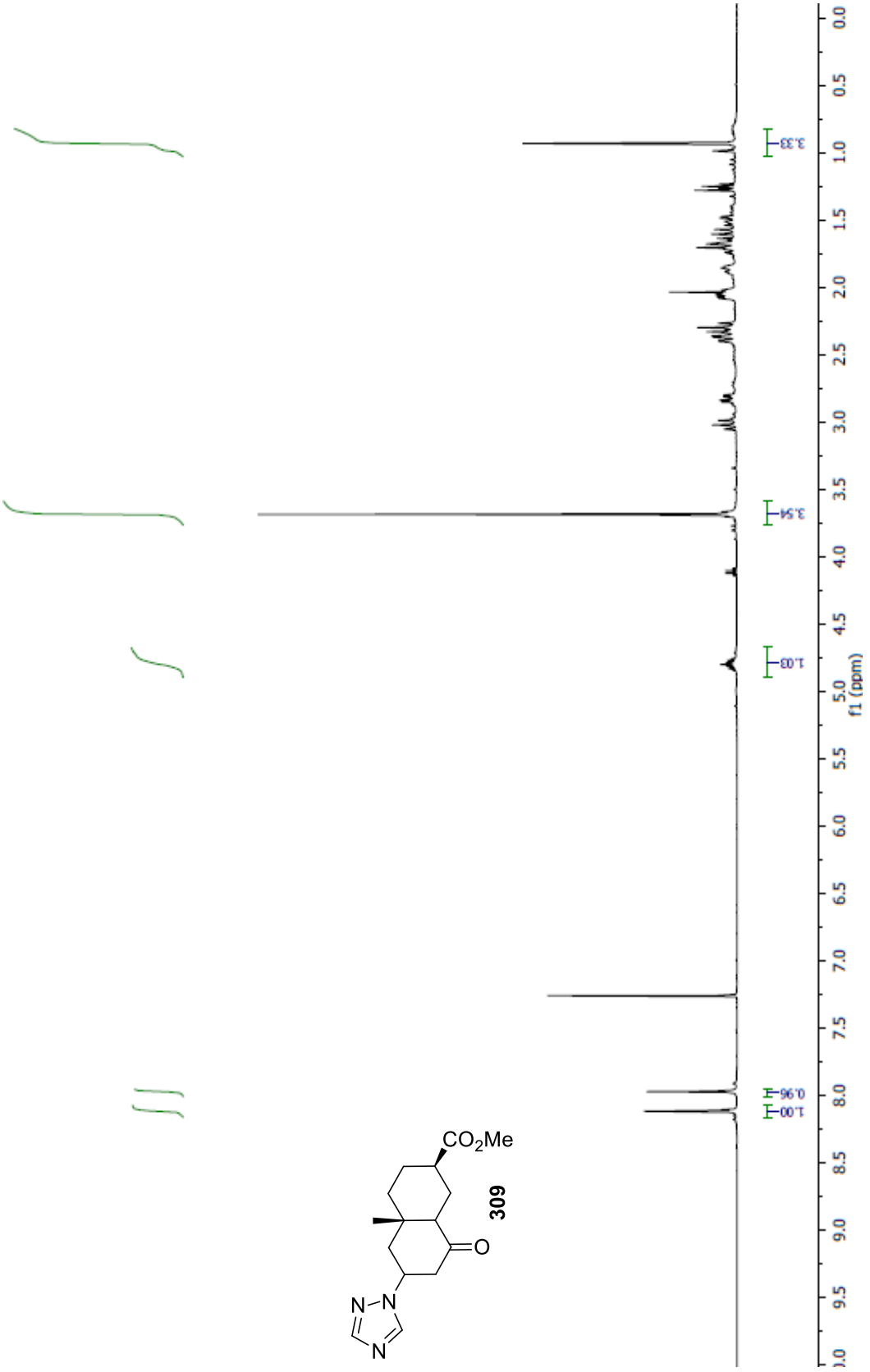
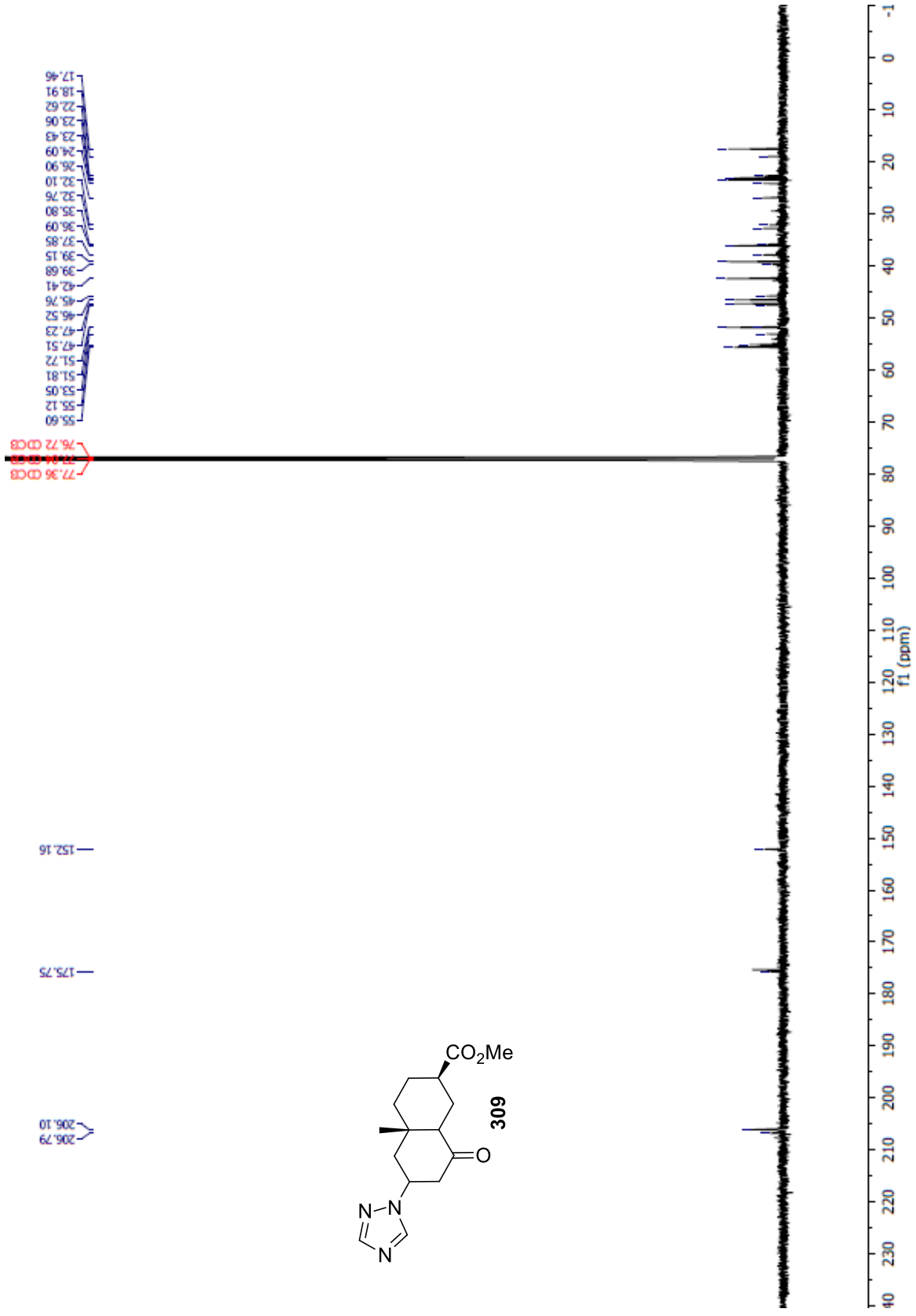
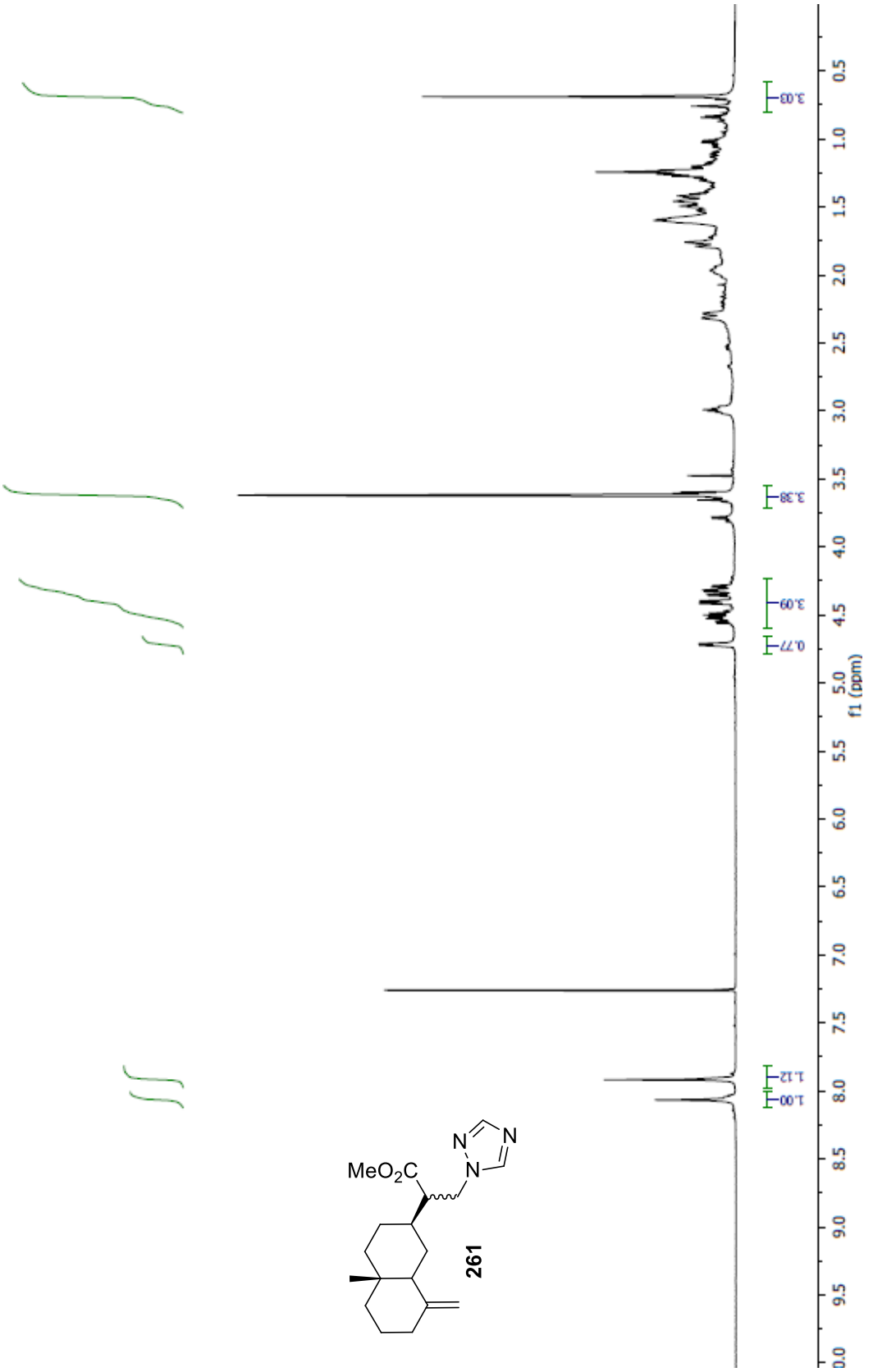
39.22
39.88
40.35
41.30
42.61
56.47
76.83 CDCl3
77.15 CDCl3
77.47 CDCl3
180.77
211.56
CO2H
O
217
f1 (ppm)

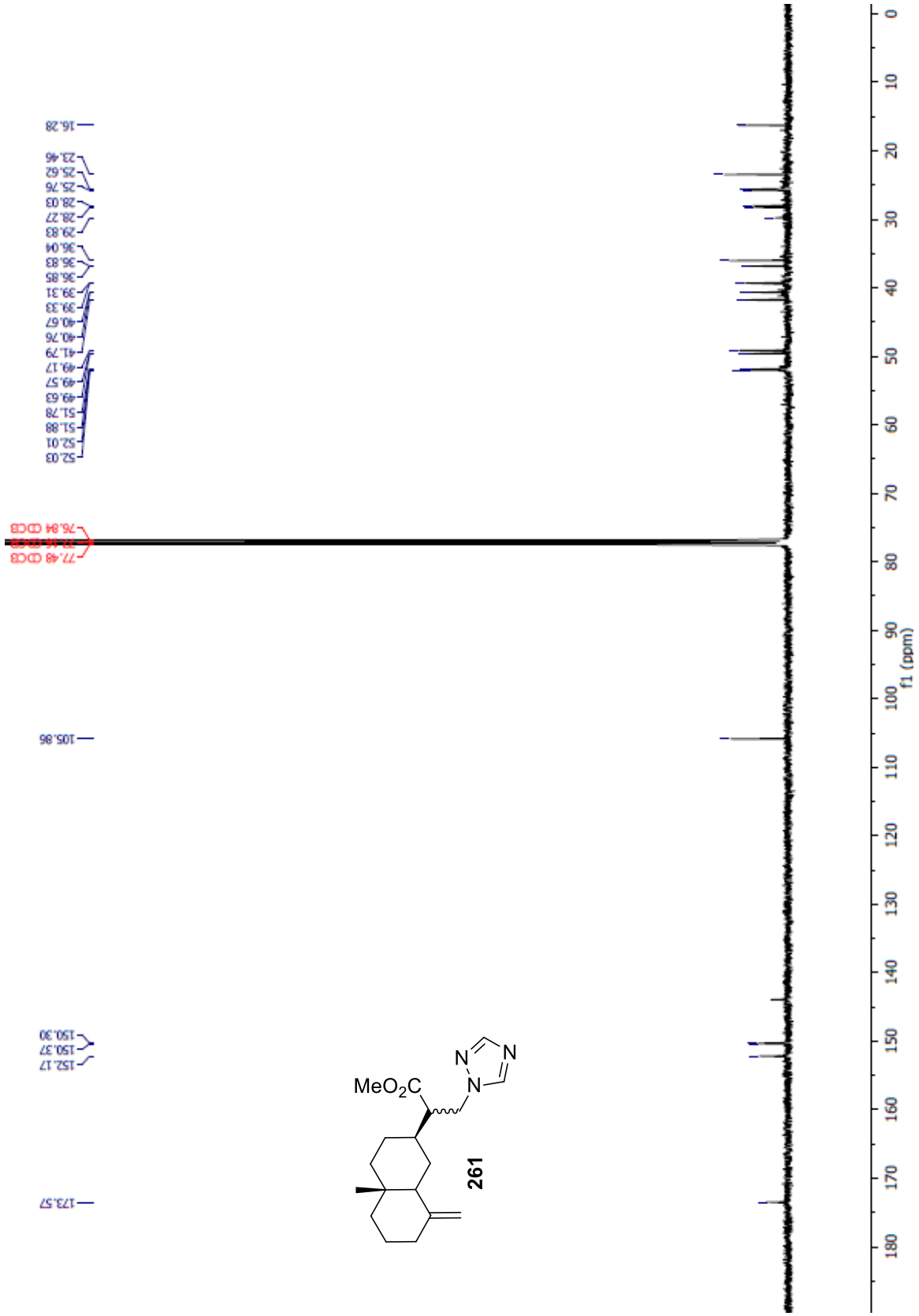
Triazole compound **309**



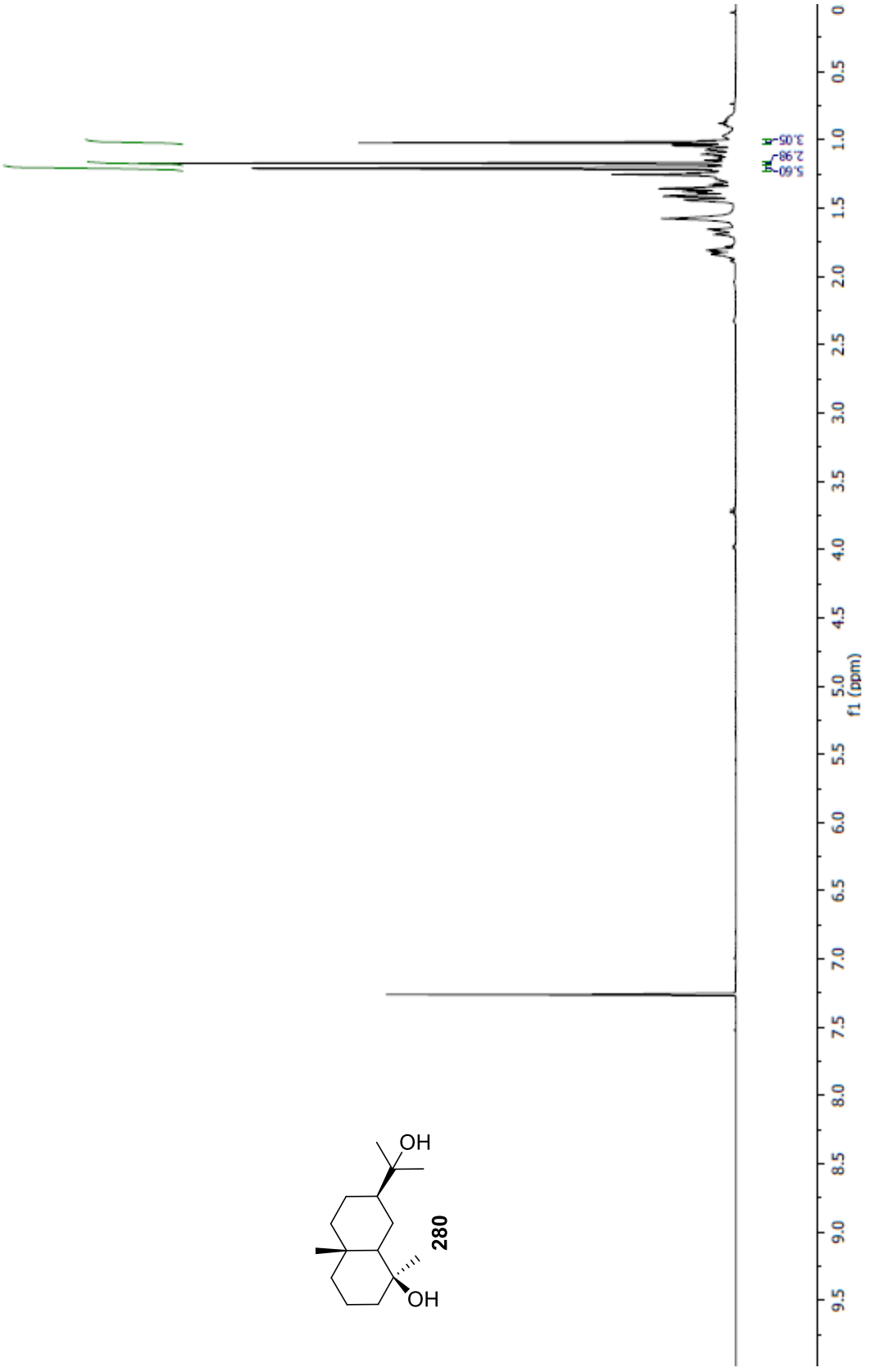

CO2Me
309
O
N
N
N
206.79
206.10
175.75
152.16
77.36 CDCl3
76.72 CDCl3
55.60
55.12
53.05
51.81
51.72
47.51
47.23
46.52
45.76
42.41
39.68
39.15
37.85
36.09
35.80
32.76
32.10
26.90
24.09
23.43
23.06
22.62
18.91
17.46
f1 (ppm)

Triazole compound **261**

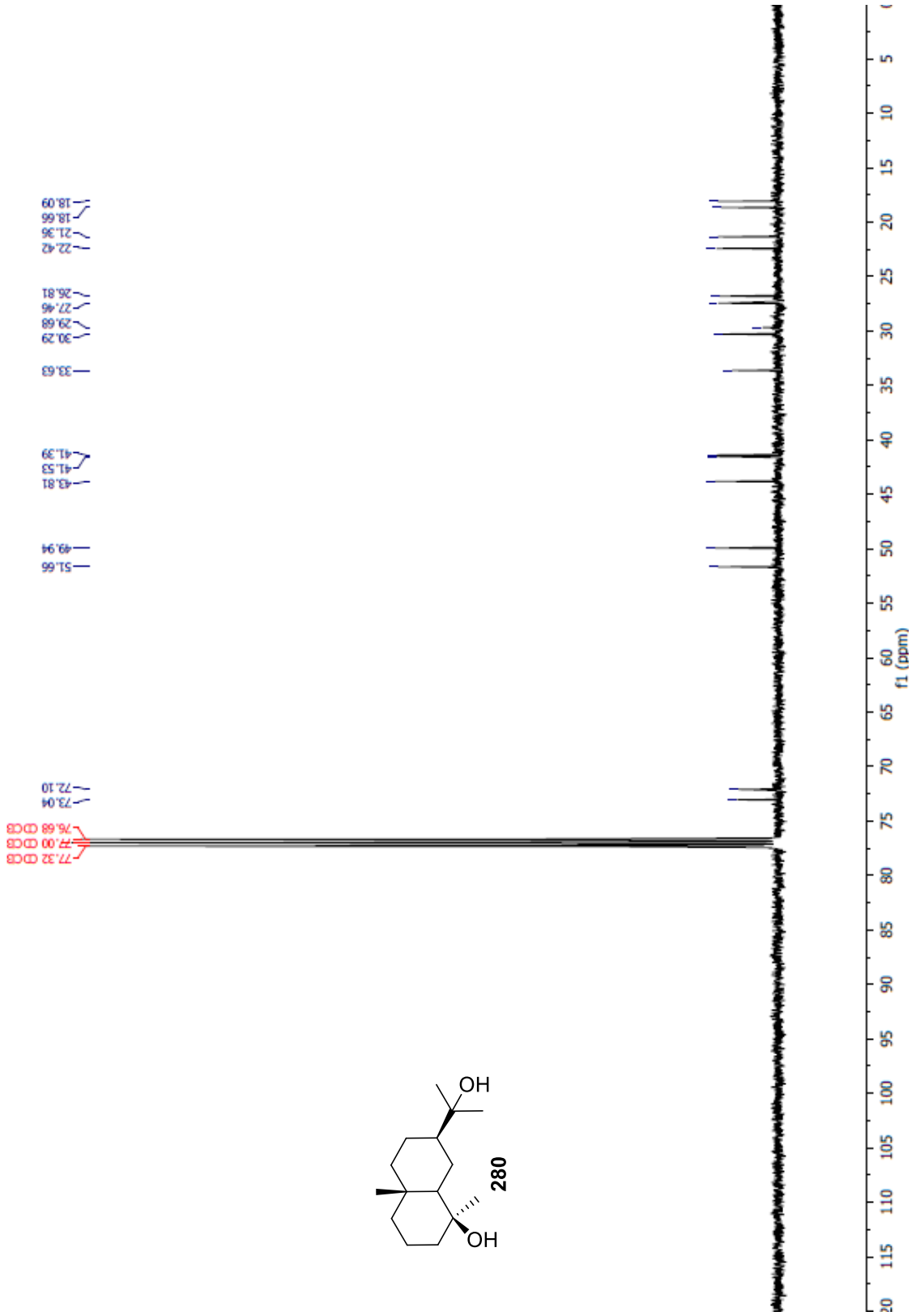



16.28
23.46
25.62
25.76
28.03
28.27
29.83
36.04
36.83
36.85
39.31
39.33
40.67
40.76
41.79
49.17
49.57
49.63
51.78
51.88
52.01
52.03
76.84 CDCl3
77.48 CDCl3
105.86
150.30
150.37
152.17
173.57
N
N
N
MeO2C
261
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

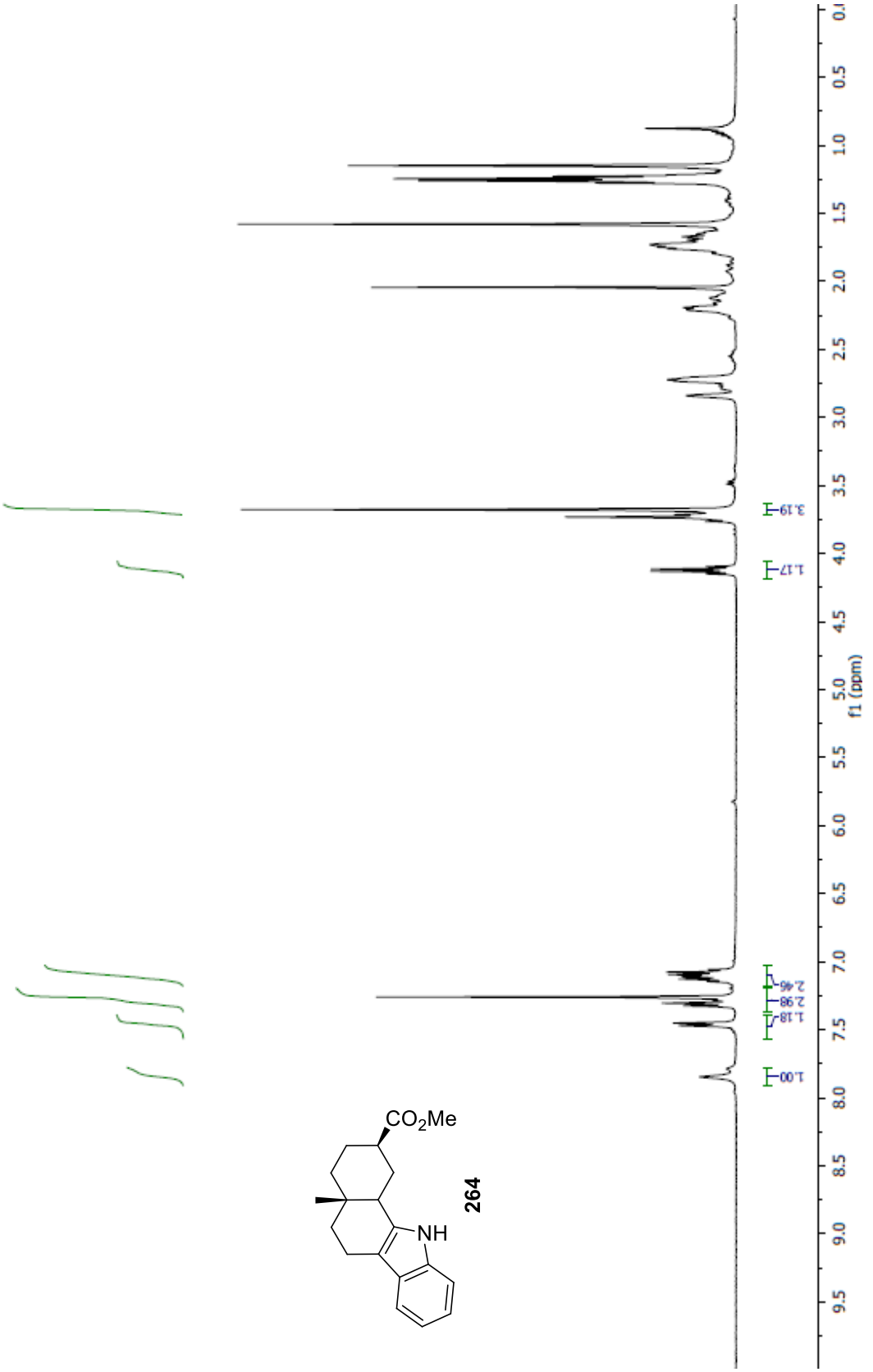
Diol **280**

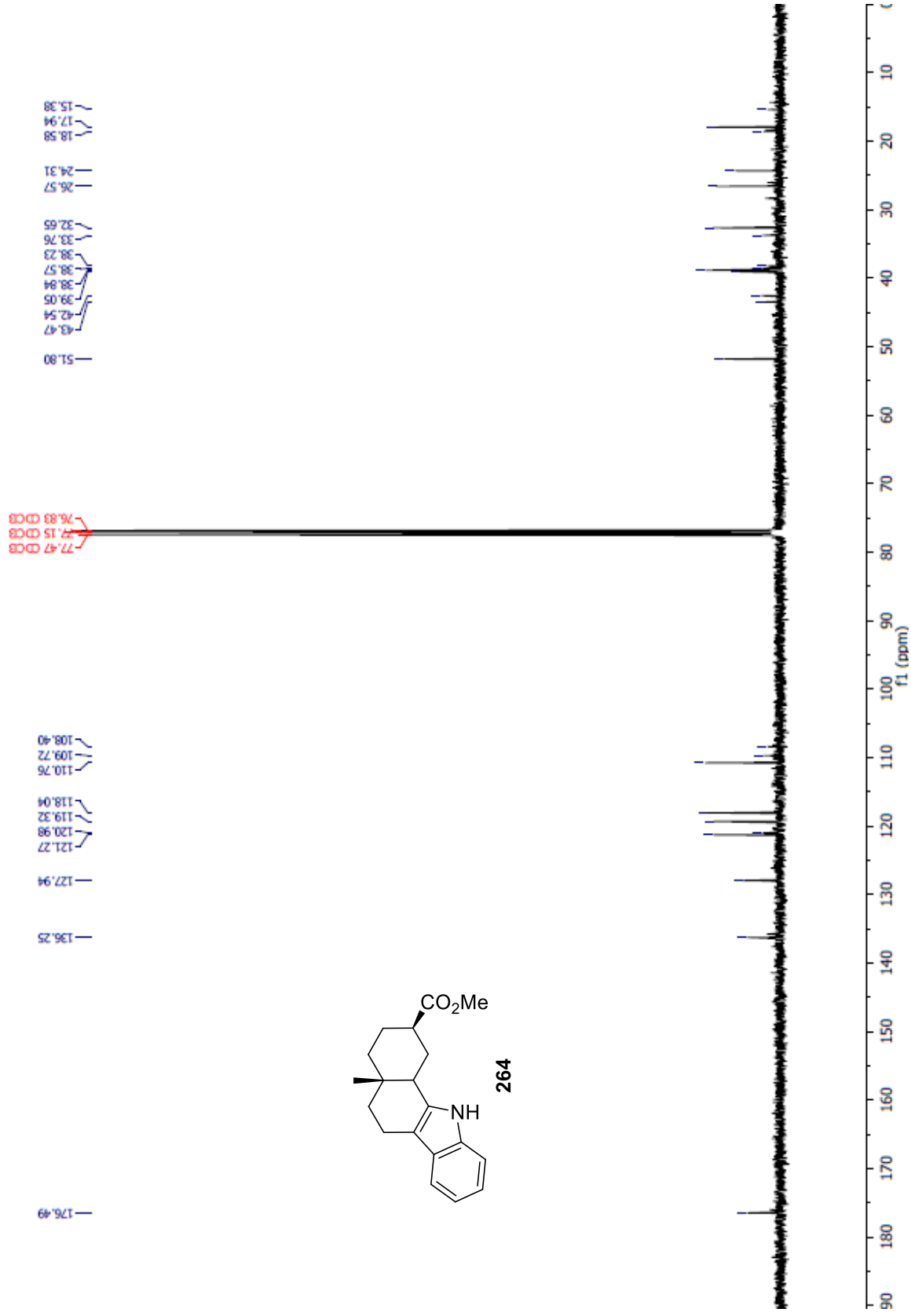


280


18.09
18.66
21.36
22.42
26.81
27.46
29.68
30.29
33.63
41.39
41.53
43.81
49.94
51.66
72.10
73.04
76.68 CDCl3
77.00 CDCl3
77.32 CDCl3
OH
280
OH
f1 (ppm)

Indole **264**


CO2Me
NH
264
3.19
1.17
2.46
2.98
1.18
1.00
f1 (ppm)


15.38
17.94
18.58
24.31
26.57
32.65
33.76
38.23
38.57
38.84
39.05
42.54
43.47
51.80
76.83 CDCl3
77.15 CDCl3
77.47 CDCl3
108.40
109.72
110.76
118.04
119.32
120.98
121.27
127.94
136.25
176.49
CO2Me
NH
264
f1 (ppm)