School of Applied Chemistry

Thermally Assisted Hydrolysis and Derivatisation Techniques for the Characterisation of Organic Materials

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This Thesis is presented as part of the
requirements for the award of the
Degree of Doctor of Philosophy
of
Curtin University of Technology

1998

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author wishes to thank the Director, Chemistry Centre (WA) for the use of equipment and time to work on the thesis, and Professor R. Alexander and Associate Professor A. Jefferson of Curtin University for their encouragement and helpful advice.

The author wishes to thank the following for supply of materials:

- Dulux Australia Pty. Ltd. and A.C. Hatrick Chemicals Pty. Ltd. for the supply of resins used in the study of alkyd resins.

- Associate Professor S Kailis, formerly of Curtin University of Technology, for the provision of numerous vegetable oils.

- Mr Z E Spadek for supply of woolgrease and data regarding the identity of its constituents.

- CETEC Australia Pty. Ltd. and A.C. Hatrick Chemicals Pty. Ltd. for the supply of resins used in the study of printing ink resins.

- Western Australian Maritime Museum, Bunnings Ltd. and the Department of Primary Industry, Queensland, Australia for the supply of woods used for characterisation purposes.

GLOSSARY OF TERMS

A.S.T.M	American Society of Testing Materials
Bisphenol-A	common name for 2,2-bis(4-hydroxyphenyl)propane
FAMES	fatty acid methyl esters
GC-MS	gas chromatography - mass spectrometry
IR	infrared
MS	mass spectrometry
PET	polyethylene terephthalate
PNP	*para* nonylphenol
PUFA	polyunsaturated fatty acid
Py-GC-MS	pyrolysis gas chromatography mass spectrometry
Pyrogram	gas chromatogram obtained by analysis using Py-GC
RAME	rosin acid methyl ester
TAAH	tetraalkylammonium hydroxide
TBAH	tetrabutylammonium hydroxide
TBP	*tert.* butylphenol
TEAH	tetraethylammonium hydroxide
THA	thermally assisted hydrolysis and alkylation
THB	thermally assisted hydrolysis and butylation
THM	thermally assisted hydrolysis and methylation
TMAH	tetramethylammonium hydroxide
TMPH	tetramethylphosphonium hydroxide
TMSH	trimethylsulphonium hydroxide
TMTFTH	trimethyl(trifluoro-m-tolyl)ammonium hydroxide

The scale on the abscissae of all chromatograms which are presented in this thesis represents the relative intensity of the detector response.

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This thesis describes the development of a novel method for the rapid identification of complex organic materials, including macromolecules, that involves a high temperature simultaneous hydrolysis and derivatisation reaction. In this procedure, aqueous quaternary alkylammonium hydroxides are made to react with a wide range of complex molecular species, including synthetic and natural polymers, under high temperature flash heating conditions. The hydrolysis products are converted to derivatives, such as alkyl esters or alkyl ethers. The reaction forms the basis for a modified pyrolysis gas chromatography (Py-GC) identification technique. Athough the process is primarily intended for the rapid identification of polymers which are susceptible to hydrolysis, it is also valuable for characterisation of a variety of hydrolysable lower molecular weight species, such as polymer additives, triglycerides and natural waxes.

The reaction takes place when an intimate mixture of an aqueous quaternary alkylammonium hydroxide solution is flash heated with the analyte in a conventional pyrolysis unit, and "on-line" GC-MS is used to separate and identify the reaction products. Analytes included synthetic polyester resins and phenolic polymers, natural products such as lipids and wood extractives, and natural polymers including lignocellulose, proteins and kerogen.

Reaction variables, such as temperature, pH, analyte particle size, substrate, and the derivatising reagent were studied, in order to find the optimum conditions for the reaction. While the reaction occurs at temperatures as low as 358°C., a 770°C. reaction temperature was adopted to allow direct comparison with Py-GC data. A high pH of the derivatising reagent was found to be necessary to achieve an efficient hydrolysis of the macromolecule. Small particle size gives better conversion to derivatised products. The nature of the heating substrate did not appear to influence the reaction. Tetraalkylammonium hydroxides (TAAH) were found to be the most

effective derivatising reagents for the reaction. Tetramethylammonium hydroxide (TMAH) was the most useful derivatising reagent, since the methyl derivatives of the hydrolysed products were conveniently chromatographed and usually had well known mass spectra. Other TAAHs were useful for (i) producing higher molecular weight alkyl derivatives of low molecular weight side chains in some polymers, e.g., acetate groups in polyvinyl acetate, (ii) the purpose of determining sites of pre-existing methylation in natural products such as lignocellulose, or (iii) cases where methylation products could be confused with existing pyrolysis products.

The reaction mechanism is believed to involve hydrolysis of the organic material, formation of the tetra-alkylammonium salt, and thermal degradation of the quaternary ammonium salt to alkylated derivatives. Some evidence is presented to support this mechanism, which is considered to be ionic in character, rather than a free radical reaction.

A detailed study of the reaction of alkyd resins indicated that polyhydric alcohols, polybasic acids, degree of cure, oil length, and rosin acid and epoxy modification could be determined. The reaction of rosin modified phenolic resins (*tert.*-butyl phenol formaldehyde and *para*-nonyl phenol formaldehyde), gave rosin acid methyl esters and easily identifiable products from the synthetic components.

Fatty acid methyl esters could be obtained directly from lipids, such as vegetable oils, without time consuming preparative steps. The problems of base catalysed isomerisation of the double bonds in polyunsaturated fatty acids were overcome by reducing the amount of base used for the reaction. The reaction facilitated the identification of fatty acids in woolwax, the triglycerides in cosmetic products, and lipids in trace quantities of human fingerprint deposits.

A more reliable representation of the chemical structure of lignocellulose in softwood and hardwood species was obtained by the reaction, as compared to conventional Py-GC which underestimates the aromatic carboxylic acid moieties. Gymnosperm or angiosperm origin was indicated by the presence of solely guaiacyl, or both guaiacyl

and syringyl derived groups, respectively. Other extraneous extractable material was identified simultaneously, including aliphatic and aromatic acids, which would not normally be detected by conventional Py-GC.

An alternative method involved extracting the wood with TMAH, followed by pyrolysis of the extract, to give less complex but more specific GC profiles. The TMAH extraction procedure also indicated some characteristic biomarker species as well as guaiacyl and syringyl derived compounds. The pyrolysis of tetraethylammonium hydroxide (TEAH) extracts revealed the sites of pre-existing methylation in the *Eucalyptus marginata* species.

The thermally assisted hydrolysis and alkylation method which has been developed is usually superior to the conventional Py-GC procedure for those polymers which are prone to hydrolysis, since it results in products which are more readily related to the polymer structure. For example, concerted hydrolysis and alkylation of polyester resins results in alkyl carboxylate esters and the alkyl ethers, whereas in conventional Py-GC the products are alkenes and carboxylic acids. Carboxylic acids are more difficult to chromatograph by GC, and aromatic carboxylic acids in particular are susceptible to decarboxylation under the pyrolysis conditions.

The reaction procedure has provided an alternative approach to the characterisation of submicrogram quantities of a range of synthetic polymers, natural products and natural polymers, which has not previously been possible without lengthy chemical degradation procedures. Although it has not displaced the conventional Py-GC technique, it has given a new dimension to the characterisation of organic materials, providing a powerful tool for forensic science investigations and the analysis of complex materials.

CHAPTER 1

INTRODUCTION

This thesis describes the development and application of a pyrolysis derivatisation method for the chemical characterisation of organic macromolecular materials. The method was developed in 1987 and the work on the applications has continued until the present time These organic macromolecular materials were mostly natural and synthetic polymers but also included lower molecular weight materials.

1.1 MACROMOLECULAR STRUCTURE DETERMINATION

The direct chemical characterisation of organic macromolecules, such as proteins, biomass and synthetic polymers, may be achieved by the use of instrumental analytical techniques which include infrared, ultraviolet and nuclear magnetic resonance spectroscopy, mass spectrometry, thermal analysis and size exclusion chromatography. An alternative approach would be to subject the polymer to prior reaction steps to reduce the molecular weight of the material by some type of degradation of the polymer with subsequent identification of the component parts in order to determine the chemical structure of the material. The chemical components of the mixture are most readily separated by chromatography techniques such as gas or liquid chromatography.

In polymer chemistry, chromatographic techniques such as gel permeation / size exclusion and field flow fractionation (Giddings, 1993) can be used to determine the molecular weight of the polymer. However, most techniques that provide information about the chemical structure of intractable materials, such as polymers, are applicable to lower molecular weight material obtained after chemical or thermal degradation of the material.

An effective identification system for natural and synthetic polymers should have:- :

- the ability to discriminate macromolecules within the same class
- adequate sensitivity and precision
- a rapid analysis time
- minimal sample pretreatment and manipulation
- an ease of interpretation of results.

In forensic science investigations, for example, it is particularly important to adopt appropriate techniques which give maximum discrimination between materials in the same class (Challinor, 1993b; Saferstein, 1982; Maehly and Stromberg, 1981). In a particular material type, the differentiating power of the technique should attain maximum sub-classification. DNA profiling, for example, provides good discrimination, with the power to unequivocally identify the source of blood, semen and hair root tissue. It can determine links in crime scene situations, and is also used for paternity testing. While tests such as solvent solubility and thermal analysis are useful for identification of physical characteristics of a paint resin, infrared spectroscopy (IR) and pyrolysis gas chromatography (Py-GC) analyses of the resin lead to a more definitive diagnosis of the paint resin composition. In some cases Py-GC analysis of paint resins can give better discrimination than IR spectroscopy (Challinor 1993b). Only traces of material are often available as forensic evidence from a crime scene and, therefore, the technique should be sufficiently sensitive to allow analysis of submicrogram size specimens. Cost effective practice requires that the methods used in a forensic science laboratory are rapid and that preparation protocols prior to analysis are not labour intensive. The data derived from the analysis is preferably not sufficiently complex that specialised data handling regimes are required for interpretation of the results.

One method used for the examination of polymers which satisfies the criteria for an effective identification system is flash pyrolysis of the macromolecular material with subsequent identification of thermal degradation products.

1.1.1 Pyrolysis Methods

Pyrolysis methods for the analysis of polymers have been developed with an increasing degree of technological sophistication. The methodology has developed to the extent that pyrolysis methods, from basic Py-GC to more complex pyrolysis-field ionisation mass spectroscopy (Py-FIMS), are now providing data about the composition of materials not generally available by other techniques. Some recent

reviews of the application of pyrolysis techniques have been published (De Forest, 1992; Blackledge, 1992, Wampler, 1989; Saferstein, 1985; Schulten, 1996).

There have been some important and novel developments in these pyrolysis methods in the last decade. End group analysis of synthetic polymers has been used for characterisation and determination of molecular weight. Polystyrene having methacryloyl end groups has been analysed (Ohtani *et al.*, 1993). Also, the number-average sequence length in the microstructure of emulsion polymers was determined by Py-GC using data from consideration of the trimer region of the pyrograms of styrene-butyl acrylate (Wang *et al.*, 1995). The characterisation of branched chain end groups in polymethyl methacrylate was undertaken by Py-GC (Ito *et al.*, 1996). A computer-based three dimensional structure method for soil organic matter and humic substances has been proposed (Schulten, 1995). The method is based on the analytical pyrolysis data derived from previously published geochemical, wet-chemical, biochemical, spectroscopic, agricultural and ecological studies. The identification of phthalocyanine type organic pigments, e.g. copper phthalocyanine blue, by Py-MS using ion trap techniques is an another example of the progress in pyrolysis technology (Private communication, J Boon).

In terms of instrument technology, packed columns have now been virtually superceded by capillary columns, allowing chromatography of higher molecular weight and more polar compounds, and improved resolution. This development has brought about a significant improvement in the performance of Py-GC by providing much more information about the composition of the analyte

1.1.2 Thermal Degradation Mechanisms

There are four major pathways for the thermal degradation of polymers (Irwin, 1982; Schnabel, 1981). These are (i) random chain scission, (ii) directed chain cleavage, (iii) side chain scission (elimination) followed by chain fragmentation and (iv) chain depropagation (depolymerisation). In many cases, more than one mechanism is possible during pyrolysis of the polymer.

(i) *random chain scission* occurs in olefinic and vinyl polymers which have a polymethylene "backbone" structure. Usually a series of oligomers is produced by random fragmentation at sites along the polymer chain. In the simplest case, polyethylene fragments to give a homologous series of alkanes, mono-olefines and di-olefines This free radical chain scission type of mechanism also takes place in the pyrolysis of polypropylene, polybutylene, polyisoprene and polystyrene (Irwin, 1979). In some vinyl polymers, such as polyacrylonitrile, the side chain does not fragment but remains attached to the polymethylene chain and a series of aliphatic oligomers are produced. However, dehydrogenation and loss of hydrogen cyanide, followed by chain scission and cyclisation gives a proportion of aromatic oligomers. (Usami *et al.*, 1990).

(ii) *Directed chain cleavage* occurs when thermolysis takes place at sites of comparatively weak bonding. For example, polyamides and polyesters undergo this type of cleavage at CO-NH and CO-O bonds, respectively. In the case of polyamides, the major pyrolysis products include fragments resulting from scission of the CO-NH bonds in the polymer chain. For example, the most abundant pyrolysis product of nylon 6.6 is cyclopentanone, derived from the "adipic acid" segment by cyclisation and loss of carbon monoxide (Senoo *et al.*, 1971). Diamines are only rarely detected as pyrolysis products in Py-GC experiments due the very polar nature of the compounds. Nitriles are common pyrolysis products of other aliphatic polyamides and these products may be formed by thermolysis of the C-N bond in the β position to the carbonyl group, followed by dehydration of the amide group (Ohtani *et al.*, 1982). Caprolactam, the cyclisation product of aminohexanoic acid, is produced on pyrolysis of nylon-6. In the case of polyesters, polyethylene terephthalate, for example, cleaves at the CO-O bond in addition to other sites in the polymer chain to give decarboxylation and recombination products (Sugimura and Tsuge, 1979).

Some examples of bond dissociation energies are given in Table 1.1 (Brydson, 1978). Low bond dissociation energies are indicative of thermal lability, e.g. O-O bonds in peroxides. Molecules having bonds with high dissociation energy are unlikely to

readily undergo thermal scission. It should be noted that the adjacent functional groups to these bonds in a polymer chain may influence the value of the bond dissociation energy. Electron withdrawing groups in close proximity to C-O and C-N bonds in polyesters and aliphatic polyamides would result in preferential thermolysis of these bonds (Schnabel, 1981).

Bond	Mean bond energy (kJ/mol)	Bond	Mean bond energy (kJ/mol)
Si-Si	178	N-H	389
S-S	220	C-H	430-510
C-S	272	O-H	464
Si-C	301	C-F	485
C-N	305	C=C	611
C-Cl	327	C=O	≈740
C-C	346	C≡N	890
C-O	358		

Table 1.1. Some examples of dissociation energies for selected bonds

(iii) *Side chain scission* takes place in some vinyl polymers where a pendant group (X) to the polymethylene chain cleaves on thermolysis The side chain radical, X·, combines with hydrogen radicals produced by thermolysis of the polymer side chain to produce HX. Aromatic hydrocarbons are produced by fragmentation and cyclisation of the backbone chain. Polyvinyl and polyvinylidene chloride undergo this type of thermolysis mechanism. Chlorine radicals are split off from the polymethylene chain and they combine with hydrogen radicals generated by thermolysis of the backbone chain, to produce hydrogen chloride. Acetic acid is a major pyrolysis product of polyvinyl acetate by a similar mechanism Fragments of the backbone chain cyclise to produce aromatic compounds including benzene, toluene and polyaromatics (Wampler, 1995).

(iv) *Chain depropagation,* or depolymerisation, takes place in polymers having polymethylene backbone chains and "unzipping" may take place to give high yields of monomer In the case of polymethylmethacrylate and poly-α-methylstyrene, the depolymerisation proceeds almost to completion, giving yields greater than 99%. These polymers do not contain any transferable hydrogen atoms which could react with a reactive radical to produce a stable tertiary radical. Thus, there is no reaction which will block the depolymerisation and prevent the unzipping process (Schnabel, 1981).

1.1.3 Relationship of pyrolysis products to polymer structure

In general, directed chain cleavage and side chain scission mechanisms result in pyrolysis products which do not have as close a structural relationship to the parent material in contrast to those pyrolysis products from random chain scission and chain depropagation processes Some examples of the pyrolysis products of selected polymers undergoing mainly directed chain cleavage and side chain scission are shown in Table 1.2 The pyrolysis products, particularly those from alkyd resins, polyvinyl acetate, polyvinyl chloride and polysaccharides do not always relate directly to the monomeric units in the polymer, in contrast to the products from pyrolysis of polymers which undergo random chain scission and chain depropagation. For example, in the pyrolysis of alkyd resins, aldehydes, alkenes and alkanes which are derived from the long chain fatty acids in the drying oils of these polymers (Challinor, 1984), do not readily reveal the identity of the fatty acids. Acetic acid, produced by side chain scission of polyvinyl acetate, and aromatic compounds (Alajberg *et al.*, 1980) are not specific diagnostic compounds for this polymer.

POLYMER	PYROLYSIS PRODUCTS	REFERENCES
Polyesters	Alkene Carboxylic acids	Sugimura and Tsuge, (1979)
Alkyd resins	Aldehydes Alkanes Alkenes Carboxylic acids Phthalic anhydride	Challinor (1984)
Nylon 6,6	Cyclopentanone Diamines	Senoo *et al.*, (1971)
Polyvinyl acetate	Acetic acid Aromatic components	Alajberg *et al.*, (1980)
Polyvinyl chloride	Hydrogen chloride Aromatic components	Alajberg *et al.*, (1980) Lattimer and Kroenke (1980)
Polyurethanes	Polyol segment products Polyester segment products Isocyanate Diamines	Lattimer *et al.*, (1990)
Polysaccharides	Furan and pyran derivatives	Helleur (1987)

TABLE 1.2. Pyrolysis products of selected polymers undergoing mainly directed chain cleavage and side chain scission mechanisms which give products which do not have a close structural relationship to the parent polymer.

1.1.4 Limitations of the Py-GC method

Many of the aforementioned pyrolysis products, e.g. carboxylic acids, amines and phenols, are polar and difficult to chromatograph by GC analysis. The aromatic carboxylic acids are susceptible to decarboxylation at elevated temperatures and are, therefore, not detected. This is a serious deficiency in the characterisation of naturally occurring polymers using pyrolysis techniques, e.g. humic substances in soils (Martin *et al.*, 1994) and aromatic carboxylic acids in lignin (Martin *et al.*, 1995b). These

polar pyrolysis products can be partly or completely adsorbed on non-polar or mid-polarity capillary gas chromatography columns. Distorted peaks of varying intensity and retention time may result. They may also be completely adsorbed in the injector of the instrument. These factors could be considered to be a limitation to an otherwise discriminatory technique used for macromolecule characterisation.

It should be noted that natural and synthetic polymers often contain small amounts of low molecular weight compounds, e.g. impurities, plasticisers. These extraneous compounds could undergo chemical reaction with the polymer when subjected to high temperatures causing main chain scission of those polymers (Schnabel, 1981). Pyrolysis processes could, therefore, result in the production of unexplained pyrolysis products which may be detected in Py-GC experiments.

1.1.5 Chemical Degradation

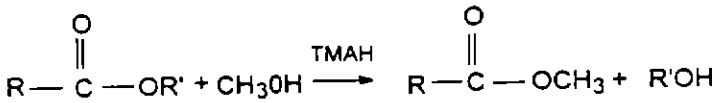
The chemical degradation of organic macromolecules often produces compounds having a close structural relationship to the macromolecule. This is the particular case with large molecules containing ester and amide linkages, i.e. those polymers which undergo pyrolysis by directed chain cleavage mechanisms. This process provides another procedure for the structural elucidation of macromolecules. Haken and Iddamalgoda (1996) reviewed the application of chemical degradation of polymers as a preliminary step to their characterisation by GC analysis. Chemical cleavage may be accomplished by fusion reactions, oxidative degradation and hydrolysis reactions.

Fusion reactions involve heating the sample with the acid, akaline, reducing or oxidising reagent at a temperature above the fusion reagent melt temperature (Frankowski and Siggia (1972), and Haken (1979, 1989)). Hydrolysis of susceptible polymers (10mg) can be achieved quantitively in a reactor attached to a GC by heating mixtures of a large excess of alkali with the polymers. The reactor can be a commercial instrument (Perkin Elmer) which has been modified to cold-trap the products and thermally desorb the products into the GC. More recently, Haken and Iddamalgoda (1995) have reported that their latest reactor may now be employed for

smaller (2-5mg) sample sizes bringing the technique closer to the capability of pyrolysis techniques

Reagents for oxidative fusion include potassium metaperiodate, lead tetraacetate, potassium dichromate and chromium trioxide. Sodium acetate is the most useful flux to lower the reaction temperature in order to avoid thermal decomposition of the sample before reaction takes place. The method has useful applications in the evaluation of the stability of a polymer to oxidation (Haken 1979). Reductive fusion gas chromatography involves the use of an organic reagent which is strongly reducing itself or slowly decomposes to release the reducing agent. Hydrazine is such a reagent, but its usefulness is limited by its volatility and instability in air.

Hydrolysis or solvolysis reactions involve the breaking of bonds between carbon and a heteroatom. The reagents include water, alcohols, ammonia, and hydrazine If the heteroatom occurs in the polymer side chain, then scission of the side chain would be expected. When the heteroatom is located in the main chain, significant rupture of the polymer would occur. In alkaline media, hydroxide ions attack the carboxyl carbon atoms and, in the case of a polyester, the ester linkages are ruptured according to the normal hydrolysis mechanism to produce the alcohol and the carboxylate salt In acid or neutral media hydrolysis is initiated by protonation of the carboxyl oxygen followed by the addition of water and cleavage of the ester linkage to produce the alcohol and the carboxylic acid. The characterisation of lipids by processes such as these are well known (Christie, 1990). The technique is a standard method for the quantitative determination and identification of polymers having hydrolysable linkages. In most cases, elevated temperatures and long reaction times are required. However, tetramethylammonium hydroxide (TMAH) was found to react very rapidly at relatively low temperatures with polyesters, alkyd resins and some polyacrylates. According to West (1975), the polymer is initially saponified by refluxing with TMAH in methanol for periods of up to 15 minutes and then esterified with methyl iodide in the presence of dimethyl formamide/methanol solvent. It appears, however, that methanolysis is accomplished in the first stage (Mosselman and de Witt, 1977) (Eq.1).



Eq 1

Hydrochloric acid hydrolysis procedures have been used in the chemical degradation of polyamides to determine their acidic and amino precursors (Mori *et al.*, 1970a and 1970b), or by alkali fusion methods (Glading and Haken, 1978) In the latter method, which employed external fusion of the polymer (100mg) with the fusion reagent, the diamine was extracted with *n*-butanol and the solution was acidified to liberate the dicarboxylic acids which were methylated by boron trifluoride/methanol reagent prior to GC analysis of both the diamine and the diacid. Hydrochloric acid hydrolysis has been used as a standard procedure for the determination of amino acids in proteins. Hydrolytic degradation processes can also be induced by enzymatic action. In some cases an accompanying pH change can bring about hydrolytic cleavage of sensitive linkages in the polymer. In other early work using hydrolysis procedures for chemical degradation, polyurethanes were cleaved by aminolysis (Wittendorfer, 1964). Also a review of degradative polymer analysis summarised how acid and alkaline fusion reactions were employed for the analysis of polyether and polyester urethanes (Haken and Iddamalgoda, 1996).

The products from these chemical degradations are often polar and require modification to permit efficient analysis by GC methods. For example, carboxylic acids and alcohols resulting from the chemical degradation of polyesters require derivatisation to methyl esters and silyl ethers, respectively. These procedures involve additional analytical steps which can be labour intensive, time consuming and, in some cases, can allow losses of product to occur.

1.1.6 Derivatisation of Polar Compounds for Analysis

Polar organic compounds are often encountered as drugs, pesticides, pollutants, etc., and it is standard laboratory practice to separately derivatise them offline prior to conventional GC analysis. The derivatised compounds then chromatograph without adverse adsorption effects. Blau and Halket (1992) have reviewed the general methods used for chemical derivatisation of polar organic compounds for chromatography. On-line methods of derivatisation, or pre-reaction of an analyte prior to GC analysis has been used to "streamline" the process of chemical structure determinations.

(i) On-line chemical reactions.

On-line methods of chemical reaction have been useful for the analysis of the components of chemical mixtures. Haken (1979) discussed the use of a pre-column as a reactor to modify the products from mixtures injected into the GC. A pre-column, coated with a reactive medium, may be used as a subtractive procedure in which a chemical compound type is selectively removed from a mixture of low molecular weight compounds. Aldehydes, ketones, alcohols and saturated, unsaturated and branched hydrocarbons may be abstracted in order to determine the presence of the respective groups. The success of this procedure depends on the rapid reaction of the target chemical compound types with the reactive medium in the pre-column.

(ii) Hydrogenation and catalysed thermolysis reactions.

While on-line hydrogenation is not a derivatisation reaction, it is a useful technique used in some pyrolysis processes. On-line hydrogenation in reactors which are coupled to GC capillary columns provides a method which can yield additional information about the composition of unknown mixtures (Schomburg *et al.*, 1982). The structural elucidation of olefinic and aromatic hydrocarbons and compounds containing polar functional groups may be carried out. The GC profiles of the mixtures, with and without this hydrogenation step, are then compared. Hydrogenation of unsaturated pyrolysis products of polyethylene and polypropylene has been used to determine the degree of branching in the polymer (Tsuge *et al.*,

1980). The flash pyrolysis products, entrained in hydrogen carrier gas, are passed over a palladium catalyst.

Sequence distributions in polyacetals were studied by the pyrolysis of the finely divided polymer in the presence of a cobalt catalyst (Ishida *et al.*, 1995) The ethylene oxide content and distributions, in relation to the basic polyoxymethylene structure, were evaluated on the basis of the cyclic ethers. These processes necessarily involved the thermolysis of the polymer with the production of smaller molecular units which were then changed by a catalytic reaction.

(iii) Pyrolytic methylation.

One of the on-line methods used for preparing methyl derivatives of acidic compounds involves a procedure termed pyrolytic methylation. The free acid, mixed with a quaternary ammonium hydroxide is injected into the heated injection port of the GC, where the quaternary ammonium salt undergoes thermal dissociation to produce the methyl derivative. Kossa and McGee (1979) have reviewed the applications of this on-line derivatisation technique. Abraham and Criddle (1985) have studied the effect of various experimental conditions in pyrolytic methylation on the conversion of carboxylic acids and phenolic compounds to their respective methyl derivatives.

Summary

Macromolecular materials could not normally be characterised until they have been converted into smaller molecular species, possibly by a chemolytic or enzymatic reaction step, prior to any derivatisation protocol. Consequently, there would be an advantage in developing a rapid single-step on-line process whereby macromolecules could be converted to products which are more representative of polymer structure and whose products may be chromatographed efficiently.

It is appropriate at this stage to outline the structure of lipids and macromolecules such as polyesters and phenolic polymers, for which such a process would be applicable. Current techniques for the characterisation of these materials and their limitations will also be discussed. These techniques always employ chemolytic degradation reactions to give smaller molecular units and these reactions are usually followed by a derivatisation step and separation and analysis by GC.

1.2 LIPIDS

Lipids are described in most texts on lipid chemistry as that fraction of any biological material extractable by solvents of low polarity. However, a narrower concept of a lipid may be defined as ".... fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds" (Christie, 1989). The more prominent lipid members may be grouped on the basis of composition as follows:-

- Fatty acids
- Lipids containing glycerol:-
  - neutral fats
  - phosphoglycerides
- Lipids not containing glycerol:-
  - sphingolipids
  - aliphatic alcohols and waxes
- Lipids combined with other classes of compounds:-
  - lipoproteins
  - proteolipids
  - phosphatidopeptides
  - lipoamino acids
  - lipopolysaccharides

In this discussion, we will be concerned with the first two groups as these are the focus of attention in the thesis.

Fatty acids serve as a source of food energy for man and animals. The types of fatty acids are an important factor in human health, particularly in respect of serum lipid concentrations and their effect on coronary heart disease. The C_{20} polyunsaturated fatty acids are important dietary precursors (Huang, 1992). Fatty acids are also significant components in commercial products, such as cosmetics and some surface coatings.

1.2.1 Structure of Fatty Acids

The fatty acids from plant, animal and microbial sources generally comprise even carbon number, straight chain carboxylic acids. Double bonds, if present, occur in the *cis* configuration in a specific position relative to the end-located carboxyl group. Common fatty acids in animal tissue vary in chain length from 14 to 22 carbon atoms and in some cases, the range can be from 2 to 36 or more. Higher plants usually have a more limited chain-length distribution. The most abundant saturated fatty acids in animal and plant tissue are straight-chain compounds with 14, 16 and 18 carbon atoms. All possible odd and even homologues with 2 to 36 carbon atoms have been found in the esterified form. Monoenoic fatty acids with 10 to more than 30 carbon atoms, containing one *cis* double bond in a variety of different positions, have been found in nature. The most abundant mono-unsaturated fatty acid is probably oleic acid (*cis*-9-octadecenoic acid). *Trans* configuration isomers are also found in nature e.g., *trans*-3-hexadecenoic acid is always present as a significant component of plant chloroplast lipids. Polyunsaturated fatty acids of animal origin can be subdivided into families according to their biosynthetic origin The families contain from 2 to 6 *cis* double bonds, separated by methylene groups (i.e. an allylic configuration). Acids with more than one methylene group between the double bonds such as the eicosadienoic acids, occur in marine invertebrates and some other organisms, but are rarely found in animals. Some plant species synthesise *trans* configuration isomers or contain acetylenic bonds. In general, polyunsaturated fatty acids are prone to oxidative degradation or autoxidation (Christie, 1989; Swern,1979).

1.2.2 Characterisation

There are two conventional approaches to the characterisation of lipids. These are a) analysis of the triglycerides and b) isolation and analysis of the individual fatty acids.

The characterisation of triglycerides by high temperature capillary GC has been reported by Chavas Da Neves and Vasconcelos (1989). Huopalahti *et al.*, (1989) describe a procedure using capillary supercritical fluid chromatography. The use of high performance liquid chromatography to separate and detect the triglycerides has been reviewed (Wojtusik *et al.*, 1989). Further discussion on triglyceride analysis is outside the scope of this study.

Lipids are generally identified by determining the fatty acid composition of their triglycerides. The generally accepted standard method of analysis of these fatty acids relies on the GC determination of the fatty acid methyl esters (FAMES) (Eder, 1995; Christie, 1993; Blau and Halket, 1992).

Fatty acids may be present in the free form or combined with glycerol as the triglyceryl ester. Free fatty acids are readily converted to their methyl esters using acid catalysts in anhydrous methanol and most lipids can be transesterified to their methyl esters without a prior hydrolysis step. Methanolic hydrogen chloride and sulphuric acid are probably the best general purpose esterifying reagents and boron trifluoride / methanol has also been used as a transesterification catalyst and, in particular, provides a rapid means of esterifying lipids (Bannon *et al.*, 1982; Christie, 1989). The use of perchloric acid as a catalyst has been reported (Maurikos and Eliopoulos, 1973).

Base catalysed transesterification procedures may employ anhydrous methanolic solutions of sodium or potassium methoxides. Methanolic TMAH was used with some success (Metcalfe and Wang, 1981). The progress of the development of these techniques is outlined in Chapter 7.

The GC determination of the fatty acid composition of lipids has also involved the methylation of the free acids using quaternary ammonium hydroxides. The

applications of the technique have been reviewed by Kossa *et al.*, (1979) The process involves the simultaneous injection of the free acids and the reagent into the the gas chromatograph where the quaternary ammonium salts are converted to methyl derivatives pyrolytically in the injection port of the GC.

Problems associated with FAME preparation include (Shantha and Napolitano, 1992)

- incomplete extraction of FAMES after transesterification
- incomplete conversion of lipids into FAMES
- formation of artefacts which overlap FAMES or may be wrongly identified as fatty acids
- contamination of the GC column resulting from residual esterification reagent
- evaporative losses of highly volatile short chain chain FAMES
- the risk of autoxidation of polyunsaturated fatty acids during the analytical procedure
- the toxicity of solvents used for extraction post transesterification
- the number of manipulative steps in the procedures which could cause losses and preclude automation
- the difficulty of analysing submicrogram quantities of material without special microchemical techniques

This latter point is particularly important in the applicability of transesterification to biological material and forensic examinations.

A single step, on-line GC microanalytical procedure which incorporates hydrolysis of the triglycerides and esterification would facilitate the identification of lipids in natural products and biological material.

1.3 POLYESTER RESINS

Commercial synthetic polyester resins have important uses as surface coatings, structural plastics and adhesives. They include alkyd resins, saturated and styrenated unsaturated polyesters. Alkyd resins are widely used in surface coating applications, including architectural oil based paints, industrial enamels and automotive body

finishes, in spite of more recent advances in coatings technology which have produced the acrylics, urethanes and epoxies. The analysis and characterisation of these polymers is an important part of materials science and forensic casework.

1.3.1 Molecular Structure of Cured Alkyd Resins

The alkyd resins are polyesters prepared by reaction of polyhydric alcohols, polybasic acids and long chain fatty acid glyceryl esters. A typical idealised part structure of an uncured resin, based on pentaerythritol, phthalic anhydride and a fatty acid glyceryl ester, having a fatty acid, RCOOH, is shown in Figure 1.1.

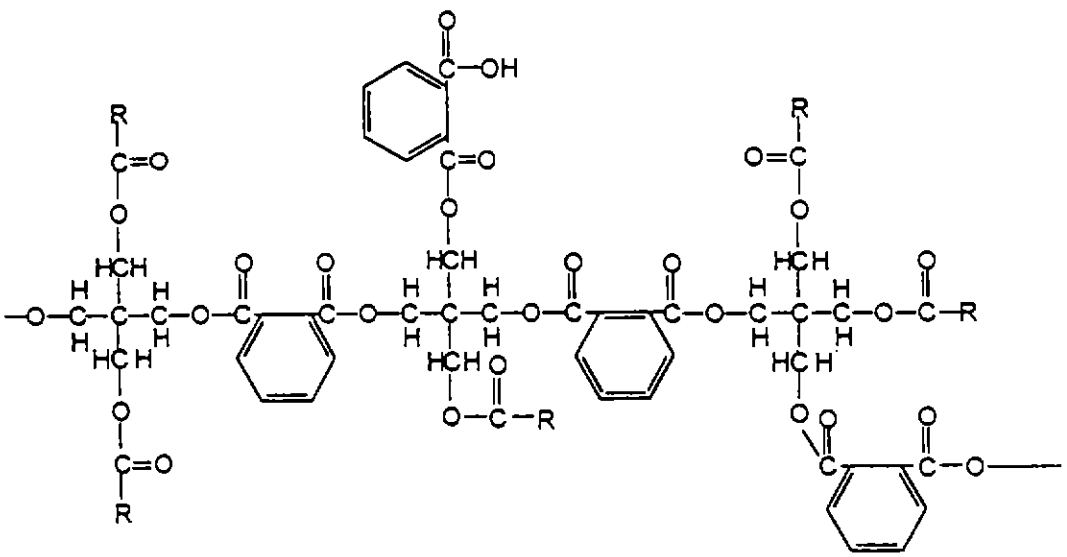


Figure 1 1 An idealised partial structure of an uncured alkyd resin.

Resin manufacturers change the type and proportion of the reactants to suit the application and to take advantage of optimum raw material costs.

The drying and performance characteristics of the alkyd can be further improved by modification with acrylics, urethanes, styrene, polyamides, silicones, rosin and phenolics.

1.3.2 Characterisation of Alkyd Resins

The chemical structure of cross-linked alkyd resins is complex. Curing takes place by autoxidation at the allylic sites in the unsaturated fatty acid chains and a complex arrangement of linkages results. Indeed, the degree of cross-linking increases with time due to the relatively slow curing reaction. Determination of the polybasic acid, polyhydric alcohol and fatty acid composition in these insoluble resins presents a formidable analytical problem. Further, modifiers such as epoxide resin, rosin-phenolic, or styrenated resins complicate the characterisation.

Infrared spectroscopy, nuclear magnetic resonance and chemical degradation are the most commonly used techniques for characterisation. Their application to the characterisation of alkyd resins is described in more detail in Chapter 5. However, in an interesting application of chemical degradation to these resins, the age of cured alkyd paint films was estimated in work published by May (1975). The coatings were saponified and then methylated with diazomethane. The ratio of saturated methyl palmitate to unsaturated methyl esters e.g., linoleneate, linoleate and oleate, was measured. It was found that whilst linoleneate was present in only trace quantities after one week of drying, linoleate falls to insignificant proportions in two to four weeks, oleate was consumed more slowly and a downward trend was just beginning to show after six weeks. Azelaic acid, $HO_2C(CH_2)_7CO_2H$, was formed by the oxidative breakdown of the unsaturated fatty acids and was found in significant proportions after one week but changed little thereafter.

Haken (1979) has reported chemical degradation / gas chromatography methods of characterising polymers which have included alkali fusion of alkyd resins. Sample sizes as low as one to four milligrams were used.

Capillary Py-GC has been used successfully to analyse alkyd and other paint resins typically found in forensic casework. Good discrimination between 20 alkyd resins of

different polyol, polybasic acid and drying oils was obtained (Challinor 1984) A summary of the pyrolysis products diagnostic for the resin components is shown in Table 1 3.

RESIN COMPONENTS	DIAGNOSTIC PYROLYSIS PRODUCTS
POLYOL	
Glycerol	Acrolein
Pentaerythritol	Methacrolein
POLYBASIC ACID	
Orthophthalic acid	Phthalic anhydride
Isophthalic acid	Benzene
DRYING OIL	Aldehydes
MODIFIERS	
Rosin-phenolic type	Substituted phenols
Styrenated	Styrene oligomers

Table 1 3 Characteristic pyrolysis products of alkyd resin components.

The advantages and disadvantages of the methods for identification of alkyd resins can be summarised as follows (Table 1.4).

METHOD	ADVANTAGES	DISADVANTAGES
IR	Rapid Sensitive	Limited within class discrimination
NMR	Good discrimination of drying oil classes	Generally only suitable for uncured resins
CHEMICAL CLEAVAGE	Components identifiable Curing can be monitored	Complex preparation and time consuming
Py-GC	Rapid Sensitive Discriminatory	Composition determination is difficult

Table 1.4. Advantages and disadvantages of commonly used methods for characterisation of alkyd resins.

A simple, rapid, sensitive and discriminatory procedure would assist in the identification of the wide variety of alkyd resins.

Saturated polyester resins are manufactured by the condensation polymerisation reaction of polyfunctional alcohols and carboxylic acids. Some typical reactants are shown in in Table 1.5 -

Polyhydric alcohols	Polybasic acids
Glycerol	Phthalic anhydride
Pentaerythritol	Isophthalic acid
Neopentyl glycol	Terephthalic acid
Trimethylol propane	Adipic acid. Sebacic acid.
	Trimellitic anhydride

Table 1.5 Starting materials for the preparation of some saturated polyesters

Saturated polyesters, are mainly used in industrial finishes on metal. The finishes are prepared *in situ* by reacting the excess hydroxyls of the polyester and baking with urea and melamine formaldehyde resins Saturated polyesters also form useful fibre-forming polymers, e.g. polyethylene terephthalate (PET). Unsaturated polyester resins have wide application in fibre reinforced plastics and automotive body fillers.

1.3.3 Composition of Unsaturated Polyesters

The unsaturated polyesters are formed by crosslinking low molecular weight polyester resins by addition polymerisation with maleic anhydride (or fumaric acid) and styrene The polyester resins are initially prepared by reacting glycols with polybasic acids.

1.3.4 Identification of Saturated and Unsaturated Polyesters

The polyesters are usually identified by IR, NMR and Py-GC techniques. In Py-GC, these polymers usually produce pyrolysis fragments not easily related to the polymer structure, since these saturated polyesters undergo a directed chain cleavage pyrolysis mechanism. In addition, those polyesters which have polyfunctional aromatic carboxylic acids often undergo decarboxylation at elevated pyrolysis temperatures. Conventional procedures for the identification of alkyd resins, saturated and unsaturated polyester polymers do not usually provide adequate information about the nature of the polyol and polybasic acids in the polymer because of the subtle differences in these components. The most effective method is chemical cleavage with subsequent derivatisation and analysis by gas chromatography However, this procedure is time consuming and not readily applicable at the submicrogram level.

1.4 PHENOLIC RESINS.

A versatile range of synthetic resins can be produced from phenols by reaction at the *ortho* and *para* sites on the aromatic nucleus and by reaction at the hydroxyl group. Phenylpropane polymeric structures also form the basis for the macromolecular matrix in wood lignin

1.4.1 Composition of Phenol-Formaldehyde Condensates

Heat reactive one step resins, known as resoles, which contain methylene, methylol and methylene ether structures are formed by an alkali-catalysed reaction of phenol and formaldehyde (Keutgen, 1968), and have the general structure shown in Figure 1 2.

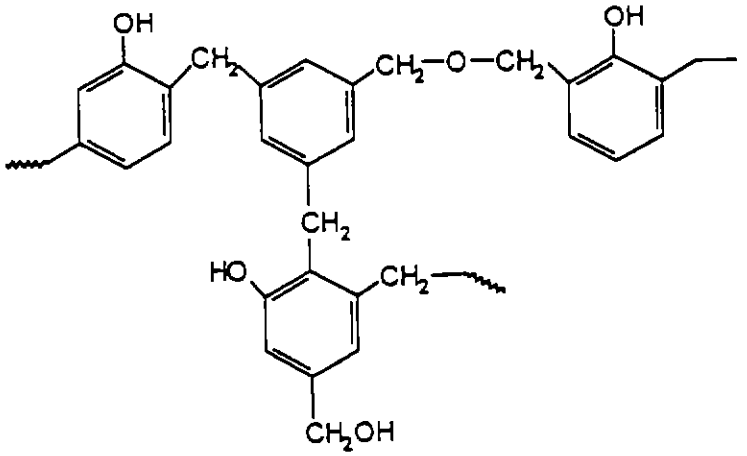


Figure 1.2. The general structure of heat reactive one step phenol-formaldehyde resins (resoles)

Acid-catalysed two step resins, known as novolacs, which require an additional curing agent and only contain methylene linkages have the general structure shown in Figure 1.3.

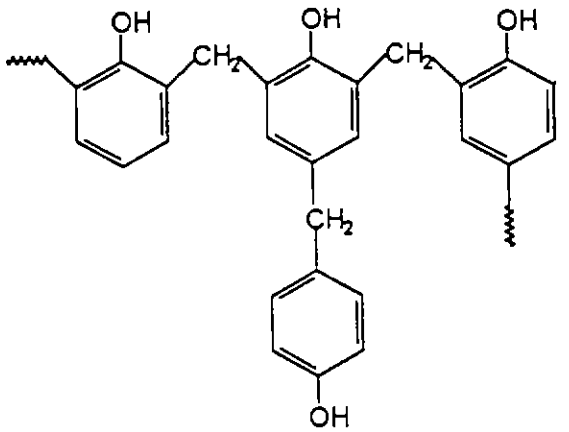


Figure 1.3. The general structure of acid-catalysed two step phenol-formaldehyde resins (novolacs).

Linear, oil-soluble resins are produced by *para* substituted alkyl and arylphenols and have the structure shown in Figure 1.4.

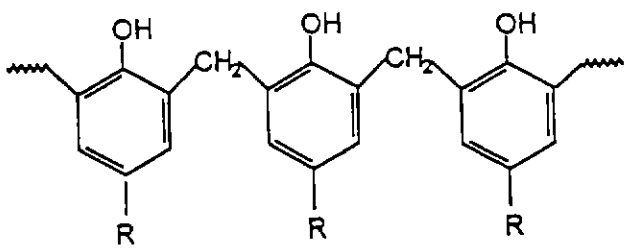


Figure 1.4. The structure of linear, oil-soluble phenol-formaldehyde resins.

This group of resins may be modified with wood rosin for special applications such as printing inks, and the identification of these materials is the subject of Chapter 6. Otherwise, the major uses for phenolics are molding compounds, coatings, and industrial bonding resins. The latter includes resins for grinding wheels and coated abrasives, brake and clutch linings, laminates, plywood adhesives, glass wool thermal insulation and other miscellaneous applications.

1.4.2 Composition of Epoxy and Phenoxy Resins

Epoxy resins are produced by a two-step reaction involving coupling and dehydrochlorination of epichlorhydrin with a polyhydric phenol or diphenol. Crosslinking can then take place at the hydroxyl groups using a curing agent. The partial-structure of a typical epoxy resin is shown in Figure 1.5..

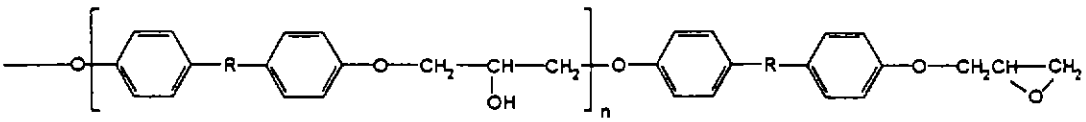


Figure 1.5. The partial-structure of a typical epoxy resin.

The repeat unit for epoxy resins, *n*, varies from approximately 0.2 to 20,000 depending on the end application (Waldie, 1974).

Phenoxy resins, produced by the same type of reaction, in which the stoichiometry is adjusted to retain phenolic end groups (n ~ 100), have the structure shown in Figure 1.6.

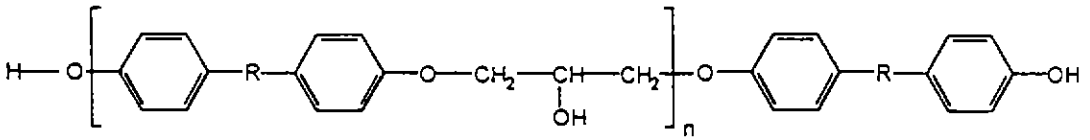


Figure 1.6. The structure of phenoxy resins produced by the same type of reaction.

Epoxy resins are mainly used in coatings whilst smaller amounts are employed in composites, casting resins and adhesives. Coatings applications include industrial, powder and solution coating. The solution coatings are excellent metal primers and are used in automotive primers because of their outstanding adhesive properties. The distinction between these epoxy resins and the higher molecular weight phenoxy resins is the ease of fabrication of phenoxy resin by ordinary thermoforming techniques and the essentially linear nature of the polymer.

1.4.3 Lignin

Lignin in wood has a chemical structure which has partial phenolic resin character. The lignin polymer comprises a complex structure containing hydroxy and methoxy substituted phenylpropane units. An idealised structure of a segment of softwood lignin (Adler, 1977) is shown in Figure 1.7.

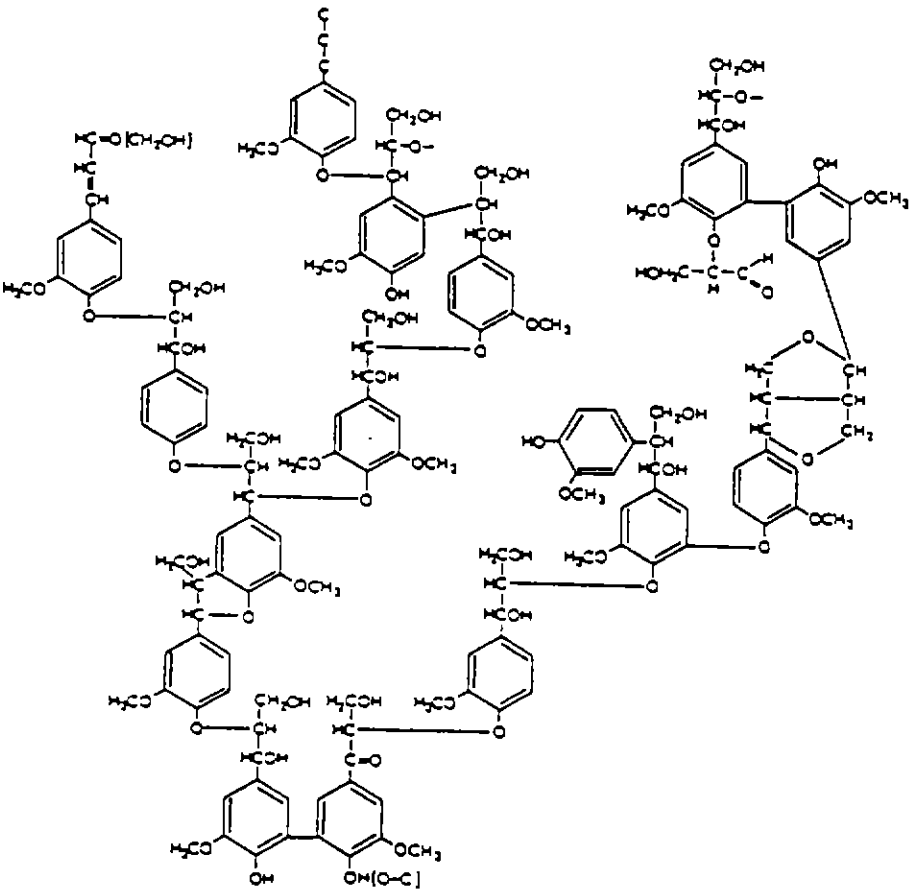


Figure 1.7. An idealised structure of a segment of softwood lignin (Adler, 1977).

This model does not show the linkages between lignin and carbohydrates or other wood constituents such as hemicelluloses. It is clear that the phenylpropane units are joined through C-O-C (ether) and C-C linkages with the ether linkages predominating. Scission of the ether bonds under acid or alkaline conditions would result in extensive fragmentation of the lignin, assisting in the identification of the respective guaiacyl- and syringylpropane units with their different functional groups.

1.4.4 Molecular Structure Determination of Lignin

While the identification of synthetic phenolic polymers by Py-GC and IR spectroscopy is readily accomplished, the characterisation of lignin phenylpropane structure in wood presents more of a challenge due the complex nature of the macromolecule. Py-GC-MS and Py-MS techniques have been used for the characterisation of lignin. These applications are briefly described in Chapter 8.

In summary, lipids, polyesters and phenolic polymers contain ester and ether functional groups These substances, on scission by a suitable nucleophile. yield fragments which can provide useful structural information. Cleavage by strong base is necessary to give carboxylic acid derivatives and hydroxy compounds (alcohols and phenols). Conversion of these polar compounds to methyl derivatives assists in their identification by gas chromatography.

1.5 SUMMARY AND CONCLUSION

From the preceding discussion it was suggested that there could be potential for a rapid pyrolysis-based derivatisation method for the chemical characterisation of some organic macromolecules. It was noted that polymers which undergo pyrolysis by a directed chain cleavage or a side chain scission mechanism give pyrolysis products that are not easily related to the molecular structure of the polymer. Chemical cleavage methods of analysis gave more diagnostic information but were lengthy and not generally suitable for microgram sized samples. Triglycerides from lipids,

polyesters and phenolic polymers were chemical classes which are advantageously characterised by chemical cleavage methods. On-line pyrolysis-based methods, which possibly involved a chemical cleavage reaction, could be appropriate for the characterisation of these product groups.

1.6 SCOPE AND SIGNIFICANCE OF THE THESIS

The Introduction outlines the procedures which are generally used for the chemical characterisation of macromolecular materials, together with an overview of the structure and specific identification techniques for materials studied in the Thesis. Method development which encompassed experimental conditions, proposed mechanisms for the pyrolysis derivatisation reactions, experimental variables and their investigation are discussed in Chapter 2. In Chapter 3, these reactions are introduced as a technique for acquiring more chemical structure information about polyesters and phenolic polymers when compared to Py-GC. The identification of vinyl acetate, methacrylic acid and cyanoacrylate polymers and specific surface coating additives is accomplished when TMAH is substituted by TBAH. Specific naturally occurring materials and their characterisation are addressed in an interim assessment of the range of applications of the technique in Chapter 4. These products include fatty acid triglycerides, natural waxes, kerogen and proteins, and complex esters in pharmaceutical preparations and UV light stabilisers. In depth studies on the determination of the chemical composition of alkyd resin coatings and monitoring of structure changes on curing are reported in Chapter 5. Chapter 6 examines the THM-GC characterisation of a range of wood rosin-based commercial resins which are the basis of printing ink, adhesive and surface coating products. The characterisation of vegetable oils and more complex lipids are discussed in more detail in Chapter 7. The sensitivity and the requirement for minimal manipulation steps are demonstrated by human skin fatty acid profiling. Studies of the structure of lignocellulose, a complex natural polymer, in timber heartwood and the chemotaxonomical advantages using the technique are described in Chapter 8. As a corollary to this, TMAH is used to

separate the extractables in plant materials in a novel departure from the direct pyrolysis methylation procedure used previously (Chapter 9) This procedure forms a basis for the chemotaxonomy of wood and other plant species. The future scope of other high temperature reactions are considered in the final chapter.

CHAPTER 2

METHOD DEVELOPMENT

2.1 INTRODUCTION

The purpose of this chapter on development of a method for on-line pyrolysis derivatisation is to describe the basic experimental conditions used for the reaction, discuss possible mechanisms for the reaction of macromolecules with tetra-alkylammonium hydroxides (TAAH), and describe the results of the optimisation of the variables in the reaction. As each macromolecular material group may require different experimental conditions, these conditions are recorded in the respective chapters where appropriate.

The experimental method which was adopted for the study of the derivatisation reaction on a number of organic chemical materials made use of conventional Py-GC instrumentation. MS was used for the identification of the products. The derivatisation reaction was considered to follow either a free radical mechanism or an ionic pathway. The influences of different experimental variables were studied including reaction temperature, pH, substrate, type of derivatising reagent and reproducibility.

2.2 EXPERIMENTAL

2.2.1 Method

Conventional Curie point Py-GC.

The sample (ca. 5 μg) was placed in the hollow of a flattened, bent 770°C, Curie-point pyrolysis wire. The prepared wire was located in the pyrolyser and flash heating was carried out at 770°C in a helium atmosphere.

Thermally Assisted Hydrolysis and Derivatisation GC.

The sample (ca. 5 μg), in finely divided form, was placed in the hollow of a flattened, bent 770°C, Curie-point pyrolysis wire with approximately 2μL of the derivatising reagent. The material to be examined was preferably scraped into a mull with the derivatisation reagent in order to maximise surface area contact. The prepared wire was located in the pyrolyser and flash heating was carried out at 770°C in a helium atmosphere. Alternatively, the mixture could be deposited in the reaction zone of an

oven type pyrolyser or coated onto the reaction tube or foil of a filament type pyrolyser. In this work, the reactions were conducted under Curie point pyrolysis conditions. Flash "pyrolysis" products were flushed into the injector of a capillary column gas chromatograph using helium carrier gas.

2.2.2 Reagents

The alkylating reagents employed were 25% w/w aqueous TMAH solution, 40% w/w aqueous tetrabutylammonium hydroxide (TBAH) solution and 25% w/w aqueous tetraethylammonium (TEAH) hydroxide solution. The reagent used for extraction of wood specimens was a 1% w/w aqueous TMAH solution.

2.2.3 Instrumentation

A Pye or Horizon Curie-point pyrolyser was fitted to a capillary column gas chromatograph via a stainless steel hypodermic needle as described by Challinor (1983). Pyrograms were obtained by interfacing the pyrolyser to a Hewlett-Packard 5730 gas chromatograph equipped with a flame ionisation detector.

The gas chromatograph was fitted with a 30m fused-silica capillary column having a (14%-cyanopropylphenyl) methylpolysiloxane bonded phase (J&W Scientific, DB 1701), 1 μm loading and 0.313mm internal diameter. Helium was used as carrier gas (linear velocity 30 cm s^{-1}). The inlet mode was splitless with an injection interval of 25 seconds and inlet pressure 1.3 kg cm^{-2}. Temperature programme settings were as follows: initial temperature 30° C, hold 2 min, increase at 8° C min^{-1} to 270° C.

All pyrolysis products referred to were identified by mass spectrometry. Mass spectral analysis was carried out with a Hewlett-Packard 5890 gas chromatograph attached to a VG TS 250 mass spectrometer using electron impact ionisation (70 eV).

2.3 RESULTS AND DISCUSSION

2.3.1 Mechanism

The reaction mechanism for the thermolysis of organic polymers generally involves free radicals. Free radicals decay by mutual deactivation by either recombination or disproportion mechanisms (Schnabel, 1981). The predicted pyrolysis products of a vinyl acetate - dibutyl maleate copolymer, for example, are butanol, acetic acid and various aromatic compounds. However, butyl acetate has been found as an unexpected product (Challinor, unpublished results) and this compound might be expected to be formed from recombination of butyl and acetate radicals. Therefore, there is potential for fundamentally changing the composition of pyrolysis products of polymers by carrying out the thermolysis process in the presence of chemically reactive materials in order to acquire more information about the structure of the polymer. In effect, a derivatising agent could be used to react with thermal or hydrolytic degradation products of a polymer.

In order to monitor the products of such a process, conventional pyrolysis instruments may be used in conjunction with GC. Low molecular weight derivatising agents would be preferred if the recombination products were to be monitored by this technique. Both quaternary ammonium salts and organometallic compounds were considered as derivatising agents in this study.

Aqueous quaternary ammonium hydroxides were found to give the most significant changes in the composition of "pyrolysis" products. However, as these compounds were strong bases and the reaction took place in aqueous medium, it was considered likely that the reaction followed an ionic pathway rather than a free radical recombination process.

Free radicals may be produced by homolytic cleavage of a bond in the macromolecule A-B, and by pyrolysis of TMAH, $R_4N^+OH^-$. The following scheme shows how radicals formed by homolytic cleavage of a bond in the macromolecule can combine with alkyl radicals formed from thermal dissociation of the TMAH :-

Thermal dissociation $A - B \rightarrow A^{\cdot} + B^{\cdot}$

Thermal dissociation $(R)_4\text{-}N^+ OH^- \rightarrow R^{\cdot} + R_3N^{+\cdot} + OH^-$

Recombination $A^{\cdot} + R^{\cdot} \rightarrow A\text{-}R$

$B^{\cdot} + R^{\cdot} \rightarrow B\text{-}R$

Attempts to test this hypothesis by designing pyrolysis experiments in the presence of tetralin, a compound which immediately blocks free radicals, were not successful due to practical difficulties.

By contrast, an ionic mechanism could involve the rapid high temperature hydrolysis of the macromolecule, formation of tetraalkylammonium salts of the hydrolysis products, followed by thermal dissociation of the salts to alkyl derivatives. The following mechanism could take place when a macromolecule A-B is converted to the alkyl derivative by the ionic mechanism:-

Hydrolysis $OH^- + A\text{-}B \rightarrow A^- + B\text{-}OH$

Formation of TAA salts

$A^- + R_4N^+OH^- \rightarrow R_4N^+A^- + OH^-$

$B\text{-}OH + R_4N^+OH^- \rightarrow R_4N^+OB^- + H_20$

Thermal dissociation to alkyl derivatives

$R_4N^+A^- \rightarrow AR + R_3N$

$R_4N^+OB^- \rightarrow BOR + R_3N$

In the case of a typical polyester, polyethylene terephthalate (PET), the mechanism of hydrolysis and alkylation with a TAAH is as follows:

Hydrolysis

$$OH^- + \text{-(-O-CH}_2\text{-CH}_2\text{-O-CO-Ph-CO-O-)}_n\text{-}$$

$$\downarrow$$

$$HO\text{-}CH_2\text{-}CH_2\text{-}OH \quad + \quad {}^-O\text{-OC-Ph-CO-}O^-$$

↓ TAAH ↓ TAAH

Formation of TAA salts

$(R_4N)^+ {}^-O\text{-}CH_2\text{-}CH_2\text{-}O^- (RN_4)^+$ $(R_4N)^+ {}^-O\text{-}OC\text{-}Ph\text{-}CO\text{-}O^- (R_4N)^+$

Thermal dissociation ↓ ↓
to alkyl derivatives

$RO\text{-}CH_2CH_2\text{-}OR + 2R_3N$ $RO_2C\text{-}Ph\text{-}CO_2\text{-}R + 2R_3N$

An ionic mechanism is favoured for the reaction of susceptible organic compounds rather than a free radical process on the basis of the following evidence provided in this work:-

(*i*) Only those macromolecules having *hydrolysable bonds* give products different to those obtained by conventional pyrolysis when flash heated with quaternary ammonium hydroxides

(*ii*) *Pyrolysis of polyvinyl acetate* (PVA) in the presence of TBAH produces butyl acetate (Chapter 3). An ionic mechanism involves hydrolysis of the acetate side chain, formation of the TBAH salt, tetrabutylammonium acetate, and thermal decomposition to butyl acetate. Although this product could be produced by a free radical mechanism, the latter appears to be less feasible.

(*iii*) *Methacrylic acid acrylic copolymers* form the methacrylic acid monomer by conventional pyrolysis in an inert gas atmosphere by a free radical mechanism. However, when these copolymers are flash heated with TBAH, butyl methacrylate is formed as expected via an ionic mechanism (Chapter 3).

(*iv*) *Pyrolysis of polyethylene terephthalate* (PET) produces benzoic acid but does not give terephthalic acid as a conventional pyrolysis product because this compound decarboxylates to benzoic acid and benzene. Flash heating of PET in the presence of TMAH gives mainly dimethyl terephthalate (DMTP) and some methyl benzoate (Chapter 3). If a free radical mechanism predominated, methyl benzoate would be the

major product and DMTP would not be produced because the terephthalic acid would be decarboxylated prior to methylation of the two carboxyl groups by methyl radicals. For an ionic mechanism to apply, hydrolysis of PET would result in the formation of terephthalic acid and ethylene glycol, which on further reaction with TMAH would give the respective methyl derivatives, i.e. dimethyl terephthalate and ethylene glycol dimethyl ether. These products are formed in good yield, and this supports the ionic mechanism.

(*v*) *Pyrolysis of alkyd resins* gives phthalic anhydride, aldehydes and alkenes as the main pyrolysis products. Pyrolysis of these resins when intimately mixed with TMAH gives a radically different range of products comprising methyl esters of phthalic and benzoic acids, methyl esters of aliphatic carboxylic acids from the drying oil components, and methyl ethers of the polyhydric alcohols used in the formulations (Chapter 3).

(*vi*) When *aliphatic esters*, present in wax esters, are flash heated with TMAH, they produce methyl esters of the respective fatty acids and methyl ethers of the aliphatic alcohols (Chapter 4). Conventional pyrolysis of esters usually causes a concerted cyclic thermolytic reaction to produce alkenes and carboxylic acids.

These experimental observations support the hypothesis that the mechanism is ionic and is based on a high temperature hydrolysis and subsequent alkylation reaction as outlined above.

In the early stages of the project, an appropriate descriptive term to convey the meaning of the process taking place was chosen as simultaneous pyrolysis methylation (SPM), in the case where TMAH was employed. The description alluded to a high temperature reaction in which conversion to methyl derivatives occurred and was intended to contrast with the term "pyrolytic methylation" (Kossa *et al.*, 1979; Abraham and Criddle, 1985; Metcalfe and Wang, 1981). However, the term did not adequately convey the meaning of the mechanism taking place, [de Leeuw *et al.* (1993) and Martin *et al.* (1994)]. A more appropriate term was then considered to be

thermally assisted hydrolysis and alkylation (THA), and in the case of methylation with TMAH, thermally assisted hydrolysis and methylation (THM).

2.3.2 Reaction Variables

Reaction Temperature

The influence of "pyrolysis" temperature on the distribution of products of the pyrolysis derivatisation reaction using TMAH was investigated. Pentaerythritol (2,2'-bis (hydroxymethyl) 1,3 propanediol), a commonly used polyhydric alcohol in synthetic resin formulations, was incompletely methylated at temperatures of 770°C, 510°C and 358°C (Figure 2.1). The four products correspond to the ter, tri, di and mono- methylated compounds (P4ME, P3ME, P2ME and P1ME). There is a trend to lower yields of the fully methylated compound (P4ME) at higher temperatures.

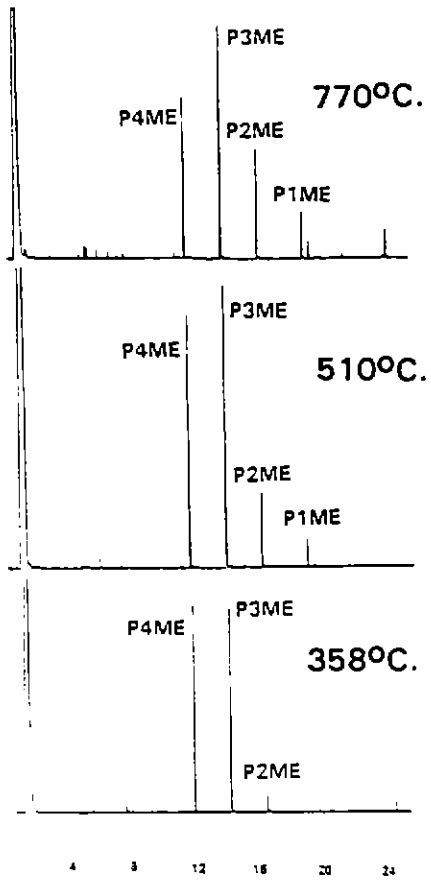


Figure 2.1. Chromatograms showing pyrolysis derivatisation of pentaerythritol with TMAH at different temperatures

Key:- P4ME = pentaerythritol tetramethyl ether, P3ME = pentaerythritol trimethyl ether, P2ME = pentaerythritol dimethyl ether, P1ME = pentaerythritol monomethyl ether.

A composite alkyd enamel, prepared by mixing alkyd resins having different formulations, which included different polyhydric alcohols, polybasic acids, drying and non-drying oils and rosin modification, was subjected to the same procedure at the different flash heating temperatures. The results shown in Figure 2.2 indicate that temperature has only a small influence on the product distribution. The most noticeable feature is that the methylated polyol products are produced in greater abundance at the lower temperature.

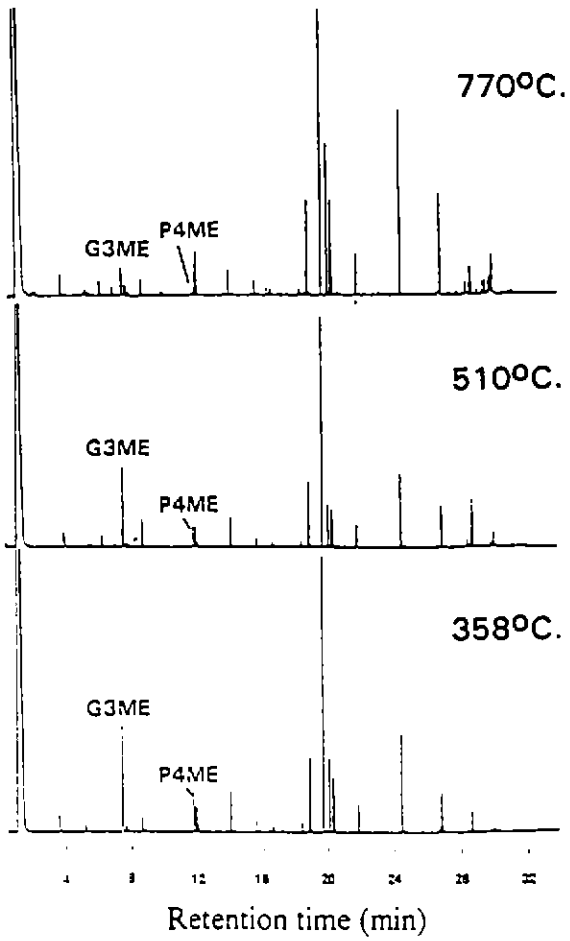


Figure 2.2 Chromatograms showing pyrolysis derivatisation of mixed alkyd resins with TMAH at different temperatures.

Key:- G3ME = glycerol trimethyl ether, P4ME = pentaerythritol tetramethyl ether.

It is likely that the reaction proceeds very rapidly at the onset of heating and the higher temperatures are unneccessary. However, as we see later, pyrolysis processes accompany the THM reaction, for example where phenolic polymers are analysed (Section 3.4.5). A routine 770°C. pyrolysis temperature was chosen as this was the standard temperature used for conventional pyrolysis. Therefore, it was necessary to carry out TMAH reactions at this temperature to enable a comparison of the results of conventional Py-GC and pyrolysis derivatisation. Further, for material having lower organic content, e.g. soils and kerogens, and other "filled" material such as highly

extended surface coatings, larger masses are required for pyrolysis, which could cause heat transfer problems at lower pyrolysis temperatures.

pH

The relative alkalinity of the quaternary ammonium hydroxide derivatising reagent may be adjusted by a) neutralising with acid or buffer and/or b) changing the substituent groups of the reagent.

During attempts to lower the pH to redress the problem of base catalysed isomerisation of polyunsaturated fatty acids in lipids (Chapter 6), it was noted that the yields of methyl ester derivatives of the fatty acids were drastically reduced, suggesting that hydrolysis of the triglycerides was incomplete. Free fatty acids (e.g. octadecadienoic acid) were methylated successfully, although the yields were also reduced.

Base catalysed isomerisation of the polyunsaturated fatty acids in triglycerides was almost eliminated by changing one substituent methyl group in tetramethylammonium hydroxide to an electron-donating trifluoromethyl-phenyl group, thereby lowering the pH of the reagent. However, the yield of fatty acid methyl esters was reduced by a factor of ten.

It was also observed that lower yields of the methyl derivatives of polyols, which have lower dissociation constants (pK_a) than carboxylic acids, were obtained when the reaction was conducted at lower pH. This could present problems in the examination of polyols in alkyd polyesters. Therefore, it is concluded that a high pH of the derivatising reagent is necessary to achieve an efficient hydrolysis of the macromolecule.

Analyte Particle Size

The surface area of the analyte in contact with the aqueous tetraalkylammonium hydroxide (TAAH) solution, prior to flash heating, has a significant influence on the degree of conversion to derivatised products. Small particle size gives a higher conversion. In extreme cases, with poor mixing of the TAAH with the analyte, the products are almost totally those obtained by conventional pyrolysis. If a free radical

mechanism applied, the product would be expected to be the same This observation provides further support for the ionic mechanism.

Substrate

The nature of the flash heating surface substrate in the reaction zone had no discernible influence on the reaction provided that the above mixing requirements were met. The heating surface substrate in commercial pyrolysers comprises an iron or alloy surface for the Curie point pyrolysis units, a quartz or platinum ribbon surface for the filament pyrolysers or a stainless steel / open tube for oven pyrolysers. Satisfactory results have been obtained with these systems. The inference is that catalytic effects do not play a significant part in the reaction.

Derivatising Reagents

TAAHs were found to be the most successful reactants for the hydrolysis and derivatisation reaction. TMAH provided the most useful derivatives, giving lower molecular weight compounds for the majority of GC applications A further advantage was that methyl derivatives have well documented mass spectra. The higher alkyl derivatives, obtained by employing TEAH and TBAH reactants, were useful for identifying compounds having sites of pre-existing methylation. Water was preferred as a solvent for the TAAH since the organic solvents such as methyl alcohol, were too volatile for use in the preparation stage of the reaction.

2.3.3 Reproducibility

Three replicate Curie point THM-GC analyses of a three year-cured, soya bean oil drying oil, long oil, pentaerythritol - orthophthalic alkyd enamel were carried out using the same batch of 25% aqueous TMAH solution. The results are presented in Figure 2.3. Peak height ratios of methyl palmitate, methyl stearate, methyl azelate, methyl suberate and dimethyl orthophthalate were reproducible to within 3% relative standard deviation. The reproducibility of the peaks for pentaerythritol methyl ethers and methyl benzoate fell outside this range. It could be postulated that the reason for this was the increased resistance to hydrolysis of ester bonding between the

polyhydric alcohol and the polybasic acid/fatty acid due to steric hindrance and the lower acidity of the hydroxyl group of the polyhydric alcohols. The variability in the peak height of methyl benzoate, originating from benzoic acid, is probably due to the effects of non-reproducible partial decarboxylation of phthalic acid.

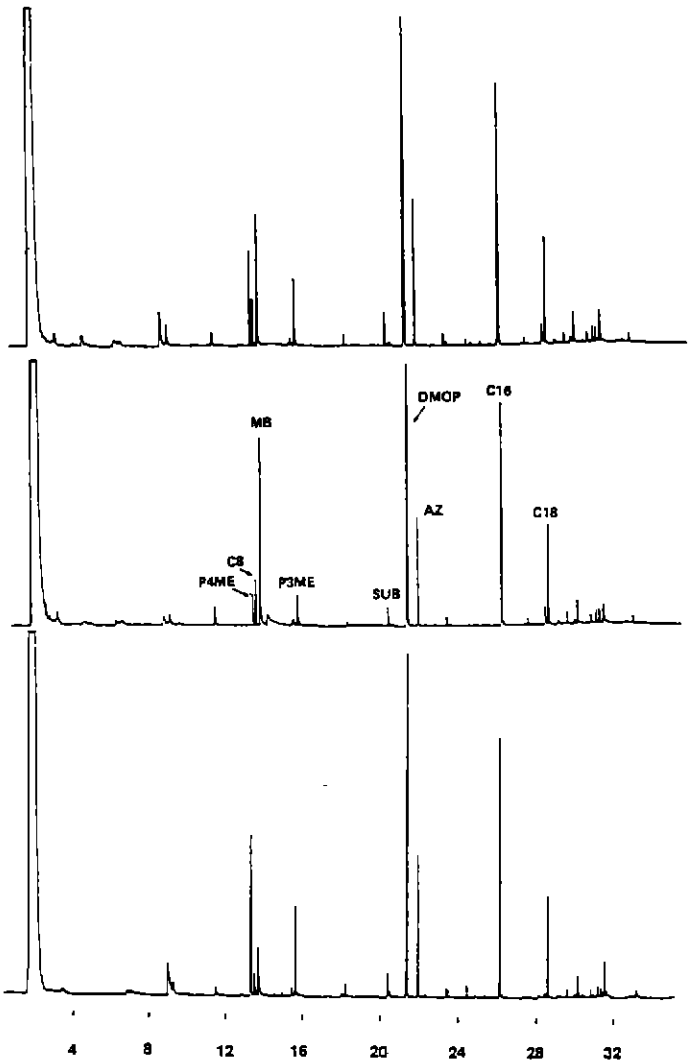


Figure 2.3 Chromatograms showing three replicate Curie point THM-GC analyses of a three year-cured, soya bean oil drying oil, long oil, pentaerythritol - orthophthalic alkyd enamel using the same batch of 25% aqueous TMAH solution.

Key: P4ME = pentaerythritol tetramethyl ether, C8 = methyl octanoate, MB = methyl benzoate, P3ME = pentaerythritol trimethyl ether, SUB = dimethyl suberate, DMOP = dimethyl orthophthalate, AZ = dimethyl azelate, C16 = methyl hexadecanoate, C18 = methyl octadecanoate.

2.4 SUMMARY

Most of the experimental evidence concerning the THM reaction indicates that the reaction is a hydrolysis and methylation process. The reaction proceeds at high temperatures ranging from 375°C to above 770°C. The flash heating temperature has only a small influence in the THM product distribution within this range. Lowering the pH of the derivatising reagent has an adverse effect on the yield of the derivatised products, however, high pH results in base catalysed isomerisation of polyunsaturated fatty acids. Small particle size was necessary to achieve acceptable yields of derivatised products. Catalytic effects from the flash heating surface substrate do not play a significant role in the reaction. Aqueous TMAH solutions provided the most useful derivatives, however, the higher quaternary ammonium hydroxide homologues were useful for identifying compounds having sites of pre-existing methylation. The method must be considered to provide very useful qualitative data; nevertheless semiquantitative results could be obtained from aliphatic carboxylic acid components in polymers.

CHAPTER 3

A PYROLYSIS-DERIVATISATION-GAS CHROMATOGRAPHY TECHNIQUE FOR THE STRUCTURAL ELUCIDATION OF SOME SYNTHETIC POLYMERS

3.1 ABSTRACT

A rapid method has been developed for the characterisation of a range of macromolecular materials including polyesters, phenolic resins and polymer additives, whereby high temperature hydrolysis and alkylation produces derivatives which can be identified by gas chromatography. The technique gives additional information about the composition of carboxylic acids, alcohols and substituted phenolic components of these polymer types. The procedure involves methylation or butylation, using TMAH or TBAH, in the pyrolysis zone of the pyrolyser with analysis by flame ionisation gas chromatography or gas chromatography / mass spectrometry. The advantages of this technique are greater chemical structure information about the polymer, minimal sample manipulation and increased sensitivity.

3.2 INTRODUCTION

In view of the intractable nature of complex polymeric resins, their chemical constitution has been determined by a diversity of analytical methods including chemical degradation (Section 1.1.6) (Haken, 1979, 1989; Haken and Iddalmalgoda, 1996). Non-destructive methods have included diamond window IR spectroscopy of automobile paint resins (Rodgers *et al.*, 1976) and proton and ^{13}C NMR of paint media (Marshall, 1983). The analysis of mixtures of additives in polymers by non-destructive methods is further complicated by the polymer matrix.

Pyrolysis-capillary gas chromatography (Py-GC) is now established as a reliable and reproducible technique for the identification and structure elucidation of surface coatings, plastics, rubbers, fibres and adhesives (de Forest, 1992; Blackledge, 1992; Wampler, 1989), particularly in materials and forensic science applications (Challinor, 1995). Analytical pyrolysis of paint resins, particularly those from automotive sources (Wampler *et al.*, 1997), can provide useful information about their composition, however, chemical structure information about polyesters can be difficult to interpret (Challinor, 1993; Wilcken and Schulten, 1996).

The pyrolysis of macromolecules produces a wide range of chemical compounds ranging in polarity from non-polar, e.g., alkanes and alkenes, to highly polar, e.g., alcohols and carboxylic acids. The polar compounds in particular give useful diagnostic information about the structure of the material. Examples of this method include the pyrolysis of polyesters to give polybasic acids and alkenes (Ohtani and Tsuge, 1995), epoxies to phenolic compounds (Bradna and Zima, 1991, 1992; Peltonen, 1986), and polyamides to amines and carboxylic acids (Senoo *et al.*, 1971). A small number of acrylic and epoxy modified melamine cross-linked polyester coatings were differentiated by Py-GC-MS and principal component analysis (Wilcken and Schulten, 1996). The major products were styrene, cyclopentanone, 2-(2-butoxyethoxy)-ethanol, di-2-propenyl hexanedioate and fragments of the neopentyl ester of adipic acid. While the non-polar components of the polymer, e.g. styrene and butyl acrylate were detected intact, the polyhydric alcohols, neopentylglycol and trimethylolpropane, and polybasic acids, adipic acid and phthalic acid were either dehydrated or decarboxylated. The phenolic residues from the epoxy resin (bisphenol-A) were present in low proportions. As a result, diligent interpretation of the origin of the pyrolysis products was necessary to determine the chemical composition of the polymer. In spite of this, the resins could be distinguished clearly with the aid of principal component analysis.

Normally, polar pyrolysis products are difficult to determine by Py-GC due to thermal degradation processes such as dehydration or decarboxylation, or their partial or complete adsorption in the pyrolysis zone, injection system or capillary column. Polar pyrolysates often show peak tailing characteristics, poor reproducibility or long elution times. Consequently, the full potential of the technique is not realised due to the limitations of the chromatographic system.

The solution to adverse chromatography problems with conventional GC is the derivatisation of the polar compounds externally, or by co-injection to give compounds which may be efficiently separated. One co-injection procedure developed earlier involves pyrolytic alkylation of carboxylic acids and phenols with derivatising

reagents which include TMAH (Kossa *et al.*, 1979; Abraham and Criddle, 1985; Metcalfe and Wang, 1981).

Polymers may be characterised by chemical degradation followed by gas chromatography of the derivatised degradation products (Haken and Iddamalgoda, 1996). Methods which have been used to modify pyrolysis product composition are limited but include the early work on the hydrogenation of olefines, resulting from pyrolysis of polyethylene, using a palladium catalyst (Tsuge *et al.*, 1980; Tsuge and Ohtani, 1995). Sequence distributions in polyacetals were studied by the pyrolysis of the finely divided polymer in the presence of a cobalt catalyst (Ishida *et al.*, 1995). The ethylene oxide content and distributions, in relation to the basic polyoxymethylene structure, were evaluated on the basis of the cyclic ethers. These processes necessarily involved the thermolysis of the polymer with the production of smaller molecular units which were then changed by a catalytic reaction.

Early work on the pyrolysis-butylation of vinyl acetate and homologous polymers has been reported (Goetz *et al* , 1985). Pyrolysis-butylation was demonstrated using flash derivatisation of a copolymer incorporating vinyl acetate, crotonic acid and a complex vinyl ester, and resulted in the formation of butyl acetate and a mixture of higher isomeric butyl esters. This brief account indicated that a filament pyrolyser was used in the work and TBAH was the derivatising reagent.

In this present work, thermally assisted hydrolysis and alkylation is achieved by intimately mixing the derivatising reagent, an aqueous solution of TMAH or TBAH, with the sample during flash heating. An overview of the application of the procedure to a range of polyesters including alkyd resins, phenolic polymers and additives in surface coating formulations is described.

3.3 EXPERIMENTAL

The experimental conditions for conventional Py-GC and thermally assisted hydrolysis and alkylation-GC are described in Section 2.2 of the thesis.

3.4 RESULTS AND DISCUSSION

In general, the mechanism of the pyrolysis of polyesters involves the scission of the alkyl-oxygen RCOO-R bond and is considered to proceed via a cyclic transition state (Ohtani and Tsuge, 1995). Pyrolysis results in the formation of the respective carboxylic acid and alkene. However, flash heating with TMAH, i.e., thermally assisted hydrolysis and methylation (THM), results in the formation of the methyl esters of the carboxylic acids and methyl ethers of the alcohols.

3.4.1 Alkyd Resins

The main pyrolysis products of alkyd resins, as detected by conventional Py-GC, have been identified as short chain aldehydes, alkenes and alkanes, benzoic acid and phthalic anhydride (Challinor, 1984). The low molecular weight aliphatic products represent only minor structural components of the whole molecule and, as a result, much of the chemical structural information about the polymer is lost as a result of pyrolysis For example, hexanal, the most abundant aldehyde resulting from pyrolysis of linoleic-rich alkyd resins, probably originates from linoleic acid chains cross-linked during the resin curing process. In contrast, THM gives products which are those resulting from hydrolysis of the ester moieties in the polymer and methyl derivatives of the carboxylic acids and alcohols produced (Fig. 3.1). The course of THM product formation is presented in Table 3.1.

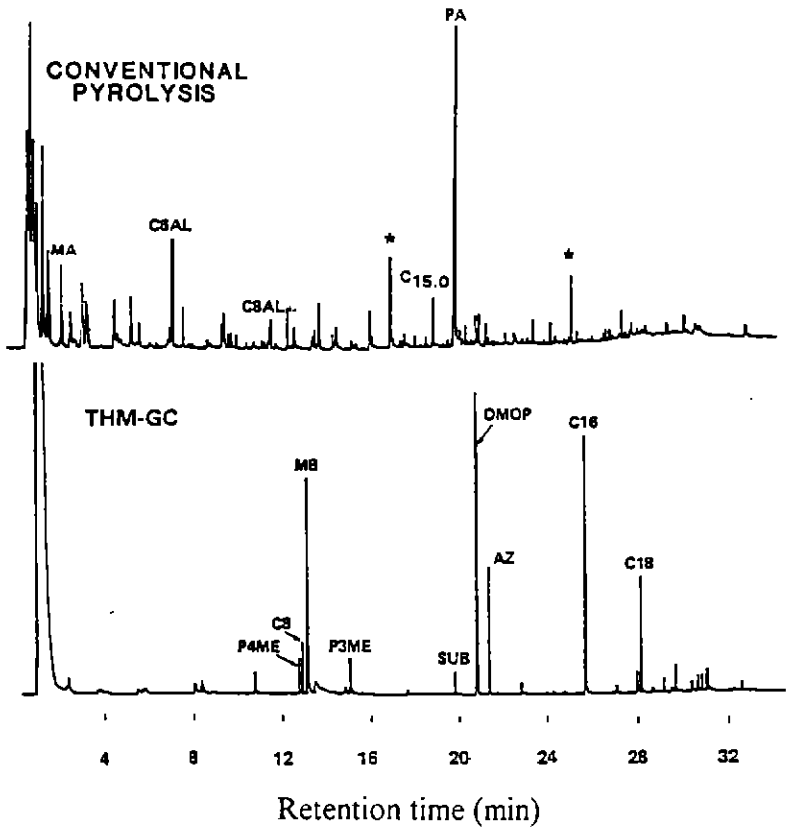


Figure 3.1. Chromatograms showing conventional pyrolysis and THM-GC analysis of a soya-bean oil-pentaerythritol-orthophthalic alkyd resin.

Key: MA = methacrolein, C6AL = hexanal, C8AL = octanal, C15.0 = pentadecane, PA = phthalic anhydride, P4ME = pentaerythritol tetramethyl ether, C8 = methyl octanoate, MB = methyl benzoate, P3ME = pentaerythritol trimethyl ether, SUB = dimethyl suberate, DMOP = dimethyl orthophthalate, AZ = dimethyl azelate, C16 = methyl hexadecanoate, C18 = methyl octadecanoate. The asterisks denote peaks attributable to unidentified long chain aldehydes.

Monomeric unit in polymer	Esterified moiety in polymer	Hydrolysis product during THM	Methylation product after THM
Phthalic acid	Phthalic acid-pentaerythritol	Phthalic acid	Dimethyl orthophthalate
Pentaerythritol	Pentaerythritol-fatty acids	Pentaerythritol	Pentaerythritol methyl ethers
Fatty acids	Fatty acids-pentaerythritol	Fatty acids	FAMES

Table 3.1. Hydrolysis and Methylation Products Derived From Structural Units in the THM Reaction of a Soya-Bean Oil-Pentaerythritol-Orthophthalic Alkyd Resin.

The detection of dimethyl orthophthalate, methyl ethers of pentaerythritol and the composition of the FAMES provides confirmation that this resin is a pentaerythritol orthophthalic alkyd enamel. A partial structure of the polymer deduced from this data is shown in Figure 3.2 The methyl palmitate to methyl stearate ratio is consistent with the alkyd enamel having a fatty acid composition similar to soya-bean oil or safflower oil. A more detailed study of the THM reaction of alkyd resins will be the subject of Chapter 5.

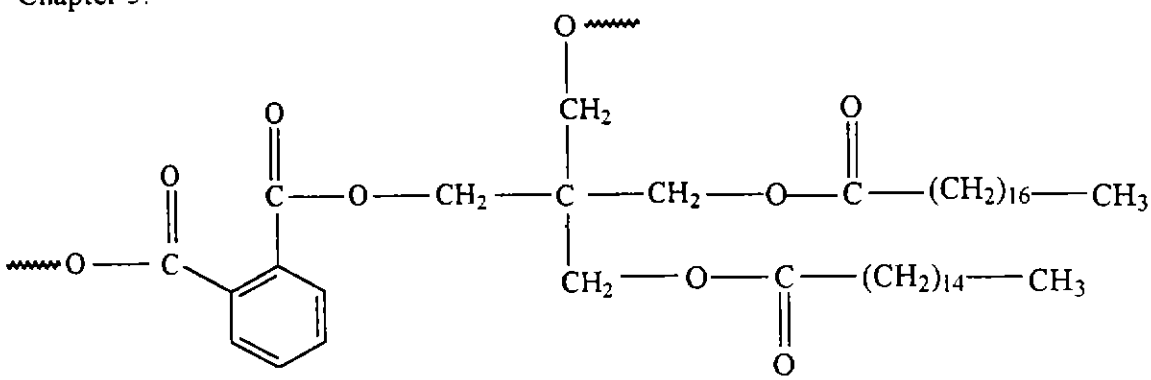


Figure 3.2. A partial structure of the alkyd resin polymer deduced from the THM-GC data. This figure does not show cross-linking between unsaturated fatty acid chains which occurs on curing.

3.4.2 Unsaturated Polyester Resins

Styrene-unsaturated polyester resins have a wide variety of uses particularly as binders in motor vehicle body fillers and glass reinforced plastics. The resins have forensic significance as evidential material from hit and run traffic accidents and, as a result, elucidation of their composition is important (Walsh *et al.*, 1986).

Styrene-unsaturated polyester resins are typically produced by esterification of ortho- or isophthalic acids, maleic anhydride and polyhydric alcohols, e.g. propylene glycol and neopentyl glycol, and can be co-reacted with modifying agents such as adipic acid. Styrene is included in the formulation to cross-link the resin.

The main products of conventional pyrolysis of a diethylene glycol-isophthalic-adipic acid-modified polyester resin include toluene, styrene, styrene oligomers and cyclopentanone. Cyclopentanone results from the scission of the C-O bonds of the adipic acid portion of the polymer with loss of carbon monoxide, similar to the pyrolysis of nylon 6.6 (Senoo *et al*., 1971). THM of the same resin gives, in addition to styrene, the products dimethyl isophthalate, methyl ethers of diethylene glycol, dimethyl adipate and methyl benzoate (Fig. 3.3). A partial structure of the polymer deduced from the data from the THM experiment is shown in Figure 3.4.

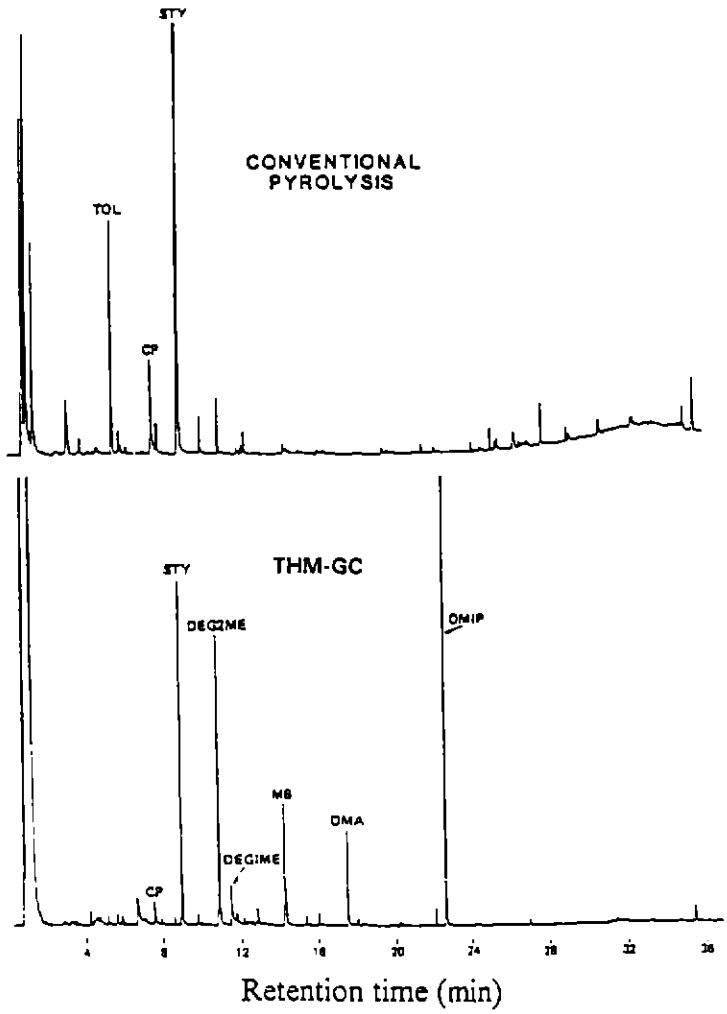


Figure 3.3. Chromatograms showing conventional pyrolysis and THM-GC analysis of a diethylene glycol-isophthalic-adipic acid modified polyester resin.

Key: TOL = toluene, CP = cyclopentanone, STY = styrene, DEG2ME = diethylene glycol dimethyl ether, DEG1ME = diethylene glycol monomethyl ether, MB = methyl benzoate, DMA = dimethyl adipate, DMIP = dimethyl isophthalate.

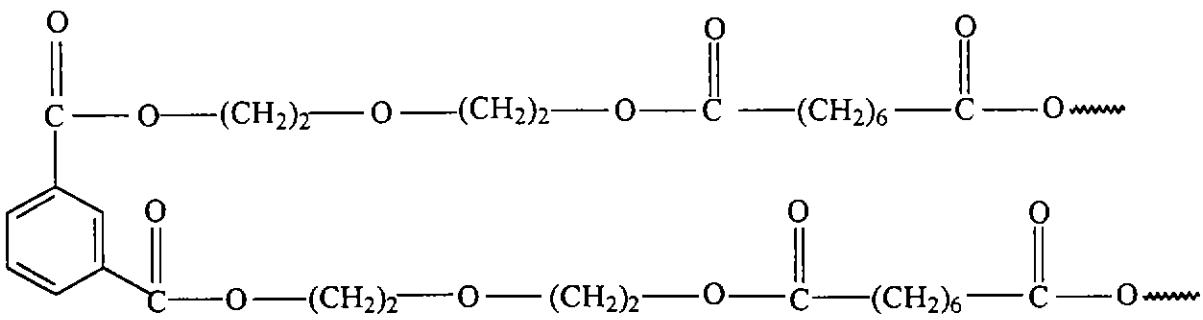


Figure 3.4. A partial structure of the styrenated unsaturated polyester polymer deduced from the data from the THM-GC experiment.

Maleic acid and styrene, which are also incorporated into the polymer and which undergo cross-linking by free radical polymerisation, are not shown in the above diagram.

The polybasic acid in these polyester resins is, therefore, generally indicated by the dimethyl phthalate isomer, the polyol by the corresponding methyl ethers and the presence of adipic acid by its dimethyl ester. Cyclopentanone is also produced, as in conventional pyrolysis, indicating that cyclisation competes with the THM esterification reaction resulting in the formation of both products.

3.4.3 Saturated Polyester Resins

Saturated polyesters are the reaction products of polybasic acids and polyhydric alcohols and are distinguished from alkyd resins which are monobasic fatty acid modified polyesters, and unsaturated polyesters which are polyesters containing unsaturated dibasic acids which are cross-linked with styrene (Section 1.3.3).

The polyhydric alcohols normally used for saturated polyesters include trimethylol propane, trimethylol ethane, pentaerythritol and 1,6-hexanediol. The acids used include isophthalic and adipic acids in formulations for appliance and automotive coatings. These polyesters have an excess of hydroxyl groups on the chain and are usually cross-linked by reacting with hexamethylol melamine, a monomeric melamine cross-linking agent (Oil and Colour Chemists Association, 1983).

Py-GC analysis of a commercial saturated polyester surface coating, described by the manufacturer as a multi-purpose polyester baking enamel, indicated that the major conventional pyrolysis products were cyclopentanone and benzene. THM-GC analysis gave products from which more diagnostic chemical information could be derived (Figure 3.5). The identity of the polyhydric alcohols was revealed by the detection of the methyl ethers of neopentyl glycol and trimethylol propane. The polybasic acids were identified as isophthalic acid and adipic acid detected as their methyl esters.

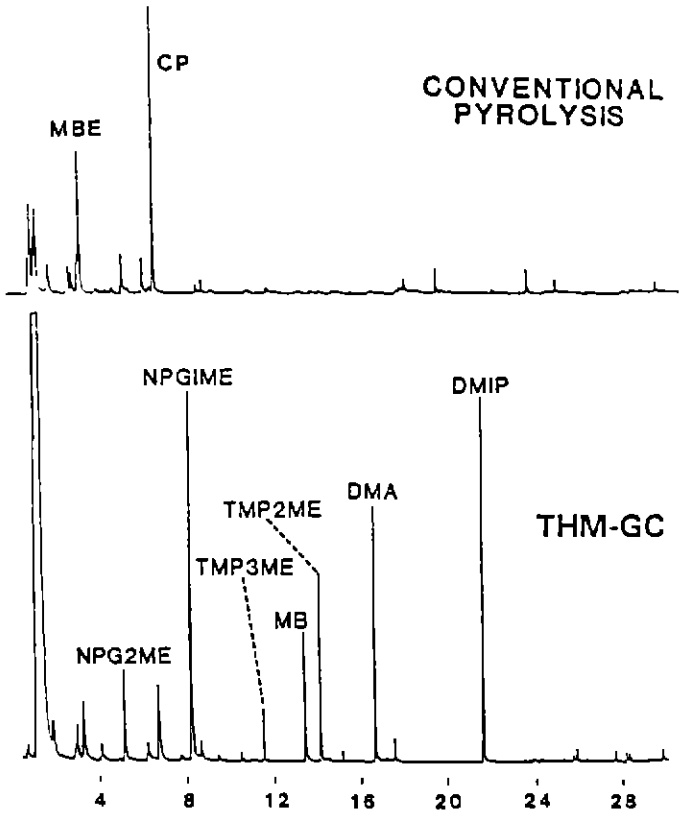


Figure 3.5. Chromatogram showing Py-GC and THM-GC analysis of a saturated polyester coating identified as a neopentyl glycol, trimethylol propane, isophthalic acid type

Key: MBE = 2-methyl-2-butenal (tentative identification), CP = cyclopentanone, NPG2ME = neopentyl glycol dimethyl ether, NPG1ME = neopentyl glycol monomethyl ether, TMP3ME = trimethylol propane trimethyl ether, MB = methyl benzoate, TMP2ME = trimethylol propane dimethyl ether, DMA = dimethyl adipate, DMIP = dimethyl isophthalate.

3.4.4 Polyester Fibres

Polyester fibres are manufactured predominantly from polyethylene terephthalate (PET). The main pyrolysis products of this polymer include benzene, benzoic acid, vinyl benzoate, biphenyl and ethylene dibenzoate (Ohtani and Tsuge, 1995). The mechanism involves a cyclic transition state to give alkenyl and carboxyl end groups in the pyrolysis fragments. At the elevated temperatures used for pyrolysis, carboxyl end groups undergo decarboxylation

In this work, THM results in the hydrolysis of the ester groups in the polyester chain and esterification of the resulting terephthalic acid to dimethyl terephthalate and methylation of ethylene glycol to give the mono- and dimethyl ethers. Methyl benzoate is also produced and this compound could result from the methylation of partly decarboxylated terephthalic acid. The product distribution may be compared to those products obtained by conventional Py-GC (Figure 3.6). The enhanced sensitivity of the procedure is demonstrated by fact that only a 1 mm length of PET fibre was used in the THM-GC experiment and the conventional pyrogram resulted from the pyrolysis of a 40 mm length of PET fibre.

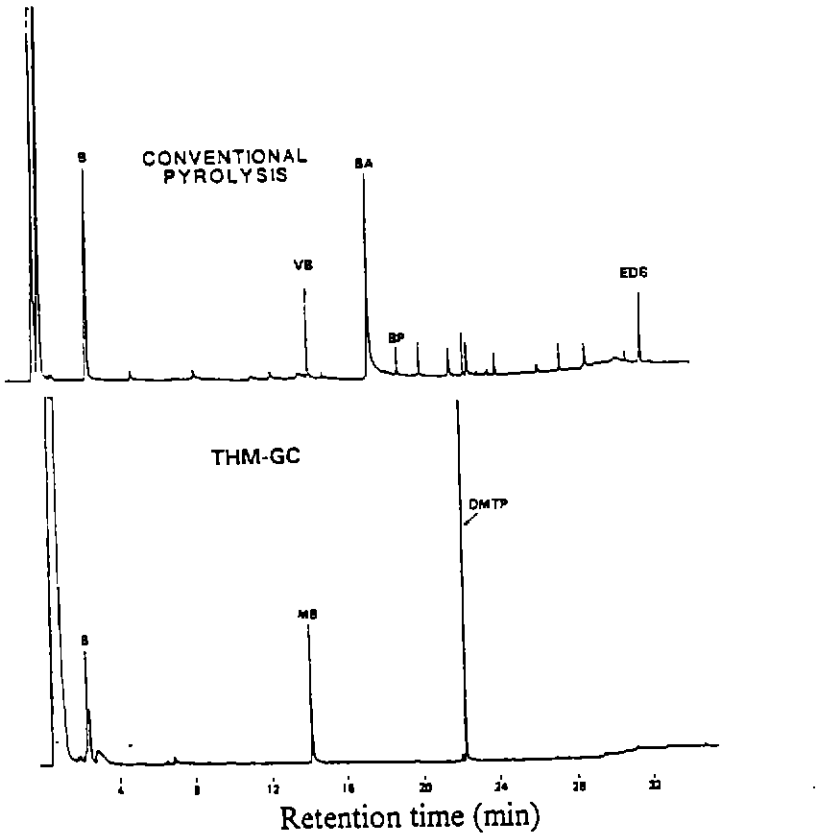


Figure 3.6 Chromatograms showing conventional pyrolysis of a 40 mm length of PET fibre and THM-GC analysis of a 1 mm length of PET fibre.

Key: B = benzene, VB = vinyl benzoate, BA = benzoic acid, EDB = ethylene dibenzoate, BP = biphenyl, EG1ME = ethylene glycol monomethyl ether, MB = methyl benzoate, DMTP = dimethyl terephthalate.

THM gives a more specific diagnosis of the polymer composition as, during the reaction, the polymer reverts to the monomeric compounds. In this case, the methyl derivatives of terephthalic acid and ethylene glycol are formed. Enhancement of sensitivity is achieved because the number of major pyrolysis products is reduced from approximately twelve compounds, including benzoic acid, in the case of conventional pyrolysis to three or four for the THM reaction. Further, benzoic acid exhibits poor chromatographic properties and, in some cases, partial adsorption on the chromatography column thus reducing the sensitivity of the conventional Py-GC method.

3.4.5 Phenolic Polymers

The phenolic polymers, in general use, include the epoxy, polycarbonate and phenol-formaldehyde resins. The pyrolysis products of epoxy resins, derived from epichlorhydrin and bisphenol-A, largely comprise substituted phenolic compounds (Peltonen, 1986). Those from polycarbonates produce similar phenolic products and diphenyl carbonate (Tsuge *et al.*, 1969). Phenol-formaldehyde resin pyrolysis products include substituted phenols, the structure of which depends on the macromolecular structure of the resin (Blazso *et al.*, 1986). THM of these phenolic polymers results in the formation of methyl ethers of phenol and the substituted phenolic pyrolysis products Fig. 3.7 shows conventional Py-GC and THM-GC of a typical epoxy resin.

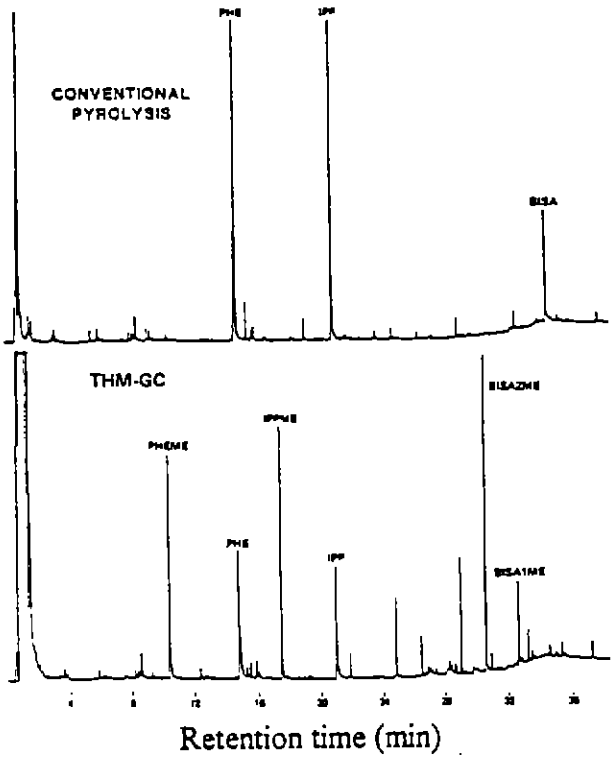


Figure 3.7. Chromatograms showing conventional pyrolysis and THM-GC analysis of an epoxy resin.

Key: PHE = phenol, IPP = isopropenylphenol, BISA = bisphenol-A, PHEME = phenol methyl ether, IPPME = isopropenylphenol methyl ether, BISA2ME = bisphenol-A dimethyl ether, BISA1ME = bisphenol-A monomethyl ether.

The major products of THM of this epoxy resin are phenol methyl ether, isopropenylphenol methyl ether and bisphenol-A methyl ethers. Incomplete conversion to the respective ethers may be caused by competing side reactions. The products are consistent with having been formed by a combination of homolytic scission of carbon - carbon bonds and high temperature hydrolysis and methylation of the carbon - oxygen ether bonds according to the following scheme (Figure 3.8).

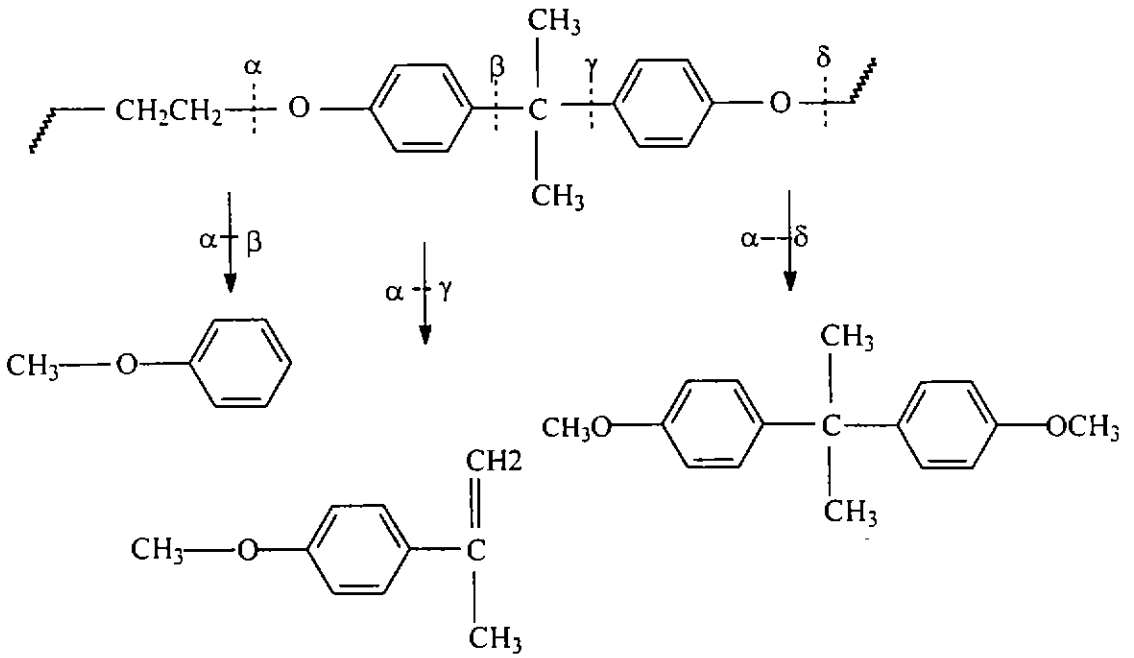


Figure 3.8. A partial structure of the diglycidyl ether of bisphenol-A moiety showing sites of homolysis and hydrolysis.

Phenol-formaldehyde resins appear to undergo a similar mechanism whereby a combination of homolytic scission of carbon - carbon bonds and high temperature hydrolysis and methylation of the carbon - oxygen ether bonds give the corresponding phenyl methyl ethers (Figure 3.9).

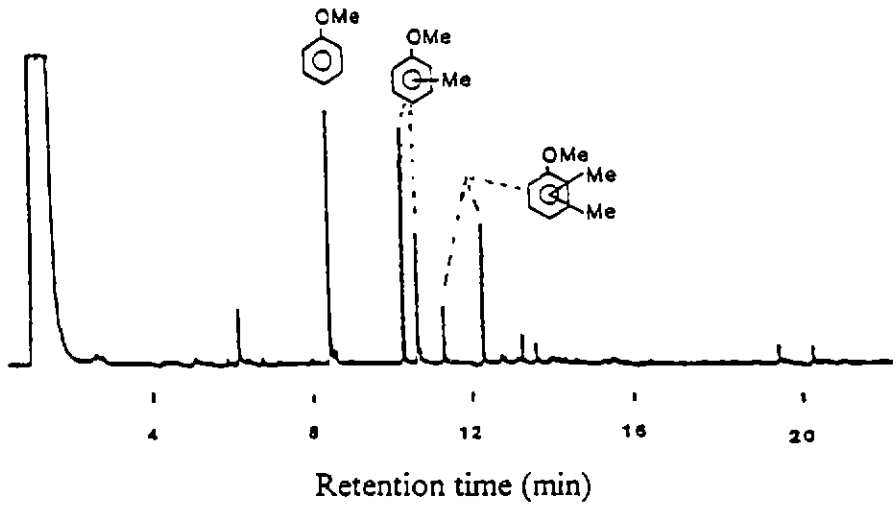


Figure 3.9 Chromatograms showing THM-GC analysis of a phenol formaldehyde resin.

3.4.5 Thermally Assisted Hydrolysis and Butylation (THB)

THM is inappropriate for the derivatisation of polymer components that give low molecular weight hydrolysis products such as acetates. Methyl acetate co-elutes with the trimethylamine peak at the commencement of the GC analysis. Reaction of the polymer with aqueous TBAH in the THB procedure results in the formation of butyl esters of carboxylic acids in carboxy-containing polymers. The THB reaction is appropriate for vinyl acetate, methacrylic acid and cyanoacrylate homopolymers and copolymers.

Vinyl acetate is usually copolymerised with other vinyl monomers in surface coatings, adhesives and in some fibres. Polyvinyl acetate pyrolyses to acetic acid and aromatic compounds which are formed by cyclisation of polymer chain fragments (Alajberg *et al.*, 1980). The detection of acetic acid in the pyrolysis products usually indicates the presence of vinyl acetate in the polymer, however, acetic acid does not usually chromatograph efficiently on GC columns normally used for pyrolysis work.

In the THB-GC analysis of this type of polymer, hydrolysis of the acetate side chain and derivatisation to the butyl ester gives the improved peak shape and sensitivity required for copolymers in which vinyl acetate is present as a minor constituent.

THB-GC chromatograms of a polyvinyl acetate paint, a vinyl acetate-containing textile fibre known as Acrilan 16, and a polyvinyl acetate adhesive are shown in Fig. 3.10. The detection of butyl acetate indicates that vinyl acetate is present in the polymer.

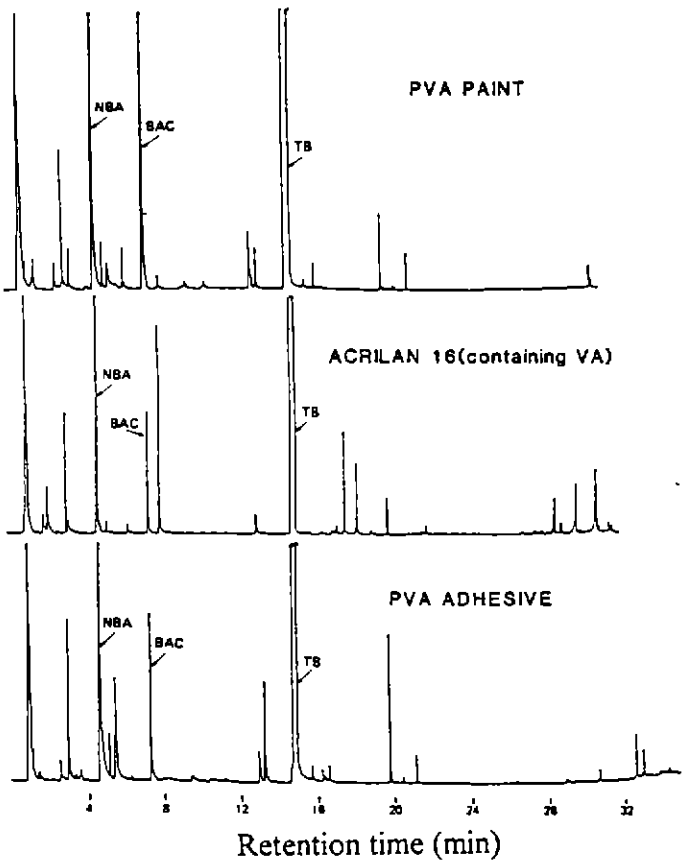


Fig. 3.10. Chromatograms showing THB-GC analysis of a polyvinyl acetate paint, a vinyl acetate-containing textile fibre and a polyvinyl acetate adhesive.

Key: NBA = n-butanol, BAC = n-butyl acetate, TB = tributylamine. The unmarked peaks represent pyrolysis products of the respective polymers.

A disadvantage of the THB-GC analysis is the appearance of by-products, n-butanol and tributylamine in the chromatogram. These products are also detected when an aqueous solution of TBAH is flash heated alone and probably arise from the thermal

degradation of TBAH by the Hofmann elimination reaction. Tributylamine, 1-butene and water are the usual products (Figure 3.11). Butanol may arise from addition of water to the alkene under the reaction conditions used. These compounds do not generally interfere with the interpretation of the pyrogram.

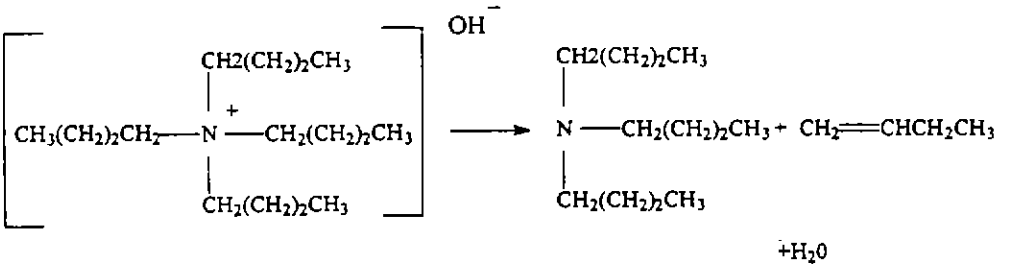


Figure 3.11. Products from the thermal degradation of TBAH.

Methacrylic acid is copolymerised with acrylic monomers in proprietary coatings and adhesives in which enhanced adhesion characteristics are required. The acid product obtained in conventional pyrolysis of this copolymer type, exhibits poor chromatographic properties. In contrast, the THB reaction of methacrylic acid copolymers results in the formation of butyl methacrylate. An example of the confirmation of the presence of methacrylic acid in a proprietary methacrylic acid copolymer adhesive by the THB reaction is shown in Figure 3 12. The mechanism of this reaction probably involves butylation of the carboxylic acid side chain in the polymer, followed by random chain cleavage of the polymethylene chain.

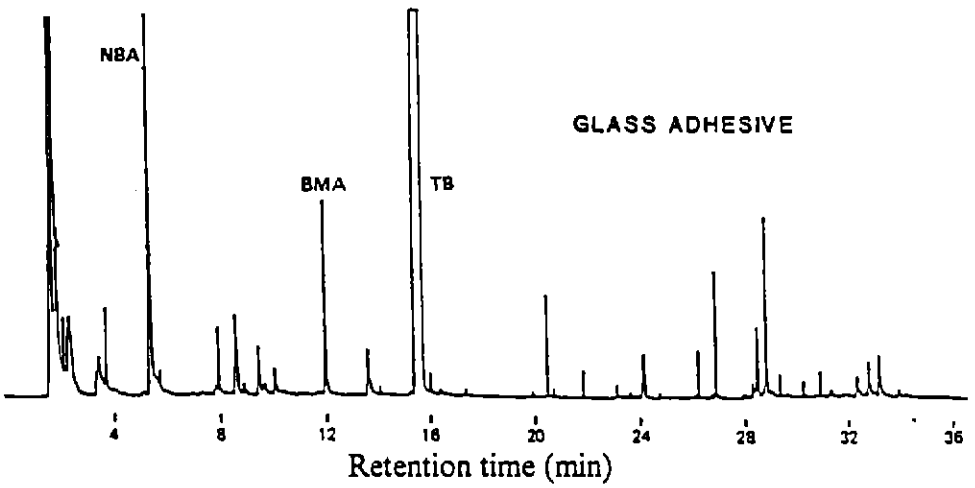


Fig. 3.12. Chromatogram showing THB-GC analysis of a commercial glass adhesive copolymer formulated with methacrylic acid.

Key: NBA = n-butanol, BMA = n-butyl methacrylate, TB = tributylamine. The unmarked peaks represent pyrolysis products of the polymer.

Cellulose acetate butyrate is a commonly used flow promoter additive in automotive acrylic lacquer formulations. It was not detected by conventional Py-GC analysis When an acrylic lacquer containing cellulose acetate butyrate was flash heated with TBAH, butyl acetate and butyl butyrate are detected as the THB products (Fig. 3.13).

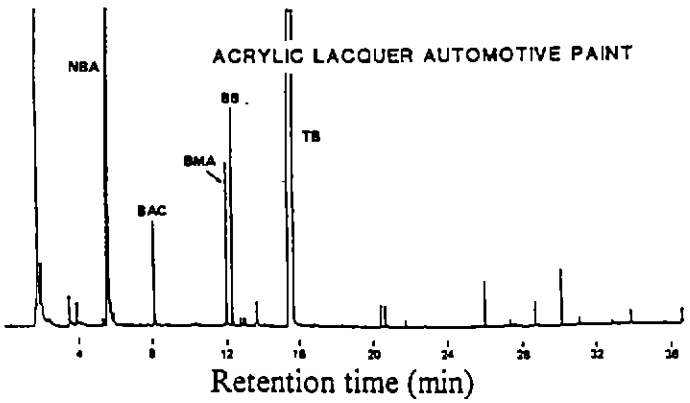


Figure 3.13 Chromatogram showing THB-GC analysis of an automotive acrylic lacquer containing cellulose acetate butyrate.

Key: NBA = n-butanol, BAC = n-butyl acetate, BMA = n-butyl methacrylate, BB = n-butyl butyrate, TB = tributylamine. The unmarked peaks represent pyrolysis products of the polymer.

Pyrolysis products of polycyanoacrylate esters (commonly marketed as 'Superglue') are difficult to separate chromatographically due to the highly polar nature of their pyrolysis products. THB gives a series of compounds, the major component having been tentatively identified as butyl cyanoacrylate (Fig. 3.14)

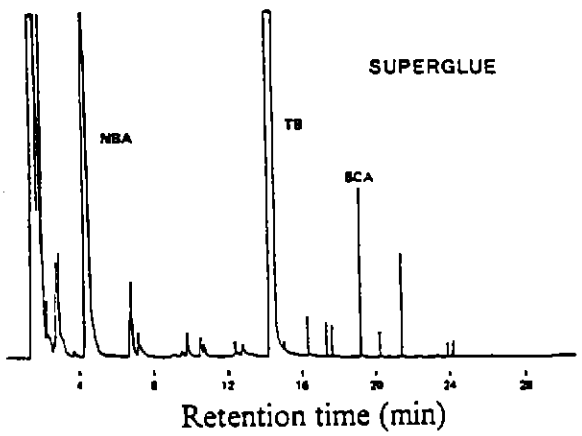


Fig. 3.14. Chromatogram showing THB-GC analysis of a cyanoacrylate adhesive.

Key: NBA = n-butanol, BCA = n-butyl cyanoacrylate, TB = tributylamine. The unmarked peaks represent pyrolysis products of the polymer.

The mechanism of this reaction is probably analogous to that of the THB reaction of methacrylic acid copolymers.

3.5 SUMMARY

The THM reaction, coupled with GC-MS separation and identification has simplified the identification of the chemical precursors of polyesters and phenolic polymers, and the identification of selected polymer additives. The products were identified as methyl derivatives of the products which would be expected to be obtained from hydrolytic degradation of the polymer and methylation of the respective components. The THB reaction produced corresponding butyl derivatives which, for some polymers, were more conveniently identified by GC, because the methyl derivatives were either too low in molecular weight for normal GC conditions, or could be confused with conventional pyrolysis products of the polymer. The THB reaction

would also be appropriate for identifying moieties in polymers which had sites of pre-existing methylation. The method involves minimal sample manipulation, uses low cost instrumentation and is more sensitive than conventional Py-GC techniques.

CHAPTER 4

FURTHER DEVELOPMENTS IN THE APPLICATION OF THE THM REACTION TO NATURAL PRODUCTS

4.1 ABSTRACT

This chapter outlines some further developments in the application of the THM pyrolysis derivatisation reaction to the characterisation of naturally occurring materials which include fats, waxes, kerogen and proteins. The method is also applied to the chemical characterisation of components of proprietary pharmaceutical preparations and polymer additives. Useful chemical structure information is obtained from these materials which is otherwise not possible without lengthy chemical degradation, derivatisation and separation techniques.

4.2 INTRODUCTION

Natural products including fats, vegetable oils, waxes and proteins are found not only in the environment but also as important components of commercial preparations. The characterisation and analysis of these materials is important in health and dietary studies, forensic science investigations, environmental protection and quality control applications. The determination of the chemical composition of petroleum bearing sediments is essential in oil exploration studies.

The analysis of lipid triglycerides by GC methods has been well established but the methods can suffer from disadvantages (Section 1 2.2) and the determination of lipid components of mixtures in proprietary products can prove unwieldy and time-consuming. Natural waxes can consist of a wide range of different lipid classes, including esters of various kinds, hydrocarbons, ketones, hydroxy-ketones, β-diketones, aldehydes, acids and terpenes (Christie, 1990). Wax esters, which are esters of long chain alcohols and fatty acids, are widespread in nature and have some commercial importance. These compounds, and the compounds with free carboxyl and hydroxyl groups in natural waxes, usually require some kind of reaction for derivatisation prior to analysis by GC The chemical nature of these components reflect their origin, for example, the fatty alcohol and acid constituents are frequently saturated or monoenoic compounds from 16 to 30 carbon atoms in length, but those of marine origin may be highly unsaturated. The relatively high molecular weight of

wax esters, their high degree of unsaturation in some cases and the branched chain nature of the fatty alcohol and acid constituents, when present, can present complications in their GC analysis.

Efforts to unravel the chemical constitution of the complex chemical nature of kerogens and other geopolymers has relied on a range of analytical techniques which have included chemical degradation procedures (Barakat, 1993; Michaelis *et al.*, 1989). Analytical pyrolysis has provided a useful alternative to chemical degradation for the study of the structure of this material, soil organic matter and other bio- and geomacromolecules (Larter, 1984; Schulten and Schnitzer, 1992). The major pyrolysis products of kerogen include saturated and olefinic hydrocarbons, aromatics, organosulphur compounds, alkyl phenols and carboxylic acids. The complex nature of the pyrolysis products and, to some extent secondary reactions, complicate the interpretation of the mode of interconnection of structural units (Larter, 1984).

Amino acids, peptides and proteins are among the most important chemical entities in life science. Conventional analytical methods for characterising components of peptides and proteins usually require sample sizes of approximately 5 mg and have to be preceded by a labour intensive hydrolysis process. There are disadvantages associated with the hydrolysis using 6N hydrochloric acid which include the decomposition of some labile amino acids, such as tryptophan. Proteins with bulky side chain amino acids such as valine and isoleucine, when linked in series, require extremely long hydrolysis times.

In the previous chapter, it was asserted that the thermally assisted hydrolysis and alkylation reaction of TAAHs with synthetic polymers having ester and ether functionality, coupled with GC, could provide additional information about chemical structure. This additional data was not possible to acquire without extensive chemical degradation procedures and had advantages when compared to conventional Py-GC. Minimal sample manipulation was required, the technique was more sensitive than existing methods and had the advantage that comparatively low cost instrumentation was employed.

This chapter outlines some of the further developments in the application of the THM reaction and to describe its potential application to naturally occurring material such as vegetable oils, waxes, kerogen and proteins, and to complex esters in drugs and U V. adsorbers

4.3 EXPERIMENTAL

The experimental procedures were similar to those described in Chapter 2 (Section 2.2). The capillary column stationary phases were selected to obtain the most efficient separations of derivatives of the different materials analysed. For example, a more polar phase e.g. DB23, (J&W Scientific) was selected for the separation of saturated and unsaturated FAMES DB23 has a (50%-cyanopropyl) methylpolysiloxane phase, whilst a dimethylpolysiloxane phase e.g. DB1 (J&W Scientific), was chosen for the separation of FAMES from alkanes and alkenes in kerogens Temperature programme settings were as follows: initial temperature 30° C, hold 2 min, increase at 8° C min^{-1} to 270° C for the DB23 column and 290° C for the DB1 column, except for the analysis of natural waxes where the initial GC temperature setting was 150° C.

4.4 RESULTS AND DISCUSSION

4.4.1 Fatty Acid Triglycerides

Animal fats and vegetable oil triglycerides are converted to their respective FAMES when subjected to THM and approximately 100 nanogram quantities are required for the determination. The results of THM-GC analysis of butter fat, showing the relative proportions of FAMES derived solely from the fatty acid triglycerides, are shown in Figure 4.1. The results obtained are in general agreement with those obtained by the BF_3-methanol GC procedure for the determination of FAMES derived from butter fat (Metcalf *et al.*, 1966).

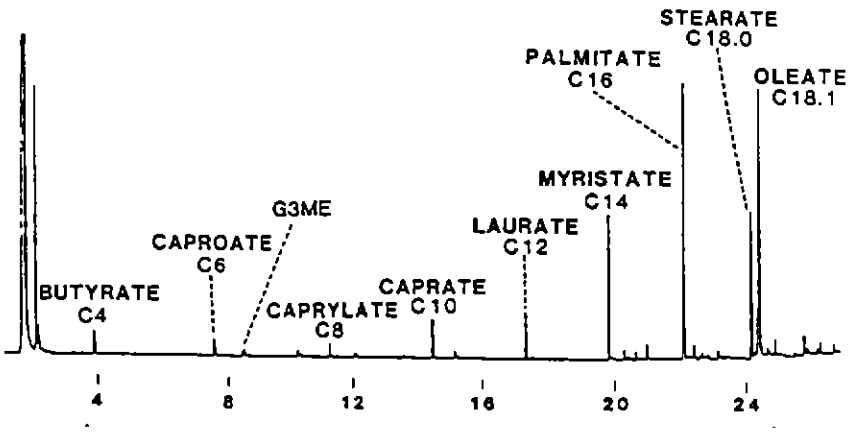


Figure 4.1. Chromatogram showing THM-GC of butter fat chromatographed on a 50% cyanopropyl - methyl silicone phase (J&W, DB 23) capillary column.

Key:-C4, C6, C8, C10, C12, C14, C16, C18.0 and C18.1 = respective fatty acid methyl esters. G3ME = glycerol trimethyl ether.

Additional compounds, eluting after methyl oleate, were detected in the chromatograms when lipids containing a significant amount of polyunsaturated fatty acids in the triglyceride, such as linseed oil, were analysed by THM-GC. These compounds were identified by mass spectrometry as isomers of linoleic and linolenic acid methyl esters These isomers were not detected in conventional analysis by transesterification using boron trifluoride/methanol. Figure 4.2 illustrates THM-GC of linseed oil indicating the isomers of the two unsaturated fatty acids.

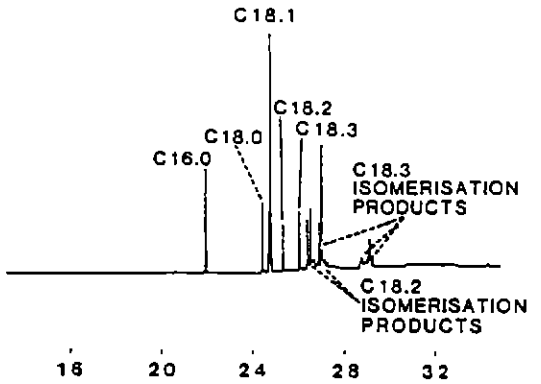


Figure 4.2. Partial THM-GC chromatogram of linseed oil chromatographed on a 50% cyanopropyl - methyl silicone phase (J&W, DB 23) capillary column.
Key:- C16 0, C18 0, C18.1, C18.2, and C18.3 = respective fatty acid methyl esters.

This isomerisation is known to occur in pyrolytic methylation of free fatty acids (Downing and Greene, 1968). The problem was overcome by neutralising excess TMAH with 5% acetic acid. In later work, methyl acetate was used to remove excess TMAH (Williams and MacGee, 1982). Attempts to adopt a similar procedure using methyl acetate to remove excess TMAH in the THM reaction failed to completely overcome the problem of base-catalysed isomerisation as the isomers were still detected. In spite of this partial isomerisation, it is possible to infer the presence of the particular polyunsaturated fatty acid based on the pattern of the artefacts produced. This subject is discussed in greater depth in Chapter 7.

4.4.2 Waxes

Natural waxes have a wide variety of uses in polishes, candles and finishes for paper, leather, textiles and wood. Cosmetics and medicinals incorporate waxes in lipsticks, creams and ointments.

Beeswax is mainly composed of myricyl palmitate $C_{15}H_{31}CO_2C_{31}H_{63}$, and contains cerotic and homologous acids, $C_{25}H_{51}CO_2H$, smaller amounts of hydrocarbons, cholesterol esters and ceryl alcohols Approximately 60% of beeswax comprises equal proportions of monohydric alcohols and acids, while hydroxy-acids are present to the extent of approximately 15% (Bennett, 1975) Jojoba oil and spermaceti, a waxy substance from the head of the sperm whale, are also used in commercial preparations including cosmetics and pharmaceuticals, and also contain higher molecular weight esters. Jojoba oil is a liquid wax ester extracted from seeds of *Simondsia chinensis* and *S. californica* desert shrubs native to Arizona, California and northern Mexico. It is composed essentially of C_{20} and C_{22} straight chain acids and alcohols in the form of esters (Budavari, 1989). Spermaceti is chiefly composed of cetyl palmitate, whilst cetyl alcohol is present in appreciable amounts and esters of lauric, myristic and stearic acids are also present. Esters of higher alcohols are also present (Budavari, 1989).

A comparison of the results of THM-GC of standard samples of white beeswax, jojoba oil and spermaceti is shown in Figure 4 3. The major product in the THM reaction of this specimen of white beeswax, methyl palmitate, is derived from myricyl palmitate. The only significant hydroxy-acid detected was the methyl derivative of 16-hydroxyheptadecanoic acid. Saturated fatty alcohols, having 24, 26, 28 and 30 carbon atoms, were detected as their methyl ethers. Saturated fatty acids possibly associated with these fatty alcohols in the form as esters were the C_{18} to C_{24} compounds, with the C_{24} fatty acid predominating, in contrast to the C_{25} fatty acid (cerotic acid) as reported by Bennett (1975). Significant proportions of hydrocarbons comprising *n*-alkanes from pentacosane to nonacosane were also found in the specimen. On the basis of the THM-GC data derived from jojoba oil, it would appear that this material is predominantly a mixture of esters of monounsaturated eicosenoic acid and C_{20} and C_{22} fatty alcohols. An interpretation of the data from the THM-GC analysis of spermaceti indicates that the material is composed of cetyl palmitate and esters of saturated C_{14} and C_{18} fatty acids.

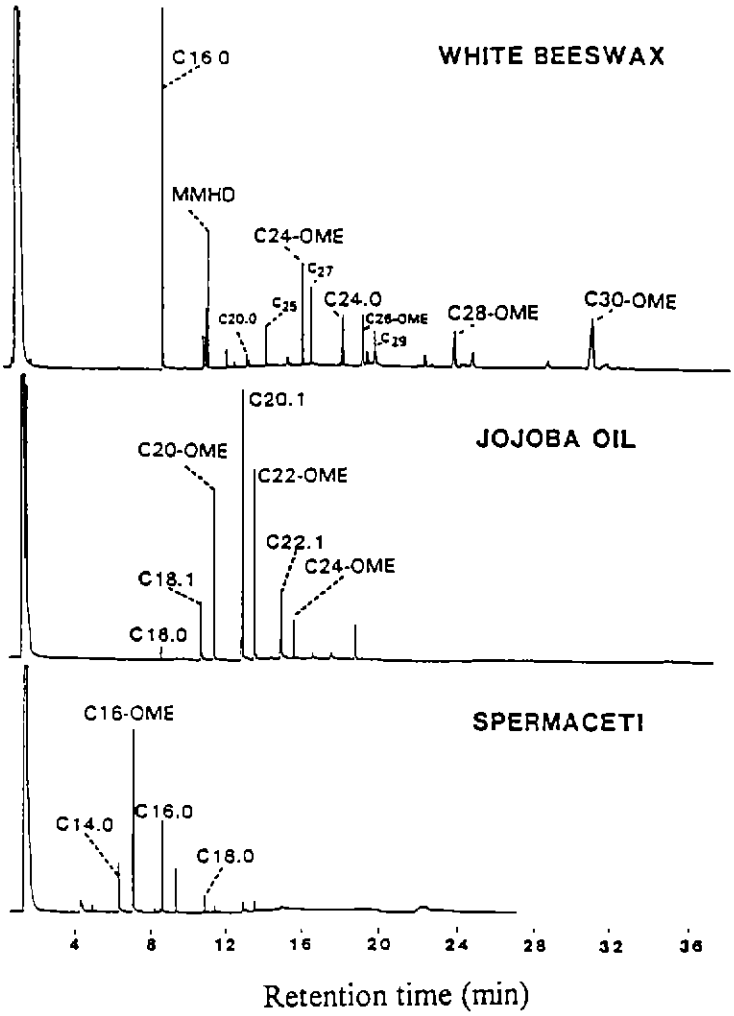


Fig. 4.3. Chromatograms showing THM-GC analysis of white beeswax, jojoba oil and spermaceti.

Key:- C14.0, C16.0, C18.0, C18.1, C20.0, C22.0, and C24.0 = respective fatty acid methyl esters; C24.OME, C26-OME, C28-OME, C30-OME = respective saturated fatty alcohol methyl ethers; MMHD = methyl 16-methoxyheptadecanoate; C_{25}, C_{27} and C_{29} = *n*-alkanes having 25, 27 and 29 carbon atoms

The different waxes may, therefore, be distinguished by the distribution of methyl derivatives of fatty acids and fatty alcohols. The results are also generally consistent with reported results for these materials.

4.4.3 Kerogen

The organic matter in kerogens is supposedly derived from terrestrial plant matter and algal, and to a lesser extent, microbial lipids (Tissot and Welte, 1984). If oxygenated species are present as esters or amides in this organic material, the kerogen would be expected to undergo hydrolytic cleavage in the high temperature THM reaction to afford the respective methyl esters. Further, if nitriles are present, these compounds would be hydrolysed under the alkaline conditions to form carboxylic acids which would be converted to the methyl esters.

The composition of the aforementioned species present in a Rundle oil shale from eastern Queensland, Australia, were monitored by THM-GC-MS using reconstructed ion chromatography for ions *m/z* 74 and *m/z* 87 for saturated FAMES (Figure 4.4). The results were compared to the pyrogram of the same oil shale obtained Py-GC with flame ionisation detection. The FAMES, presumably derived from the lipid fraction in the kerogen, comprised a homologous series ranging from $C_{4.0}$ to $C_{25.0}$. Compounds C8.0, C12 0, and C16.0 were dominant in contrast to C6, C10 and C14 *n*-alkenes in the conventional Py-GC determination. Doublets and/or triplets of associated peaks were detected in the FAME series. The mass spectra of these minor components indicated that the compounds were branched chain and/or olefinic in nature.

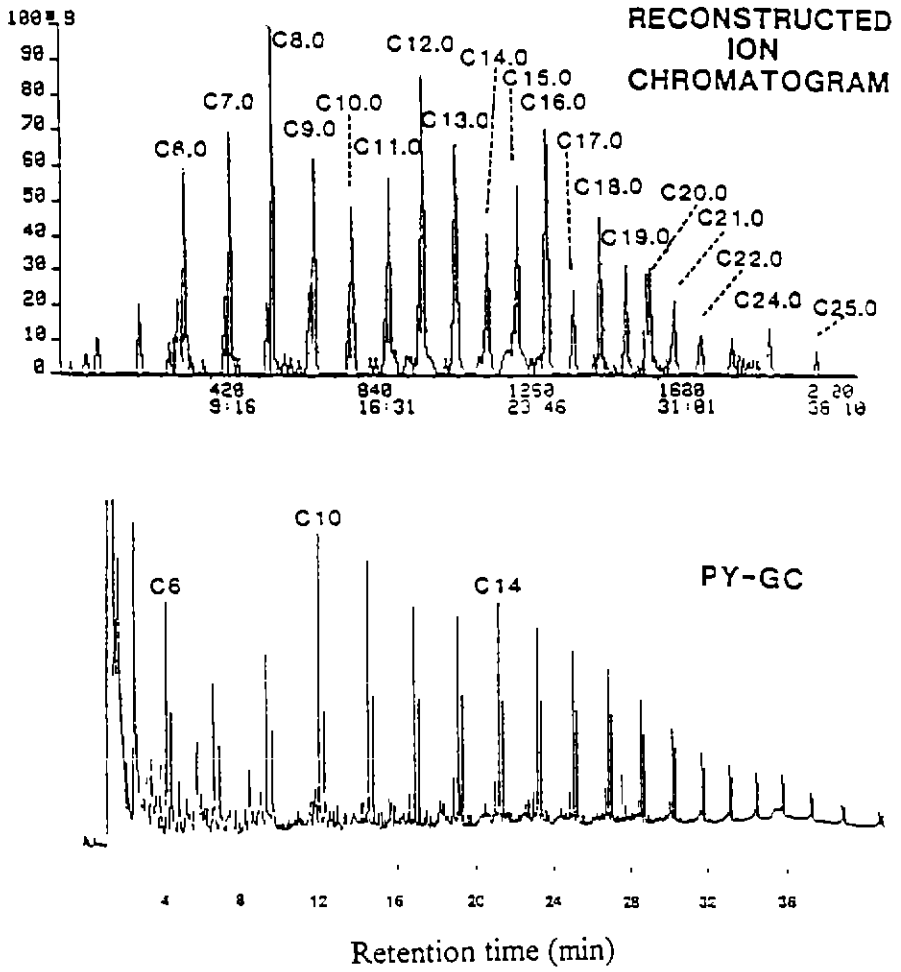


Figure 4.4 THM-GC-MS reconstructed ion chromatogram (m/z 74 and 87 for FAMES) and Py-GC of Rundle oil shale from Queensland, Australia.

Key:- C6.0 to C25.0 = respective saturated fatty acid methyl esters, C6, C10, C14 = respective n-alkenes

These results may be compared to the work of Regtop *et al.* (1982) in which straight chain unsubstituted alkanamides ranging from C_9 to C_{28} were identified in a Rundle shale oil together with smaller quantities of an associated homologous series of 2-methyl alkanamides using chemical separation and GC-MS. Nitrile compounds were extracted from, and identified in Rundle shale oil by hydrolysis under alkaline conditions, extracted as the carboxylic acids and subsequently identified as their methyl esters (Harvey *et al.*, 1988). The major products were a series of C_4 to C_{34} *n*-alkanoic acid methyl esters and the minor components were two olefinic series and

alkanoic acid methyl esters, and the minor components were two olefinic series and one phenyl-alkyl series. Therefore, the origin of the acids, detected in the THM-GC experiment, from the above sources, rather than from esters cannot be discounted and remains to be confirmed.

These results may also be contrasted to those obtained from the chemical degradation study on Monterey kerogen where mild alkaline hydrolysis conditions were used to obtain fatty acids from which methyl derivatives were prepared (Barakat, 1993). The study revealed ester functionality in this immature kerogen which is derived from organic matter from marine (algal) organisms deposited in a highly reducing environment The fatty acids detected in the work by Barakat included saturated normal monocarboxylic acids (C_8-C_{36}), saturated normal α, ω-dicarboxylic acids (C_7-C_{29}), isoprenoid acids (C_{14}-C_{17}, C_{19}-C_{21}), iso- and anteiso acids (C_{11}-C_{18}), unsaturated acids ($C_{16.1}$, $C_{18.1}$) and aromatic acids. The assumption is made that these fatty acids are derived from esters in the kerogen. Kawamura *et al.* (1985) also reported the detection of a series of long chain carboxylic acids in Green River kerogen which had been subjected to anhydrous and hydrous pyrolysis. The C_{10}-C_{32} fatty acids were released by saponification with 0.5N KOH/methanol and esterified with BF_3/methanol. GC analysis showed a strong even/odd predominence with a maximum at C_{16} with lesser amounts of C_{18} and C_{22} acids.

4.4.4 Proteins

The identification of proteins is important in:-

- art conservation and restoration work (Mills and White, 1994)
- speciation of edible products
- characterisation of bacteria and higher plants, enzymes and sediments (Saiz-Jimenez and de Leeuw, 1986)
- soils
- leathers and parchments and
- human hair.

In connective tissue, protein exists in the the form of collagen and is found in skin, bone, muscle and hide Collagen contains frequently repeating glycine-proline-hydroxyproline amino acid sequences. Keratin is the structural protein of hair, horn and feather and has a high content of the sulphur-containing amino acid, cystine, which is responsible for the dithio (-S-S-) linkages between the protein chains. Globular proteins are present in albumins, which are water soluble but can easily lose hydrogen bonding on heating to become insoluble. The proteins in egg albumin contain moderate amounts of aspartic and glutamic acids, and leucine.

Differences in Py-GC profiles between these specific protein types would therefore be expected Amino acid sequences in protein have been investigated by Py-GC-MS (Boon and de Leeuw, 1987) and aromatic and aliphatic amino acid components in various enzymes were determined (Tsuge and Matsubara, 1985). The Py-GC characterisation of human hair protein for forensic purposes has received considerable interest (Munson and Vick, 1985) The work concluded that the method did not provide a useful technique for forensic hair comparisons nor was there justification for further research.

In this work, the potential of the THM reaction for the hydrolysis fragmentation and derivatisation of proteins was examined. The THM-GC profiles of bone collagen from different sources (cow, sheep, elephant tusk [ivory]) have not shown significant differences. Keratin from human hair, bird feather and human nail have similarly been difficult to distinguish, although they were readily distinguishable from collagen profiles whilst egg white albumin was different. THM-GC profiles of collagen (bone), keratin (human hair) and albumin (egg white) are shown in Figure 4.5. The compounds corresponding to the peaks marked with an asterisk were detected in corresponding conventional Py-GC experiments. The identity of many of the THM products could not be determined from their mass spectra after reference to the mass spectral libraries and require more investigation to determine their identity.

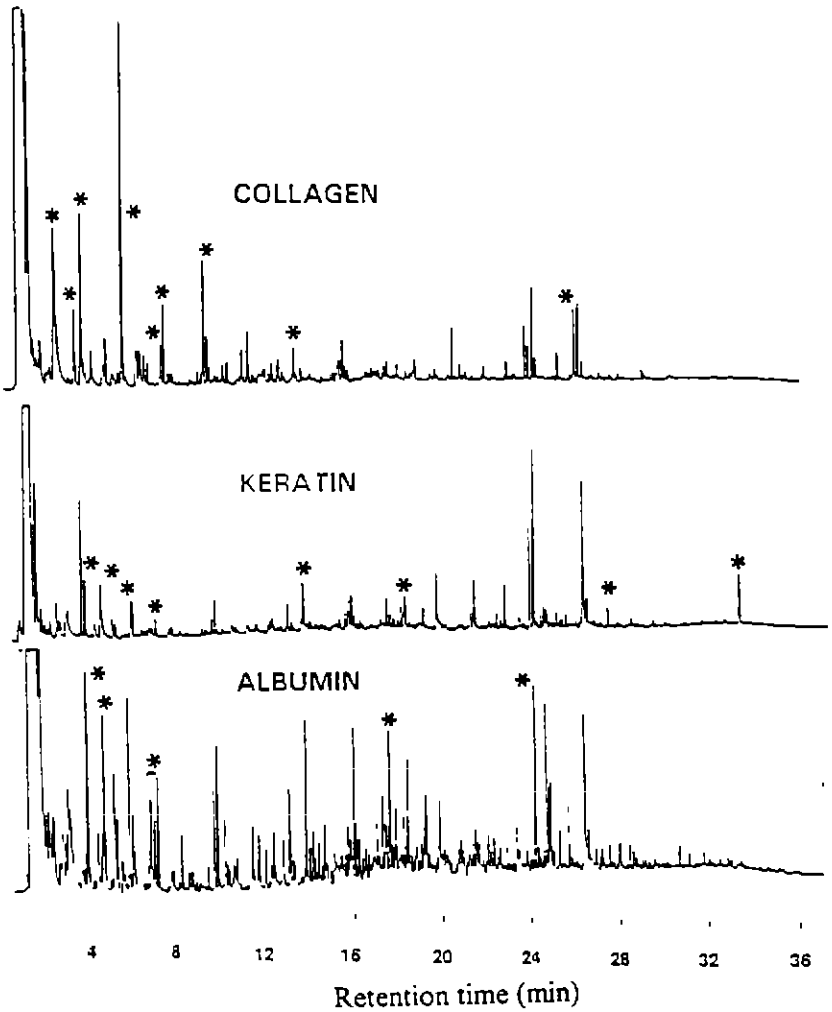


Figure 4.5 Chromatograms showing THM-GC of collagen (cow bone), keratin (human hair) and albumin (denaturated egg white).

The peaks marked with an asterisk correspond to those compounds detected in corresponding conventional Py-GC experiments

The THM reaction of structural proteins

Fibroin in the form of silk and keratin as wool are two structural proteins which have important uses in the textile industry. Standard reference materials of wild silk and Merino wool were analysed by THM-GC (Figure 4.6) and the products were identified by MS. The lipid constituents of these naturally occurring materials were also simultaneously identified by referring to the FAME composition in the chromatograms, without resorting to separate analysis strategies. Tentative identifications of the peaks corresponding to the THM products, mass spectral data

and structure of the lesser known compounds, and proposed amino acid origin are shown in Table 4.1.

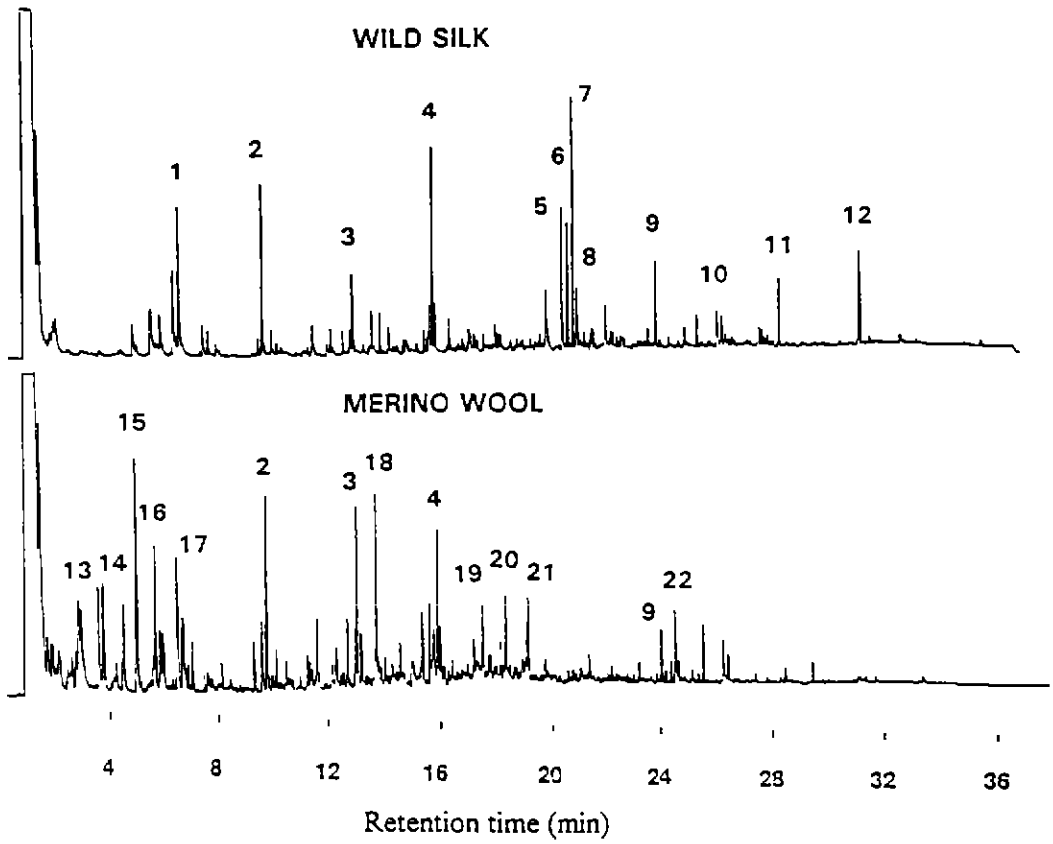
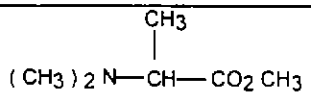
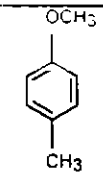
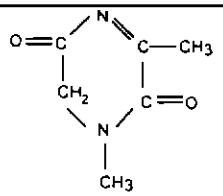
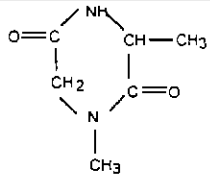
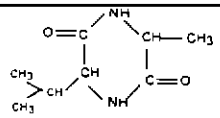
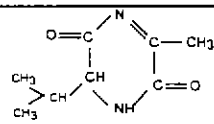
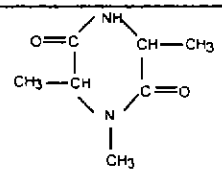
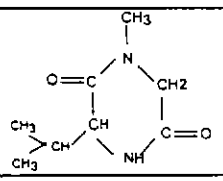
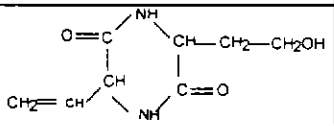
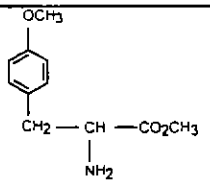
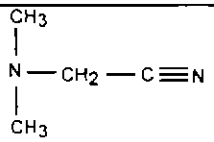
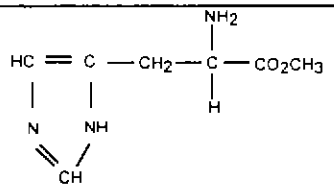
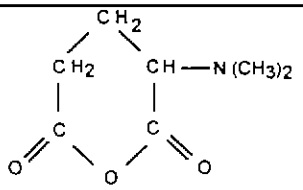


Figure 4.6 Chromatograms showing THM-GC analysis of standard reference materials of wild silk and Merino wool.

Tentative identifications of the peaks, mass spectral data and amino acid origin are shown in Table 4.1.

PEAK NO.	PROPOSED IDENTITY	MASS SPECTRAL DATA	PROPOSED STRUCTURE	AMINO ACID ORIGIN
1	N, N-dimethyl alanine methyl ester	72, 42, 56, 131		ALA
2	1-methoxy-4-methyl benzene	122, 77, 121, 107		TYR
3	1,3-dimethyl 3,4-dehydro-2,5-piperazinedione	56, 140, 42, 91, 134		GLY-ALA
4	1,3-dimethyl-2.5-piperazinedione	42, 142, 127, 56. 57		GLY-ALA
5	3-methyl, 6-(2-propyl)-2,5-piperazinedione	127, 58, 42, 170, 85		ALA-VAL
6	3-methyl, 6-(2-propyl)3,4 dehydro-2,5-piperazinedione	125, 168, 153, 55, 42, 56		ALA-VAL
7	3,4,6-trimethyl-2,5-piperazinedione	113, 156, 42, 71, 56		ALA-ALA
8	isomer of compound peak 5	127, 58, 42, 170, 85		GLY-VAL
9	methyl palmitate	-	-	-

PEAK NO.	PROPOSED IDENTITY	MASS SPECTRAL DATA	PROPOSED STRUCTURE	AMINO ACID ORIGIN
10	methyl oleate	-	-	-
11	3-(2-hydroxyethyl)-6-methylene-2,5-piperazinedione	127, 58, 170, 85, 56		SER-SER
12	4-methoxyphenyl-2-aminopropanoic acid methyl ester	121, 78, 91, 42		TYR
13	dimethyl disulphide	94, 45, 79, 46, 96	-	CYS
14	toluene	-	-	PHE
15	dimethylamino - acetonitrile	42, 58, 83, 84, 43		GLY
16	ethyl benzene	-	-	PHE
17	histidine methyl ester	69, 70, 42, 53, 72, 131		HIS
18	3-dimethylamino - 2,6-diketo-tetrahydropyran	56, 126, 42, 68, 69		GLU
19	related to compound peak 21	128, 42, 69, 179, 194	-	-

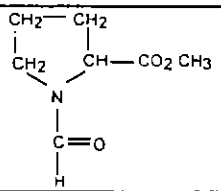
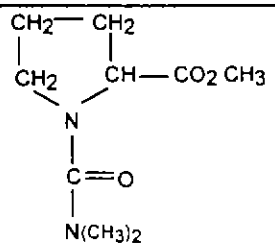
PEAK NO.	PROPOSED IDENTITY	MASS SPECTRAL DATA	PROPOSED STRUCTURE	AMINO ACID ORIGIN
20	formyl proline methyl ester	98, 42, 70, 68, <u>157</u>		PRO
21	related to compound peak 19	128, 127, 42, 56, <u>186</u>	-	-
22	related to compound peak 20	98, 42, 70, 68, <u>156</u>		PRO

Table 4.1. Tentative identifications, mass spectral data, structure and proposed amino acid origin of the peaks corresponding to the THM products of standard reference materials of fibroin (wild silk) and keratin (Merino wool).

Key: ALA = alanine, TYR = tyrosine, GLY = glycine, VAL = valine, SER = serine, CYS = cystine, PHE = phenylalanine, HIS = histidine, GLU = glutamic acid, PRO = proline. Mass numbers underlined indicate tentative molecular ion.

The THM reaction products of silk fibroin and wool include methylated derivatives of individual amino acids including alanine (peak marked 1), tyrosine (peaks marked 2 and 12), glycine (peak marked 15), and proline (peaks marked 20 and 22). These compounds are produced by alkaline hydrolysis of the peptide chain and methylation of amino and carboxylic acid groups in the amino acids by TMAH. The relative peak areas reflect the concentration of the respective amino acids in the protein molecules. For example, the THM products of tyrosine, 1-methoxy-4-methyl benzene (peak marked 2) and 4-methoxyphenyl-2-aminopropanoic acid methyl ester (peak marked

12) are both detected in silk and the former compound only is detected in wool. The ratios of the summed peak areas for the tyrosine related compounds approximate to the published figures in which approximately 12% tyrosine is reported to be present in silk and approximately 5% of this amino acid in wool. The reported compositions of other amino acids of fibroin in the form of silk and keratin as wool are summarised in Table 4.2. Dimethyl disulphide (peak marked 13), a product derived from the amino acid, cystine, was only detected in the products of the Merino wool fibre. As expected, this compound was not detected in the THM chromatogram of silk as this protein does not contain this amino acid.

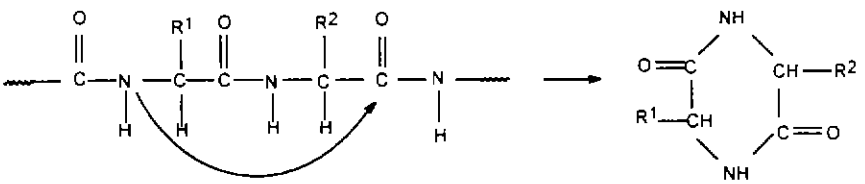
AMINO ACID	FIBROIN (silk) [weight %] (1)	KERATIN (wool) [weight %] (2,3)	
Glycine	42.8	6.0	5.6
Alanine	33.5	3.9	4.1
Valine	3.3	5.5	5.7
Leucine	0.9	7.9	8.6
Isoleucine	1.1	3.8	3.6
Proline	0.5	6.7	6.6
Phenylalanine	1.3	3.7	40
Tyrosine	11.9	5.2	6.5
Tryptophan	0.9	1 9	*d*
Serine	16.3	8.4	9.3
Threonine	1.4	6.6	6.7
Cystine	0.0	12.8	11.9
Methionine	0 0	0.6	0 7
Arginine	1.0	9.9	10.5
Histidine	0.4	3.0	1.1
Lysine	0.6	0.9	3.6
Aspartic acid	2.2	6.9	7.4
Glutamic acid	1.9	14.5	15.3

Table 4 2. Amino acid compositions of fibroin (silk) and keratin (wool). Results are taken from Lucas *et al.*, (1958) (1), Ward and Lundgren (1954) (2) and Mclaren and Milligan (1981) (3) and converted to weight per cent. *d* = decomposed.

Heterocyclic compounds, which appear to be formed by ring closure of two adjacent amino acids in the peptide chain, are also produced. The identity of these compounds was not clearly indicated by the Wiley and NBS mass spectral library searches. Hypothetically, these compounds could be partly N-methylated derivatives of 2,5-

piperazinediones substituted in the *1* and *4* positions by groups characteristic of the respective amino acid. The mass spectra were not consistent with 2,4 imidazolinediones substituted in the *5* position, proposed as pyrolysis products of proteins (Munson and Vick, 1985). Mass spectra of compounds represented by peak 4 to peak 8, characteristic of silk, indicate that the compounds are related and, in the absence of confirming library search spectra, the structures are consistent with amino acid pairs in the peptide sequence. Further work to determine or confirm the identity of many of these compounds will be the focus of future research.

The mechanism of the formation of 2,5-piperazinediones by cyclisation of portions of the peptide chain under basic conditions with aqueous TMAH in the THM reaction of proteins is proposed as follows :-



Eq. 4.1

R^1 and R^2 are groups characteristic of the respective amino acids, e.g. $R^1 = R^2 = H$ or CH_3 for glycine or alanine, respectively.

Pyrolysis reactions also appear to play a minor role simultaneously to the THM reaction. For example, toluene and ethyl benzene may be reasonably expected to be pyrolysis products of phenylalanine amino acid units and dimethyl disulphide from pyrolysis of cystine segments. The heterocyclic products formed in the THM reaction can be compared and contrasted to the alkyl pyrollidinediones which were proposed as thermolysis products of some proteins by Boon and de Leeuw (1987).

In summary, high temperature reactions of aqueous TMAH with peptides produce compounds that are consistent with a chemolysis process rather than thermolysis.

The mechanism of the formation of the products appear to involve scission of the CO-NH peptide linkage together with other reactions such as cyclisation and dehydration. These reactions may be contrasted to the THM reaction of polyester polymers discussed in Chapter 3 which involve hydrolysis of the CO-O ester bonds.

4.4.5 Complex Esters

Esters formed from complex alcohols and simple carboxylic acids may be incorporated as additives into proprietary drug formulations and modifiers in polymeric products, together with natural products and materials closely associated with natural products. Conventional procedures for the identification of these additives would usually require a separation step.

The application of THM-GC to the structural identification of complex esters used in some proprietary pharmaceutical products has proved useful in forensic science. The drug, haloperidol, is formulated as the decanoate ester, (Figure 4.7) dissolved in sesame seed oil and contains approximately one percent benzyl alcohol as a preservative.

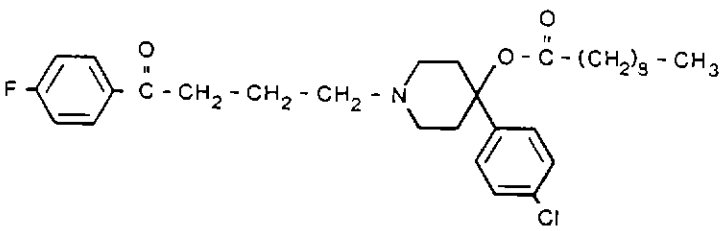


Figure 4.7. Chemical structure of the drug, haloperidol, formulated as the decanoate ester.

THM results in the formation of benzyl methyl ether from the preservative, methyl decanoate from the active ingredient, and FAMES (Figure 4.8). The relative composition of the FAMES was consistent with a known pure sesame seed oil standard. The higher molecular weight fragments of the haloperidol molecule

resulting from the hydrolysis reaction were not detected under the GC conditions used in the experiment.

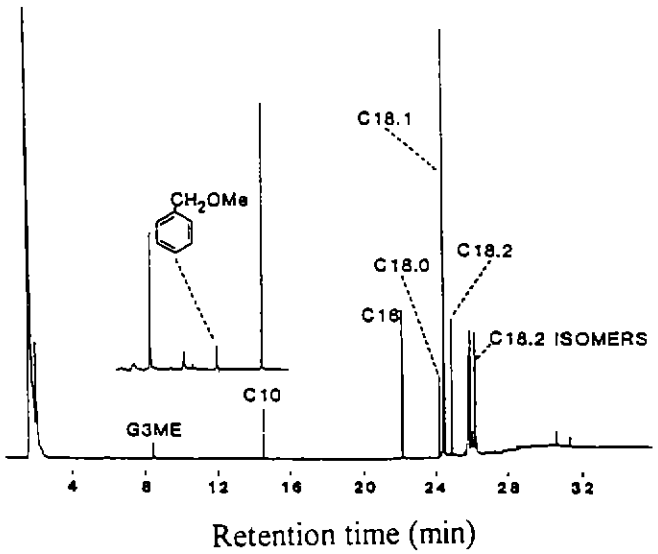


Figure 4.8. Chromatogram showing THM-GC analysis of a proprietary drug (haloperidol) formulation chromatographed on a DB23 type column.

Key:- BME = benzyl methyl ether, G3ME = glycerol trimethyl ether, C10, C16, C18.0, C18.1, C18.2 = respective fatty acid methyl esters.

The inset shows a scale expanded section of the chromatogram indicating the peak attributed to benzyl methyl ether.

Some of the hindered amine type U.V. absorbers used in surface coatings and plastics are also esters having a complex composition. One such product, Tinuvin 292, manufactured by Ciba-Geigy Ltd., has the structural formula shown in Figure 4.9.

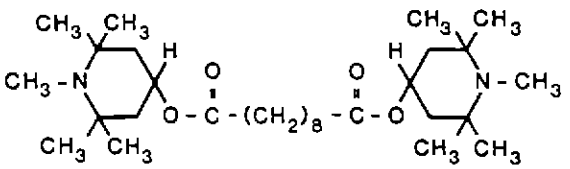


Figure 4.9. Structural formula of a hindered amine type U.V. absorber, Tinuvin 292.

When subjected to THM, octanedioic acid dimethyl ester (dimethyl sebacate), pentamethylpiperidol and its methyl ether were detected (Fig. 4.10).

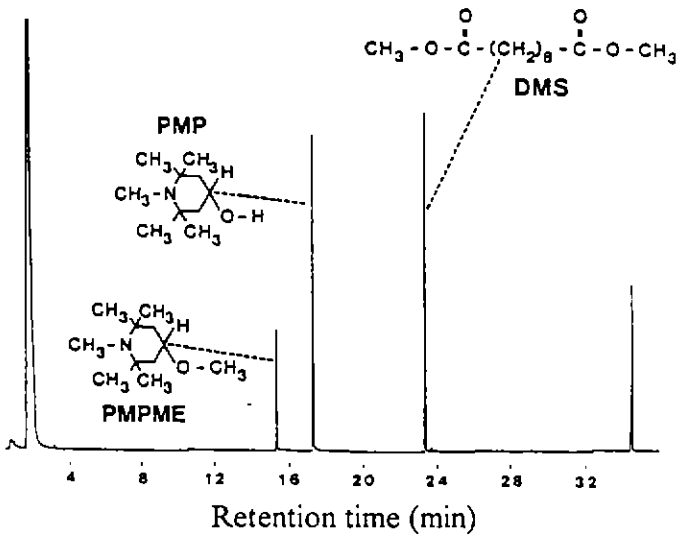


Fig. 4.10. Chromatogram showing THM-GC of a hindered amine light stabiliser, Tinuvin 292.

Key:- DMS = dimethyl sebacate, PMP = pentamethylpiperidol, PMPME = pentamethylpiperidol methyl ether.

4.5 SUMMARY.

In this chapter, the application of the THM reaction has indicated that :-

(i) ester components in animal fats and vegetable oils, natural waxes and ester functionality in kerogen can be determined with minimal manipulation of the material. The application of the THM reaction to the characterisation of lipids is discussed in more detail in the next chapter.

(ii) collagen, keratin and albumin protein profiles were distinguished without difficulty. The THM reaction has the potential to afford a useful method for the estimation of amino acids in silk fibroin and wool fibres.

(iii) polyhydric alcohols, phenols and polybasic acid components in esters in pharmaceutical drug formulations and industrial additives can be rapidly identified.

CHAPTER 5

STRUCTURE DETERMINATION OF ALKYD RESINS USING THE THM REACTION

5.1 ABSTRACT.

Several different types of alkyd resins have been analysed by thermally assisted hydrolysis and methylation - gas chromatography (THM-GC). The process, considered to be a high temperature hydrolytic methylation reaction using aqueous TMAH, gives rise to methyl esters of carboxylic acids and methyl ethers of polyhydric alcohols. The identification of dibasic acid, polyol, drying oil type and rosin and epoxy modification is assisted by the procedure. The degree of cure and the oil length can also be estimated. The procedure is sensitive and involves minimal sample manipulation.

5.2 INTRODUCTION

Alkyd resins are widely used in surface coating applications, including architectural oil based paints, industrial enamels and automotive body finishes, in spite of competition from more recently developed coatings such as the acrylics, urethanes and epoxies. Consequently, the analysis of alkyd resins is important in materials and forensic science investigations

Alkyd resins are polyesters prepared by reaction of polyhydric alcohols with polybasic acids, usually phthalic acid, and modified with vegetable or marine oils. These resins cross-link by a free radical process which involves autoxidation at allylic sites in the unsaturated fatty acid chains in the drying oils. The degree of cure describes the extent to which this cross-linking process has occurred. The term "oil length" is used to describe the relative proportion of the drying oils to the synthetic resin (Oil and Colour Chemists Association of Australia, 1983). Resin manufacturers change the type and proportion of these components to suit the application and to take account of raw material cost. The drying and performance characteristics of the resins can be further improved by modification with acrylics, styrene, polyamide, silicone, rosin and phenolic resins.

Infrared spectroscopy is one of the most commonly used techniques for characterisation of the generic type of paint. The high pressure diamond cell is an

accessory which has been adopted in many forensic laboratories for the IR examination of microgram samples of automotive paint (Rodgers *et al.*, 1976). Fourier transform IR spectroscopy is now a popular technique, especially when used with a microscope attachment. However, IR cannot differentiate the polyhydric alcohol and drying oil types in alkyd resins.

Proton and ^{13}C nuclear magnetic resonance is also used for the identification of polyhydric alcohols and polybasic acids. Further, it was possible to classify the drying oils into linoleic, linolenic, ricinoleic and eleostearic rich types but was limited to uncured resins (Marshall, 1983).

Chemical degradation methods, with subsequent analysis by GC, have been used to determine the composition of polyols, polybasic acids and drying oil fatty acids (Haken, 1979). A.S.T.M. methods provide procedures for determination of polyols by acetylation (ASTM, 1991) and carboxylic acids by transmethylation (ASTM, 1989) in uncured resins. A rapid method for the preparation of methyl esters of drying oil carboxylic acids and aromatic polybasic carboxylic acids in cured resins used TMAH as the saponification reagent and methyl iodide as the esterification reagent (West, 1975). In one of a number of methods for analysing paint, the age of cured alkyd paint resins has been monitored by measuring the proportion of saturated methyl palmitate to unsaturated methyl esters, e.g. linolenate, linoleate and oleate. The cured resins were saponified and then methylated using diazomethane (May and Porter, 1975).

Capillary Py-GC has been used successfully to characterise alkyd and other paint resins typically found in forensic casework (Challinor, 1983). Good discrimination between twenty alkyd resins of different polyol, polybasic acid and drying oil was achieved using the same conditions (Challinor, 1984). Polyols were identified by the formation of pyrolysis products such as acrolein from glycerol, and methacrolein from pentaerythritol. Orthophthalic and isophthalic acid alkyds were determined by the presence or absence, respectively, of phthalic anhydride in the pyrolysis products. The composition of the drying oil was reflected by the relative proportions of aldehydes which were formed. Modified alkyd resins were distinguished by characteristic

pyrolysis products from the modifier, e.g. substituted phenols from rosin-phenolic and styrene oligomers from styrene modified alkyd resins. Subsequently, a range of 25 alkyd resins were examined by Py-GC and the performance of packed and capillary columns were compared. Both systems proved to have a high level of reproducibility (Bates *et al.*, 1989). The capillary GC method was superior since it assisted in the detection of additional compounds which could be directly related to particular features of the resin composition. Although some modified alkyds could be identified by the presence of characteristic peaks in the pyrogram, others such as silicone, polyamide and rosin-modified alkyds did not give pyrolysis products directly attributable to the modifiers. The method did not give a reliable indication of oil length.

In more recent work on a group of resins related to alkyd resins, a small number of cross-linked polyester coatings including neopentyl glycol - adipic acid based resins, were differentiated by Py-GC-MS (Wilcken and Schulten, 1996). While the non-polar components of the polymer were detected intact, the polyhydric alcohols, neopentylglycol and trimethylolpropane, and polybasic acids, adipic acid and phthalic acid were either dehydrated, decarboxylated or detected as fragments of the polyester chain such as di-2-propenyl hexanedioate. As a result, diligent interpretation of the origin of the pyrolysis products was necessary to determine the chemical composition of the polymer. In spite of this, the resins could be distinguished clearly with the aid of principal component analysis.

As described in Chapter 3, THM-GC was used for the chemical structure elucidation of a range of polymeric materials including alkyd resins (Challinor, 1989). A comparison was drawn between the results obtained by the conventional Py-GC of a soyabean alkyd resin and the changes that took place when this alkyd was flash heated with aqueous TMAH.

This chapter describes the application of this technique to alkyd resins of different composition, and shows how polyols, polybasic acids and drying oils may be

identified. Some interpretation of the condition of the drying oil is also made, e.g., degree of cure and proportion of oil in the resin.

5.3 EXPERIMENTAL

The experimental conditions were as outlined in Chapter 2 (Section 2.2).

Materials.

Alkyd resins were obtained from Dulux, Australia and A.C. Hatrick Chemicals Pty. Ltd.

5.4 RESULTS AND DISCUSSION

Conventional pyrolysis of alkyd resins at 770^{o}C gives a mixture of products which include aldehydes, carboxylic acids, alkanes, alkenes and benzoic acid. The major pyrolysis product of orthophthalic alkyds is phthalic anhydride, whilst isophthalic alkyds are characterised by a relatively high proportion of benzene (Challinor, 1984). Pyrograms of four resins of different composition obtained by conventional Curie point Py-GC show that these may be clearly distinguished on the basis of pattern recognition in the sample set used in the study (Fig.5.1). The polyhydric alcohol and the fatty acid composition are not readily deduced from the pyrograms. The detection of phthalic anhydride indicates that the resins are all orthophthalic types.

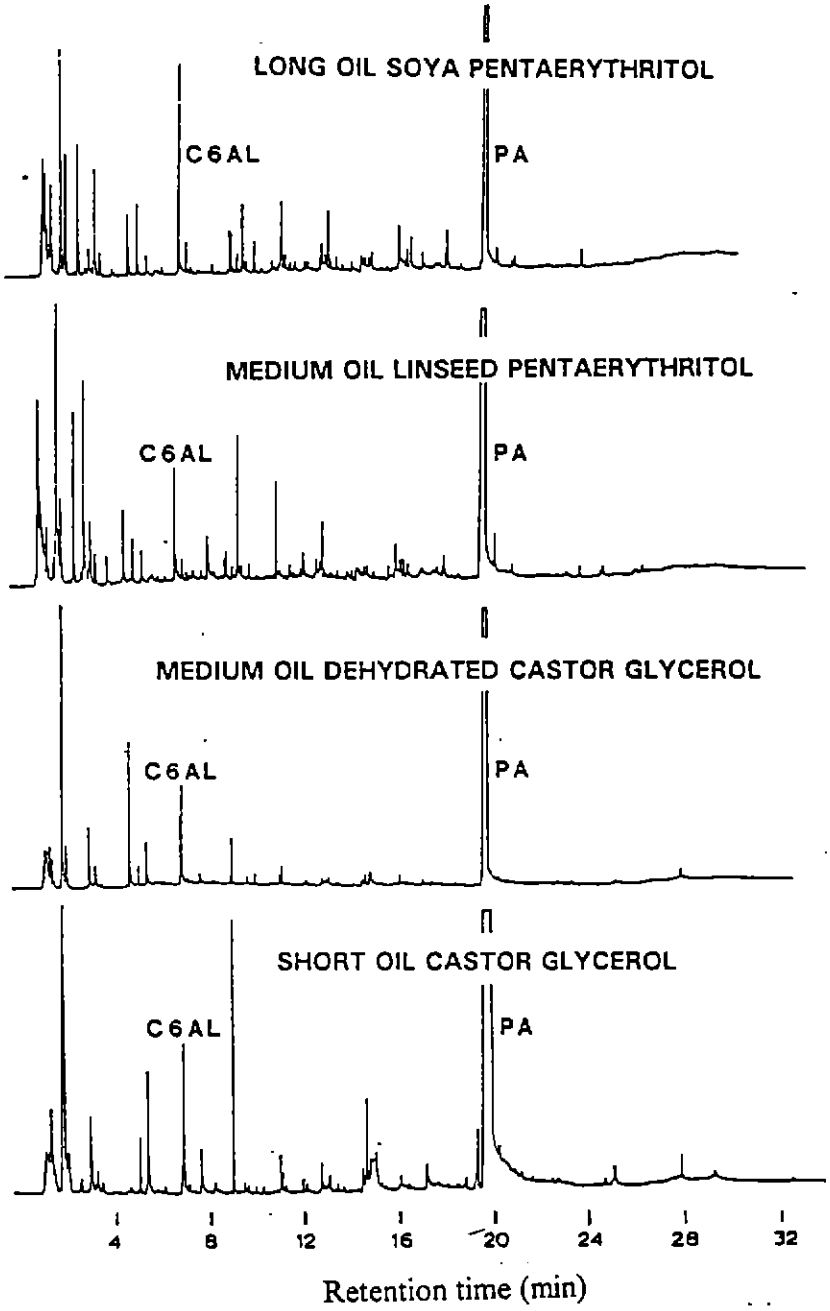


Figure 5.1. Pyrograms of four alkyd resins of different composition.

Key: C6AL=hexanal, PA=phthalic anhydride.

In contrast, the THM-GC analysis of alkyd resins was superior to conventional Py-GC because it provided a ready identification of the polyhydric alcohol, polybasic acid, drying oil, degree of cure and oil length. Further, the identity of modifying agents could be established by the same method.

5.4.1 Polyhydric Alcohol Identification

Glycerol and pentaerythritol are the two most common polyhydric alcohols used in alkyd resin production (Oil and Colour Chemists Association of Australia, 1983). THM results in the formation of methyl ethers of the respective polyols. The chromatograms of two resins based on glycerol and pentaerythritol are shown in Fig.5.2. The identities of the unmarked peaks are given in later sections for the purpose of clarity.

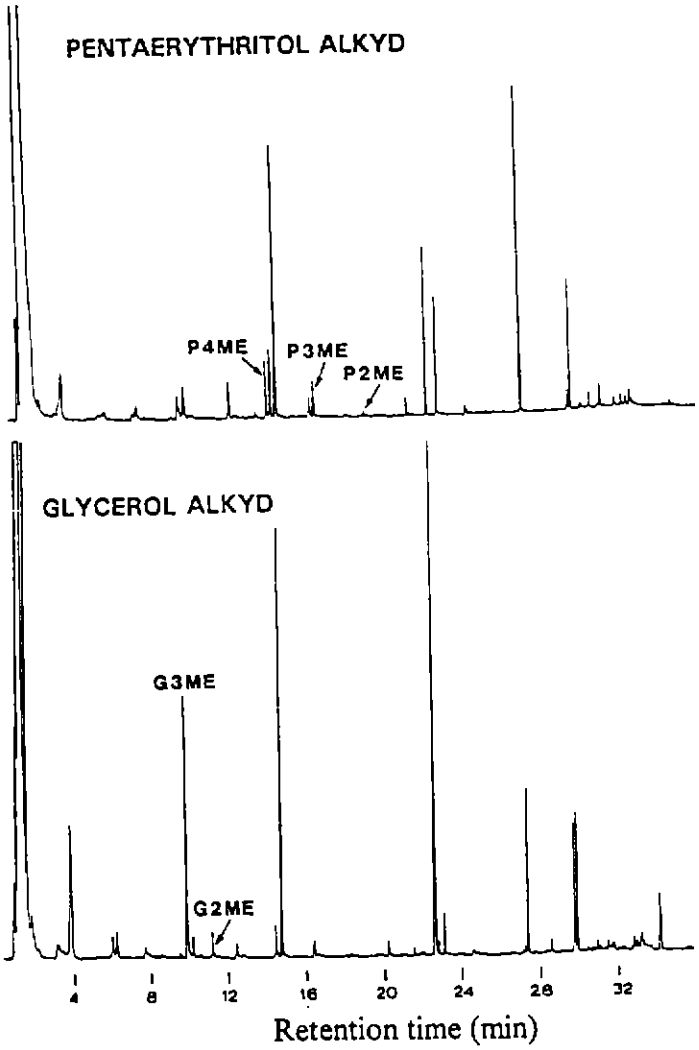


Figure 5.2. Chromatograms showing THM-GC of two orthophthalic alkyd resins containing different polyhydric alcohols.

Key: P4ME, P3ME, P2ME = pentaerythritol tetra-, tri- and di-methyl ether, and G3ME, G2ME = glycerol tri- and di-methyl ether, respectively.

It is apparent from the above chromatograms that complete conversion to the methyl ethers is not achieved using the THM reaction. As discussed in Chapter 1, it was observed that a high proportion of the tetramethylated ether was formed when pentaerythritol alkyd resins were subjected to lower reaction temperatures. Similarly, in glycerol alkyd resins a greater proportion of the glycerol trimethyl ether was formed. In spite of this incomplete conversion, it is possible to positively identify the polyhydric alcohol in an alkyd resin.

5.4.2 Polybasic Acid Identification

The two most common dibasic acids used in alkyd resins are orthophthalic and isophthalic acids, with the majority of the resins being based on the orthophthalic type The dibasic acid is converted by the THM reaction to the respective dimethyl ester. Chromatograms of a mixture of an orthophthalic alkyd, isophthalic alkyd and polyethylene terephthalate show that each of the phthalic acid isomers can easily be distinguished. (Fig.5.3).

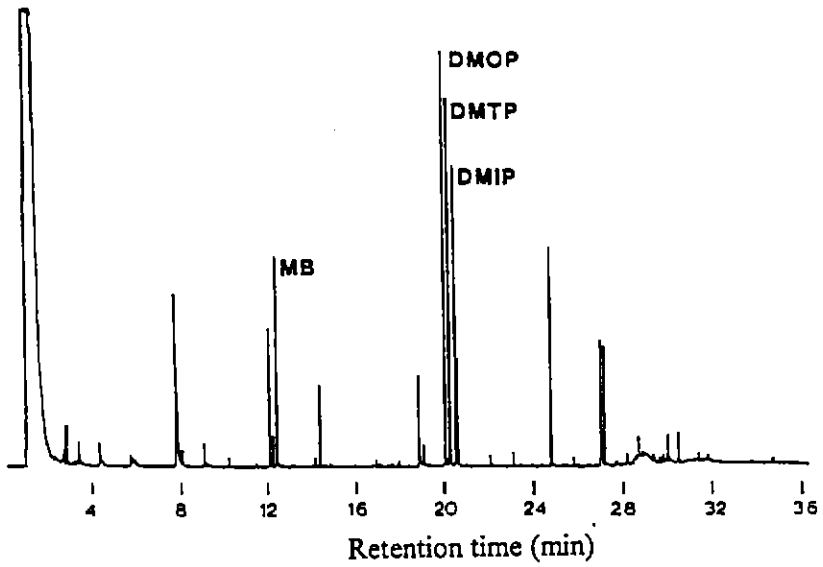


Figure 5.3. Chromatograms showing THM-GC of orthophthalate, isophthalate and terephthalate polymers.

Key: DMOP = dimethyl orthophthalate, DMIP = dimethyl isophthalate, MB = methyl benzoate, DMTP = dimethyl terephthalate.

Benzoic acid is converted to methyl benzoate. The benzoic acid originates from decarboxylation of phthalic acid or as a modifying agent e.g. chain stopper.

Unfortunately, the yield obtained in the conversion of the phthalic acid to the dimethyl ester is variable. The reasons for this are still under investigation, but it could be due to varying losses of dibasic acid by decarboxylation.

5.4.3 Drying Oil Identification

The drying oil types which have been identified using the THM reaction include linoleic acid rich types (soya bean oil, safflower, sunflower), uncured linolenic rich (linseed), ricinoleic rich (uncured castor, hydrogenated castor), marine oils and coconut oil types. The products of the reaction are long chain saturated fatty acid methyl esters ranging from methyl octanoate to methyl octadecanoate and higher molecular weight methyl esters. Unsaturated fatty acid methyl esters are also detected. The distribution of the methyl esters reflects the composition of the drying oil. Figure 5.4 shows examples of typical THM chromatograms obtained from the five different classes of modifying drying oils. The chromatogram obtained from the soya bean alkyd is typical of a linoleic-rich alkyd. Little remains of the linoleic acid derived portion in the cured resin due to crosslinking. However, compounds eluting after C18.0 have been identified as methyl esters of octadecenoic acid, and these are considered to be due to thermal isomerisation of any remaining linoleic acid. A characteristic of this soya bean alkyd is the high proportion of azelaic acid (nonanedioic acid) methyl ester which is obtained. Uncured linolenic rich alkyds e.g., linseed oil, are characterised by the presence of a significant proportion of octadecatrienoic acid methyl ester, which has been thermally isomerised to the all-*trans*- form. On curing this disappears and the drying oil loses some of its identity, although the ratio of palmitic to stearic acid is characteristic of linseed oil. Hydrogenated castor oil alkyd resin, a typical ricinoleic rich alkyd, is characterised by the presence of a compound which has been tentatively identified as ricinoleic acid methyl ester. Fish oil alkyd resins are characterised by pamitoleic acid, C16.1, present

in varying proportions depending on the degree of cure, and a significant concentration of tetradecanoic acid. Coconut oil alkyds have a fatty acid composition typical of lauric acid oils with significant proportions of octanoic (caprylic), decanoic (capric), dodecanoic (lauric) and tetradecanoic (myristic) acid methyl esters.

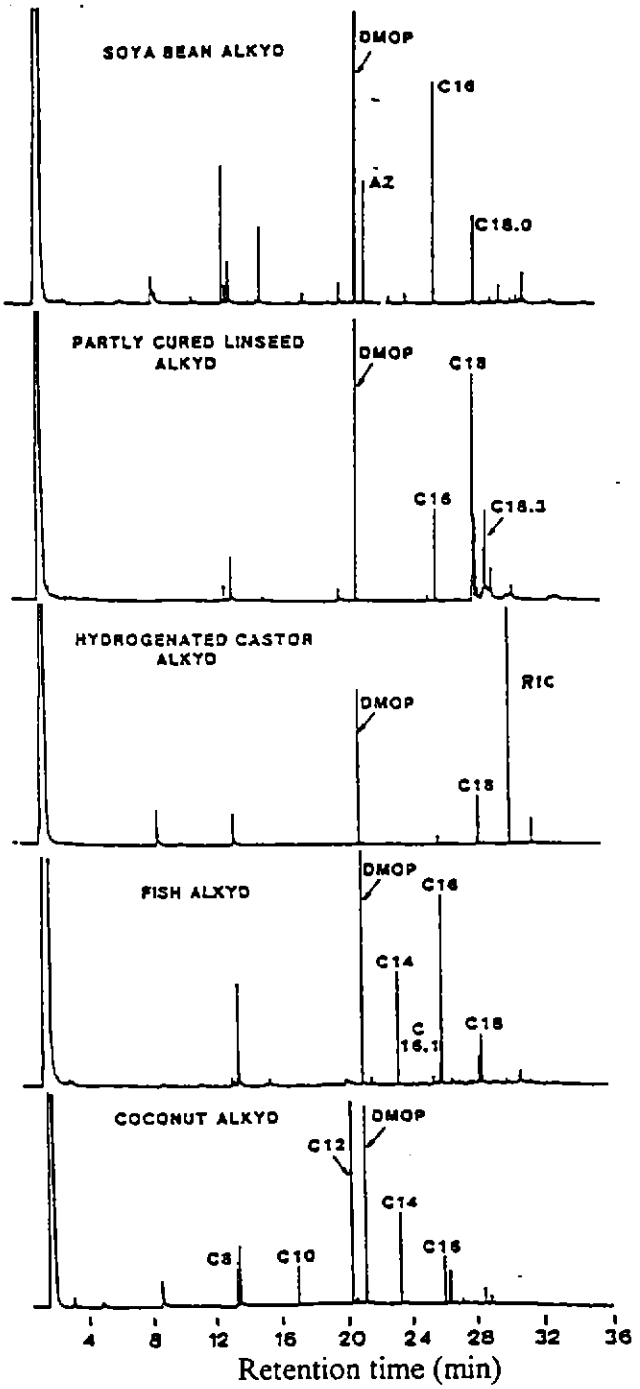


Figure 5.4. Chromatograms showing THM-GC of five orthophthalic alkyd resins having different drying oil modification.

Key: DMOP = dimethyl orthophthalate, C8, C10, C12, C14, C16, C18 = respective

alkanoic acid methyl esters, C18 3 = octadecatrienoic acid methyl ester, RIC = ricinoleic acid methyl ester.

5.4.4 Degree of Cure

The relative proportion of unsaturated to saturated fatty acid methyl esters gives an indication of the degree of cure, or age, of the alkyd resin. Linseed oil alkyd resins, for example, have a high proportion of (Z,Z,Z)-9,12,15-octadecatrienoic acid (linolenic acid), (Z,Z)-9,12-octadecadienoic acid (linoleic acid) and (Z)-9-octadecenoic acid (oleic acid) present in the uncured resin. The chromatograms in Figure 5.5 show the trends which developed in a five month trial in which a linseed oil- pentaerythritol- orthophthalic alkyd resin was allowed to cure indoors at room temperature. The results show that:-

(i) before curing commences the relative proportion of linolenic acid, C18.3, to the saturated palmitic acid, C16.0, is significant. After two days of curing the linolenic acid disappears due to rapid cross linking with other sites of unsaturation in the resin.

(ii) after two weeks the proportion of oleic acid C18.1 (*trans* isomer) to stearic acid C18.0 is similar but slowly reduces with time until the oleic acid falls to approximately one-third of its original concentration after about four months.

(iii) azelaic acid (nonanedioic acid) begins to appear in the reaction products after three days cure and increases to a maximum after one month.

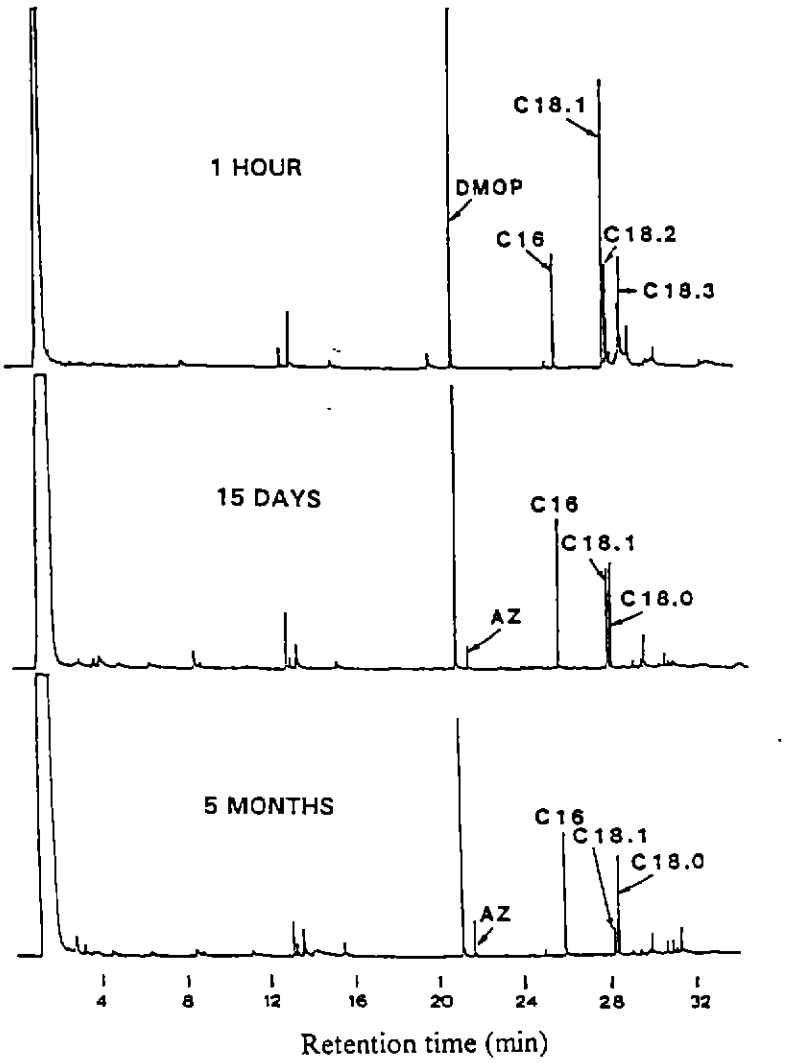


Figure 5.5. Chromatograms showing THM-GC of a linseed oil alkyd resin cured over a 5 month period showing the variation in unsaturated fatty acids with time.

Key: C16.0, C18.0, C18.1, C18.2, C18 3 = respective alkanoic and alkenoic acid methyl esters. AZ=azelaic acid methyl ester

Similar results are observed in other alkyd resins which have unsaturated drying oils present. Linoleic rich alkyds exhibit a gradual reduction in concentration of linoleic acid, (C18.2), and oleic acid, (C18.1) with time. Fish oil alkyds lose palmitoleic acid, (C16.1), and oleic acid, (C18.1). This procedure of following the reduction in

concentration of unsaturated acids and appearance of azelaic acid is therefore useful for estimating the age of an alkyd paint for up to about four months.

5.4.5 Oil Length

The oil length of an alkyd resin is the percentage of fatty acid triglyceride present in the total solid resin. During the course of these studies it became apparent that it was possible to obtain an approximation of the oil length of an alkyd resin by comparing the ratio of products from the drying oil to the total products from the THM reaction. Alternatively, the proportion of aromatic products (from phthalic acid) to aliphatic carboxylic acid products reflects the oil length of the resin. The results of THM on a short oil and a long oil alkyd resin are shown in Figure 5.6.

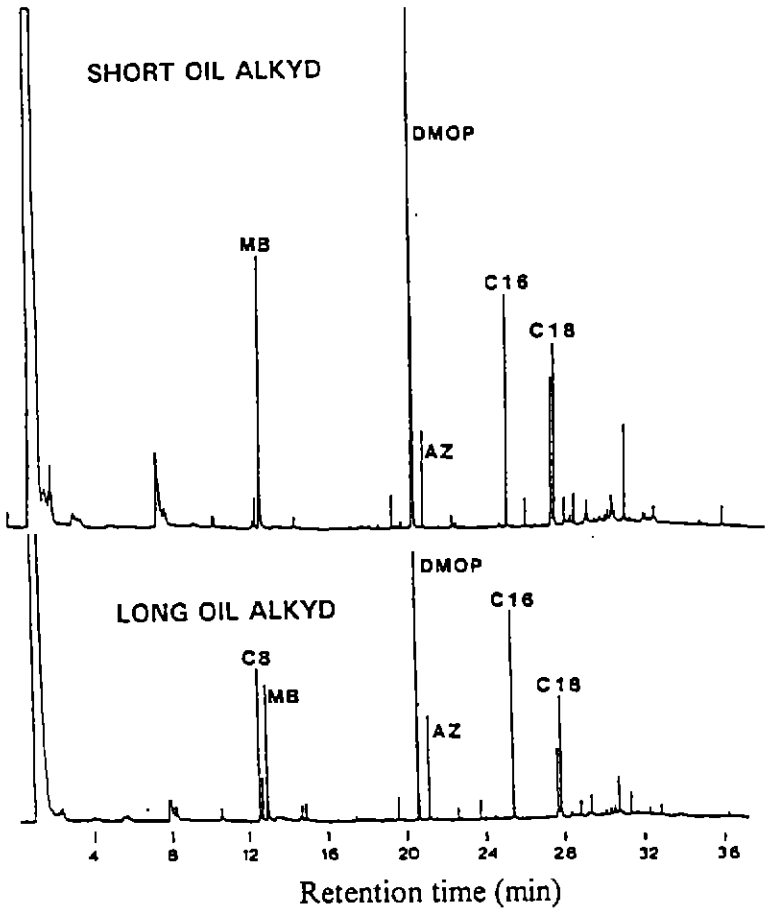


Figure 5.6. Chromatograms showing THM-GC of short oil and long oil alkyd resins. *Key*: DMOP = dimethyl orthophthalate, MB = methyl benzoate, C8, C16, C18 = respective alkanoic acid methyl esters, AZ = azelaic acid methyl ester.

As discussed previously, phthalic acid undergoes some decarboxylation, giving variable yields of the dimethyl ester, and consequently, the results are not quantitative. However, some indication of oil length is possible by the application of this method

5.4.6 Modified Alkyds

Alkyd resin modifiers which are amenable to alkaline hydrolysis and alkylation include wood rosin and epoxy resin. The presence of these modifiers can be detected by THM. Rosin contains a high proportion of abietic acid (Structure of methyl ester XV, Chapter 6, section 6.4.3) and related compounds including dehydroabietic acid (Structure of methyl ester XIV, Chapter 6, section 6.4.3).

The methyl ester of dehydroabietic acid is detected in THM-GC analysis of a rosin modified alkyd resin. However, it is not possible to detect the presence of rosin in alkyd resins by Py-GC.

Epoxy constituents in alkyd resins are converted to methyl ethers of phenol, isopropenyl phenol and bisphenol-A. Figure 5.7 shows examples of chromatograms from the THM of the reaction of rosin modified and epoxy modified alkyd resins.

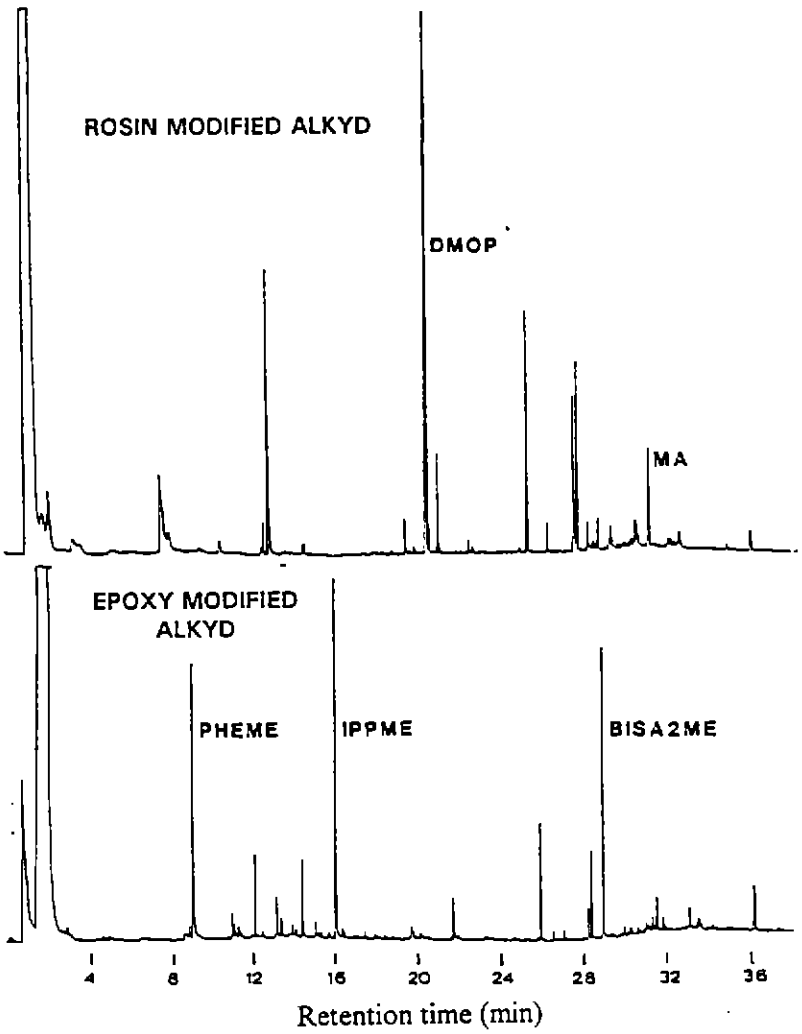


Figure 5.7. Chromatograms showing THM-GC of rosin modified and epoxy modified alkyd resins.

Key: DMOP = dimethyl orthophthalate, MA = methyl abietate, PHEME = phenol methyl ether, IPPME = isopropenyl phenol methyl ether, BISA2ME = bisphenol-A dimethyl ether.

5.5 SUMMARY.

THM-GC analysis of alkyd resins gives chemical structure information about the identity of polyol, drying oil and modification which is not readily obtainable by conventional Py-GC. The derivatised products more directly reflect the components of the polymer. The amount of the remaining unsaturation in the drying oil gives an

indication of the degree of cure, and the proportion of aromatic to aliphatic products reflects the oil length of the resin.

CHAPTER 6

CHARACTERISATION OF ROSIN - BASED COMMERCIAL RESINS BY PYROLYSIS- AND THM - GAS CHROMATOGRAPHY - MASS SPECTROMETRY

6.1 ABSTRACT

Rosin-based resins are utilised commercially in printing ink, and adhesive and coating applications. They may incorporate polyhydric alcohols and polybasic acids, and are often modified with phenol-formaldehyde resins. The composition of *tert*-butyl phenol- and *p*-nonyl phenol-formaldehyde condensates and their rosin ester derivatives have been determined by Py-GC and THM-GC-MS. The corresponding mass spectral data of these resin precursors are also reported.

6.2 INTRODUCTION

Blends of rosin-containing natural resins and synthetic components are used to manufacture a wide range of commercial products which include printing inks, floor polishes, adhesives and surface coatings. The identification of these commercial products is necessary for product development, quality control, and forensic science and material science investigations.

Rosin is derived commercially as wood rosin from the stumps of pine trees and as gum rosin from the tapping of living trees. The amber coloured solid consists of approximately ninety per cent rosin acids and ten per cent neutral material (Keutgen, 1969; Leach, 1988). The principal acid, abietic acid, (Structure of methyl ester XV, section 6.4.3) contains conjugated diene and carboxylic acid functionality which can be reacted on a commercial scale to prepare products having improved properties such as adhesion, gloss, hardness, or plasticiser function.

Rosin esters are produced by esterifying the carboxylic acid group with polyhydric alcohols, such as glycerol and pentaerythritol, to give resins of lower acid value and higher melting point (Keutgen, 1969). Commercially useful resinates are prepared by reaction of the rosin acids with zinc and calcium oxides. Cobalt, lead and manganese oxides are reacted with the rosin acids to give resinate driers. Plasticisers, used in some lacquers, can be produced by hydrogenating the unsaturated sites in rosin and rosin esters to give products which are less susceptible to oxidation. The diene function is also reacted to produce dimers which have the same acid value but a

higher melting point. The unsaturated function provides sites for Diels-Alder addition reactions with polybasic compounds such as maleic anhydride, fumaric acid and trimellitic anhydride. They are also reacted with phenol-formaldehyde resins to convert them to oil soluble resins, which are of particular use in the printing ink industry (Leach, 1988).

Py-GC has been used as an identification technique for phenol-formaldehyde resins. The pyrolysis product distribution of phenol-formaldehyde condensates has been investigated by Py-GC (Blaszo and Toth, 1986). The proportion of alkylbenzenes and alkylphenols in the pyrolysis products were related to the structure of the polycondensate. The sequence of phenolic units in phenol formaldehyde condensates have also been studied by Py-GC-MS (Blaszo and Toth, 1991). The distribution of phenols in the pyrolysate reveals the presence of terminal phenolic units coupled at different positions by the methylene bridges.

The Py-GC profiles of rosin-modified phenol-formaldehyde condensates may be expected to be more complex because of the polyhydric alcohols and diterpenoid fatty acids present in the formulated products. As observed in previous chapters, ester linkages in this type of product , such as those in abietic acid esters, would be hydrolysed and methylated in the THM reaction to form methyl derivatives. In particular, the THM reaction is useful to determine the presence of rosin-phenolic modification in some alkyd resins (Chapter 5). The carboxylic acids and polyol composition of polyesters, fats, vegetable oils and oil shales have been identified (Chapter 4). Some natural resins, including rosin containing materials such as amber, have been examined for forensic purposes by the THM technique (Challinor, 1990). The identification of resins and resinites in the geosphere has been undertaken using pyrolysis techniques, including the THM technique (Anderson and Winans, 1991).

This chapter describes the characterisation of commercial rosin based-resins and their precursors by Py-GC-MS and THM-GC-MS techniques.

6.3 EXPERIMENTAL

The methods and conditions used in this study were the same as those which were described in Chapter 2 (Section 2.2).

Materials.

Resins were industrial grade quality, obtained from A.C. Hatrick Chemicals Pty. Ltd.. and CETEC Pty. Ltd., Melbourne.

6.4 RESULTS AND DISCUSSION

The rosin based commercial resins are reaction products formed from polyhydric alcohols, polybasic acids, rosin acids, and modifying agents including phenol-formaldehyde resins. These individual precursors were initially examined by THM-GC-MS in order to determine their GC characteristics and the individual mass spectra of the methyl derivatives.

6.4.1 Polyhydric Alcohols

Glycerol and pentaerythritol are the most commonly used polyols in rosin esters. The THM reaction of the glycerol unit results in the formation of the corresponding glycerol trimethyl ether (G3ME), as described in Chapter 5.

THM of the pentaerythritol group gives the pentaerythritol tetramethyl ether (P4ME) and the pentaerythritol trimethyl ether (P3ME). Both of these compounds are detected in pentaerythritol-based products and their relative proportion does not seem to have any particular significance. This phenomenon has been observed in the THM reaction of alkyd resins (Chapter 5). Bisphenol-A is also used as a starting material in some resins and the monomethyl and dimethyl ethers of bisphenol-A (BISA1ME and BISA2ME) are found as products in the THM reaction of those resins.

The THM-GC profile of a prepared mixture of glycerol, pentaerythritol and bisphenol-A is shown in Figure 6.1.

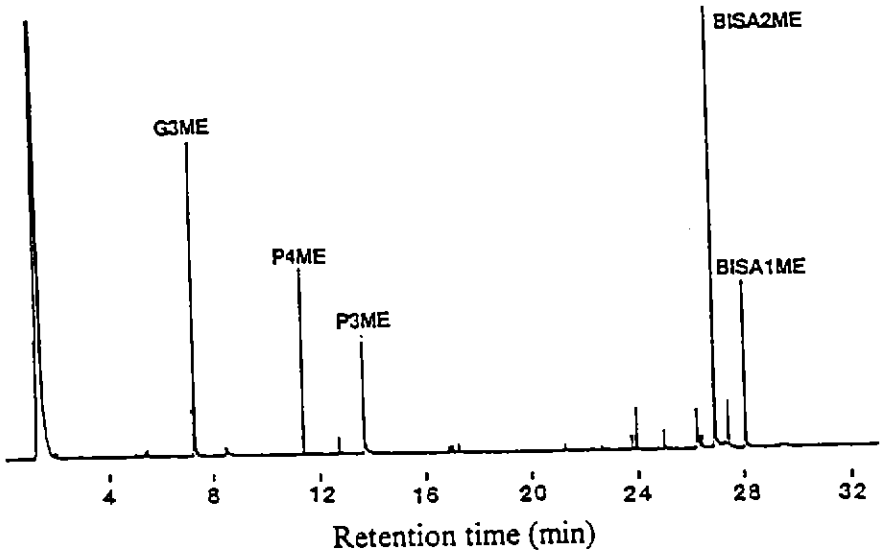


Figure 6.1. Chromatogram showing THM-GC of a mixture of polyhydric components found in rosin modified resins. The acronyms are defined in the above text.

Trihydroxymethylpropane and trihydroxymethylethane are known to have been used in some rosin-based products. These reactants have not been detected as pyrolysis products of resins in this study.

6.4.2 Polybasic Acids

The polybasic compounds used in commercial resins of the type used in this study include maleic anhydride, trimellitic anhydride and phthalic acids. The detection of these compounds by conventional Py-GC is difficult because of their polar nature. However, the acids and anhydrides which are present as esters in the resins may be converted by the THM reaction to methyl esters, which are more efficiently chromatographed.

The THM-GC profile of a prepared mixture of these acids, detected as their methyl esters, dimethyl fumarate (DMF), dimethyl maleate (DMM), dimethyl orthophthalate (DMOP), dimethyl isophthalate (DMIP), and the trimethyl ester of trimellitic acid (TMA3ME) is shown Figure 6.2. Methyl benzoate (MB) is also detected as a partly decarboxylated by-product of the polybasic aromatic acids. Dimethyl fumarate (DMF) might be expected to result from *cis-trans* isomerisation of methylation derivatives of maleic acid.

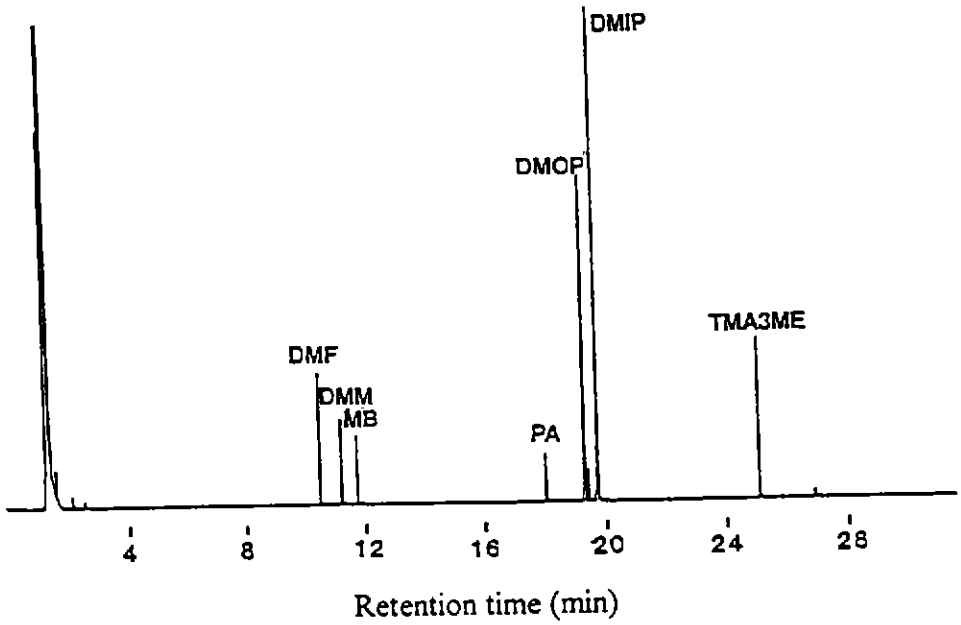


Figure 6.2. Chromatograms showing THM - GC of polybasic acids typically found in rosin modified resins. The acronyms are defined in the above text.

6.4.3 Rosin Acids

Wood rosins, which contain a high proportion of diterpene carboxylic acids, have been characterised by THM-GC and their components identified by mass spectrometry (Challinor, 1990b). Wood rosin is also combined with other reactants to produce commercial resins. The components of five rosins from different sources have been identified by this technique. The THM-GC profiles are shown in Figure 6.3. The compounds were identified as rosin acid methyl esters (RAMES) by comparison with standard mass spectra (EPA/NIH and NBS mass spectra libraries and mass spectral data reported in the work by Anderson and Winans, (1991)).

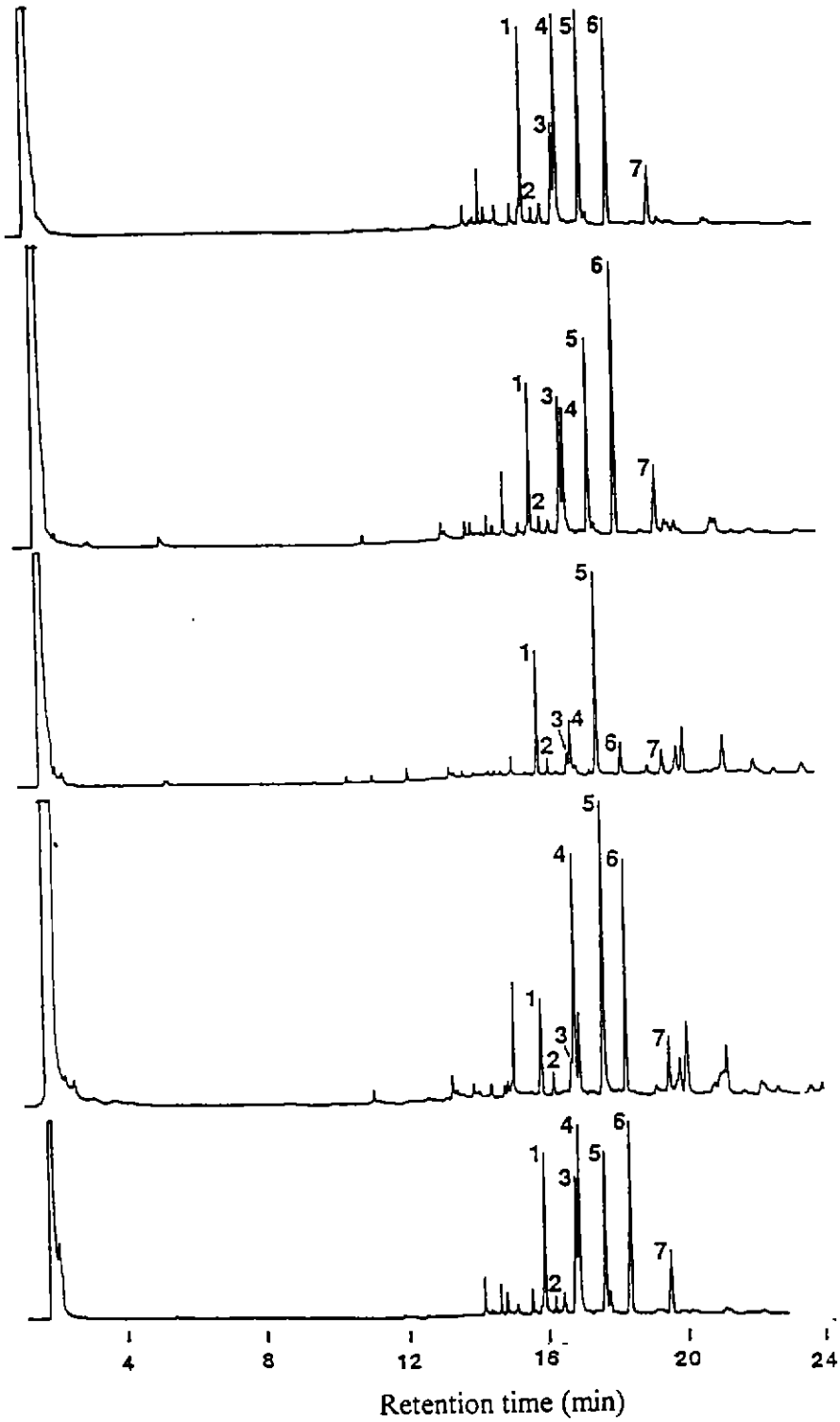


Figure 6.3. Chromatograms showing THM - GC profiles of five wood rosins. Mass spectra of the compounds represented by peaks 1 to 7 are given in Fig. 6.4.

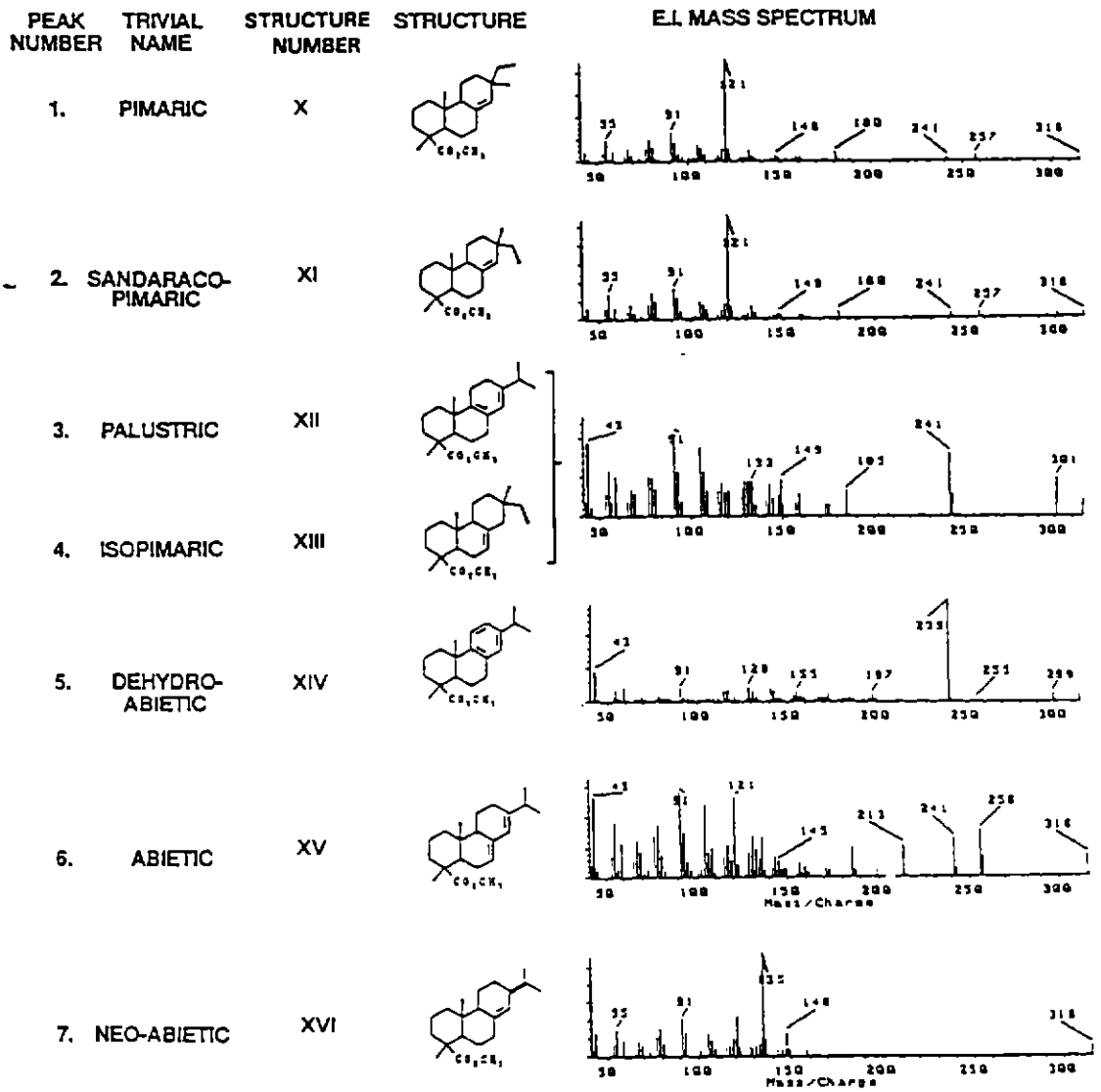
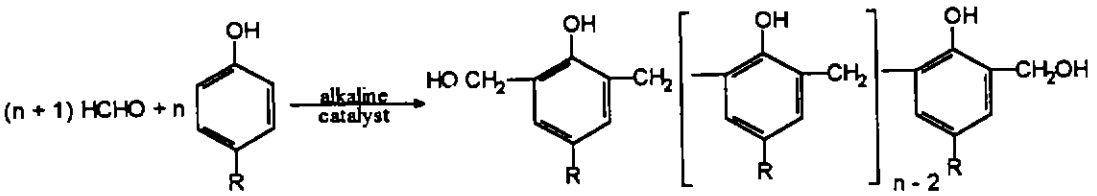


Figure 6.4. Molecular structures and mass spectra of rosin acid methyl esters (RAMES) present in wood rosins. Peak numbers refer to Fig. 6.3.

Peaks 3 and 4 (Figure 6.3) were not resolved in the total ion chromatogram obtained from the THM-GC-MS examination of the five rosins

6.4.4 Phenolic Resins

Phenol-formaldehyde resins may be used either alone, or modified with rosin and a polyol, to prepare commercial resins for formulating adhesives, printing inks and coatings. The unmodified phenolic resins incorporate a *para*-substituted alkylphenol, which for oil soluble resins have an alkyl group R, more than four carbon units long. Resins produced with an alkaline catalyst result in a product having free hydroxymethyl groups, which may be reacted with unsaturated sites in materials such as tung oil or rosin:



The commercial phenolic resins examined in this study have C4 and C9 alkyl groups in the *tert*-butylphenol (TBP) -formaldehyde resin, and *p*-nonylphenol (PNP) - formaldehyde resins.

Py-GC and THM-GC chromatograms of a TBP-formaldehyde resin are shown in Figure 6.5. Py-GC of this unmodified TBP-formaldehyde resin gives a chromatogram showing peaks for TBP (peak 2) and methyl and dimethyl substituted TBP isomers (peaks 3 and 4). Xylene isomers (peak 1) probably originate from the residual solvent present in the resin. The mass spectral data of the compound corresponding to peak 5 suggested an alkyl-substituted TBP, whilst peak 6 was consistent with an hydroxymethyl, ethyl-TBP isomer. The THM reaction of the TBP-formaldehyde resin results in the formation of the respective methyl ethers of TBP (peak 7), a methyl TBP isomer (peak 8) and a dimethyl TBP isomer (peak 9), which elute before the free phenols Peaks 10 and 11 have not been identified but may be related to peaks 5 and 6.

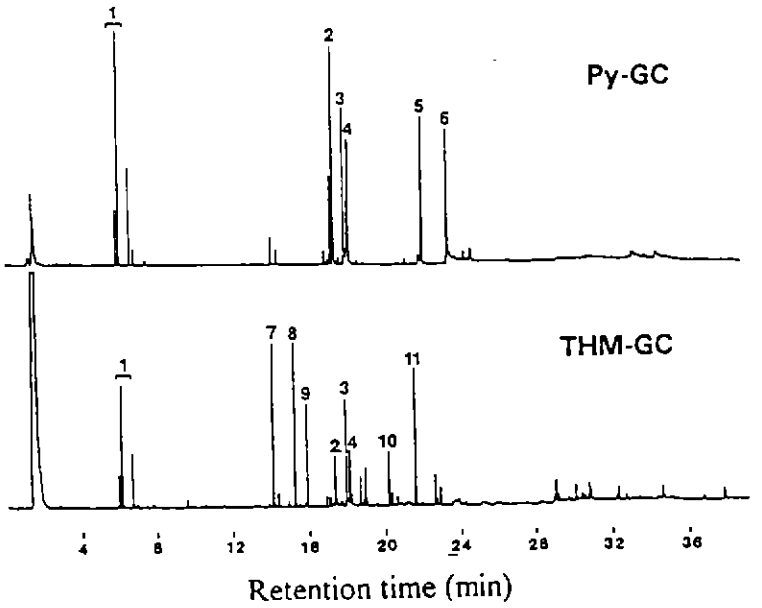


Figure 6.5. Chromatograms showing Py-GC and THM-GC of a TBP - formaldehyde resin. The identity of the peaks are detailed in the above text

The Py-GC data indicates that it may be possible to determine the proportion of hydroxymethyl containing end-groups in the phenol-formaldehyde polymer used for preparing modified resins.

Py-GC and THM data for a PNP - formaldehyde resin are shown in Figure 6.6. Py-GC of this unmodified PNP - formaldehyde resin results in a complex mixture of branched chain nonenes (peaks 1), free *p*-nonylphenols (peaks 2), and the methyl substituted *p*-nonylphenols (peaks 3). THM reaction of the same resin gives an additional corresponding group of *p*-nonylphenol methyl ethers (peaks 4), and methyl substituted *p*-nonylphenol methyl ethers (peaks 5). The mixture of *p*-nonylphenol methyl ethers which were detected have electron impact (EI) mass spectra which include major ions m/z 121, 135, 149 and 163 and a molecular ion, m/z 234.

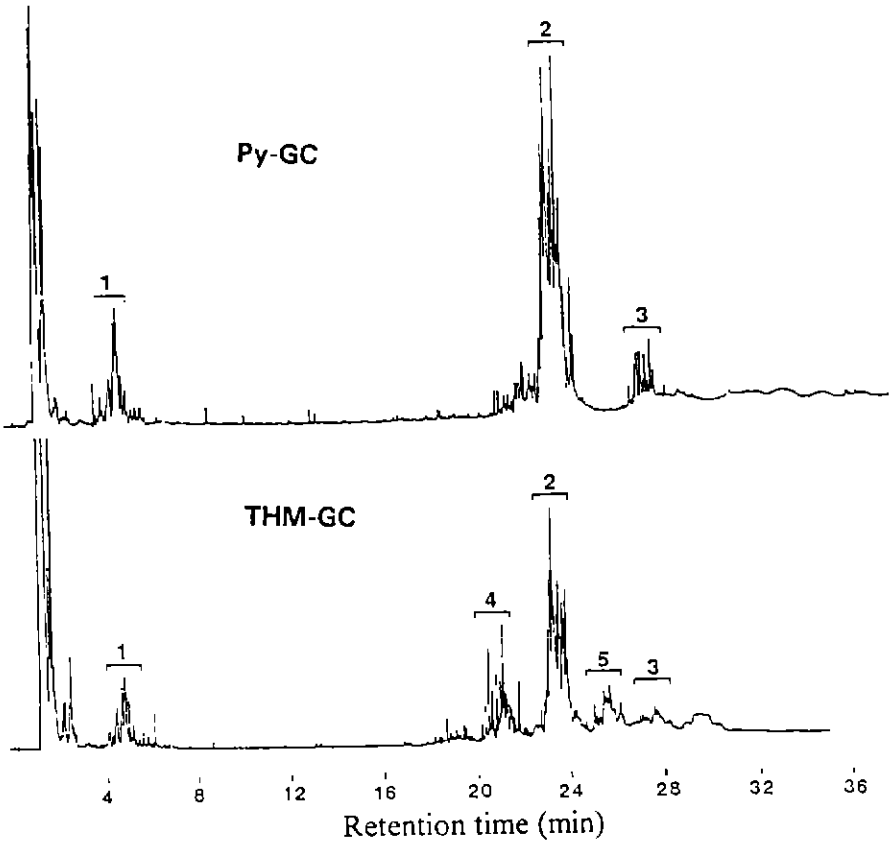


Figure 6.6. Chromatograms showing Py-GC and THM-GC of a PNP-formaldehyde resin. The identity of the peaks are detailed in the above text.

6.4.5 Phenol Formaldehyde Modified Rosin Esters

When the phenol-formaldehyde condensate, P, is combined with rosin esters, R, an idealised structure for the rosin modified phenolic resin, having pentaerythritol as polyol, is shown in Figure 6.7.

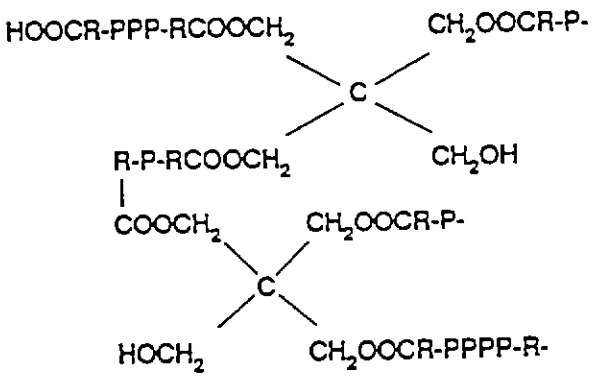


Figure 6.7. An idealised structure for a rosin modified phenolic resin, having pentaerythritol as polyol.

The Py-GC and THM-GC profiles of Resin A, a *p*-tertiary butyl-phenol (TBP) formaldehyde condensate - modified pentaerythritol rosin ester is shown in Figure 6.8. Py-GC gives a clear indication of the substituted phenol composition of the resins. A mixture of tertiary butylphenols, TBP (peak 1), methyl substituted TBPs (peak 2) and dimethyl TBPs (peak 3) are detected in resin A, reflecting a similar composition to that displayed in the Py-GC profiles of the unmodified phenol-formaldehyde condensate (Figure 6.5). The complex group of compounds eluting later are pyrolysis products of the rosin acids. The THM-GC profile of Resin A indicates the presence of RAMES, with dehydroabietic acid and abietic acid methyl esters predominating (peaks 10 and 11, respectively). The polyol in the resin is identified as pentaerythritol by the presence of the tetra- and tri-methyl ethers (peaks 5 and 6, respectively). Dimethyl fumarate (peak 4) is also detected in the TBP resole modified rosin indicating the presence of maleic or fumaric acid. A proportion of TBP, methyl and dimethyl TBP methyl ethers (peaks 7, 8 and 9, respectively) are formed in the THM reaction of resin A.

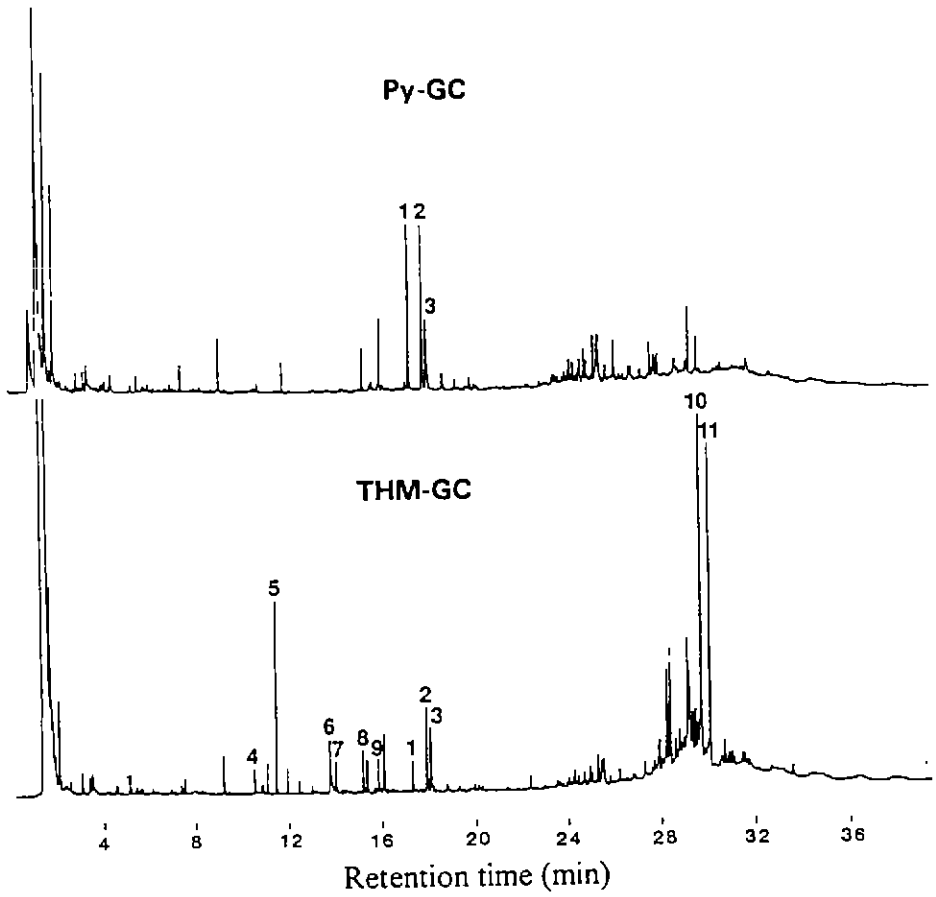


Figure 6.8. Chromatograms showing Py-GC and THM-GC of a TBP-formaldehyde modified rosin ester, Resin A. The identity of the peaks are detailed in the above text.

Py-GC and THM-GC profiles of Resin B, a PNP-formaldehyde condensate-modified pentaerythritol rosin ester, are shown in Figure 6.9. Py-GC of Resin B gives a mixture of *p*-nonylphenols (peaks 1), similar in general profile to the PNP-formaldehyde condensate (Figure 6.6). THM-GC profiles of both resins indicate the presence of RAMES with dehydroabietic acid methyl ester (peak 5) and abietic acid methyl ester (peak 6), predominating. The polyol in both resins is identified as pentaerythritol by the presence of the tetra- and trimethyl ethers (peaks 2 and 3, respectively). Substituted PNP methyl ethers (peaks 4) are formed in the THM reaction of Resin B. The smaller proportion of methyl ethers formed in the THM reaction contrasts with the results of the THM reaction of the free phenols, TBP and PNP, where conversion to the respective methyl ethers is significant.

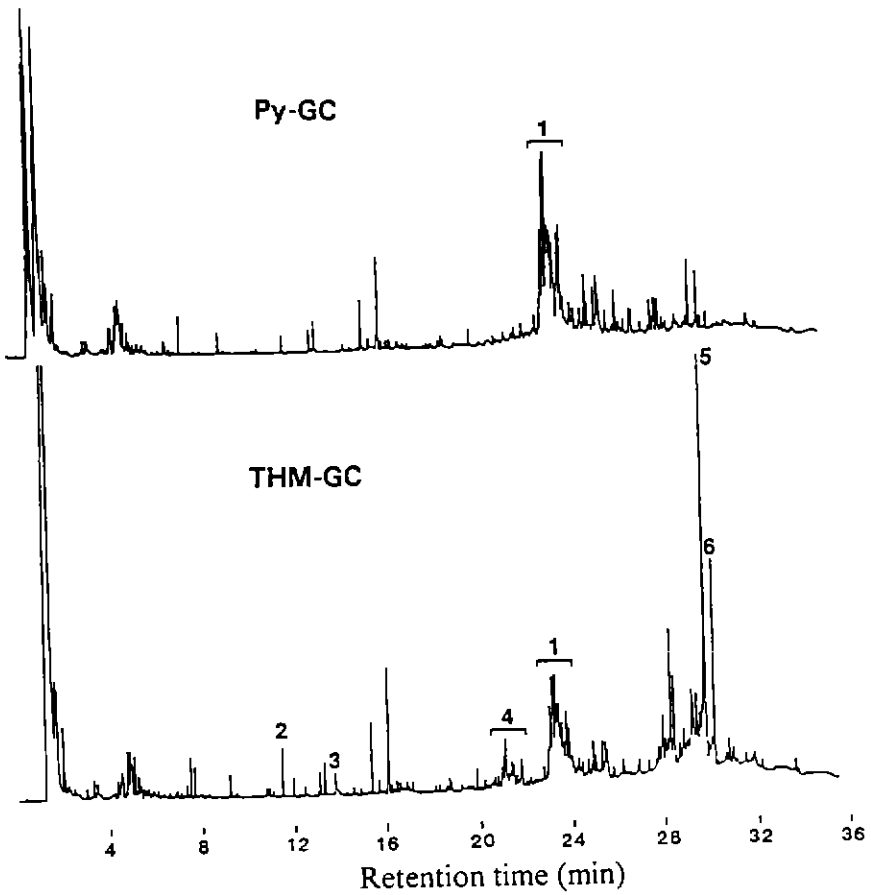


Figure 6.9 Chromatograms showing Py-GC and THM-GC of a PNP-formaldehyde rosin ester, Resin B. The identity of the peaks are detailed in the above text.

6.5 SUMMARY

Commercial rosin-based synthetic resins are formulated using reactants which may include varying proportions of polyols, polybasic acids, phenol-formaldehyde condensates and rosin of wood origin. Pyrolysis techniques have been shown to be of value in the characterisation of these resins, and the identification of the resin precursors. Py-GC reveals the composition of *tertiary*-butyl and *para*-nonyl phenol condensate modification in rosin-based resins. Thermally assisted hydrolysis and methylation gives more complex profiles. However, the THM reaction assists in the identification of the polyol, the polybasic acid and RAMES in the modified rosin esters.

CHAPTER 7

THE CHARACTERISATION OF FATTY ACIDS IN TRACE QUANTITIES OF LIPIDS

7.1 ABSTRACT

The scope of a rapid derivatisation method was examined for the identification of the fatty acid composition of lipids by flash heating with aqueous TMAH. The FAMES were identified by GC-MS. The THM pyrolysis derivatisation method enabled a distinction to be made between a number of vegetable oils and animal fats. The samples included human skin surface lipids, vegetable oils and waxes in cosmetics, wax esters in jojoba oil and fatty acids in woolgrease, a source of lanolin. The problems of base catalysed / thermal isomerisation of polyunsaturated fatty acids in linseed oil were also addressed. The procedure was suitable for submicrogram quantities of lipid materials and did not require any preliminary separation or derivatisation steps.

7.2 INTRODUCTION.

Fatty acids are present in saturated and unsaturated fats and oils, waxes, oil shales, soaps, biological tissue and the phospholipids in plants and animals. They originate in the cytoplasm of the plant or animal, where they mainly function as a reserve food source. Fatty acids have nutritional importance in the human diet for preventing many disorders. New roles for biologically important fatty acids are still being discovered. For example, γ-linolenic acid in evening primrose oil has been said to be beneficial to health and polyunsaturated fatty acids (PUFA) in fish oils and certain plants may prove important agents in controlling cardiovascular and inflammatory diseases. The function, biosynthesis, taxonomical significance and analysis of fatty acids in bacteria are also being studied (Kaneda, 1991; Welch, 1991; Vasyurenko and Frolov, 1986). Petroleum geochemical studies of oil shales reveal the existence of fatty acids derived from algae, plant or bacterial origin. The composition of the fatty acids may be expected to give an indication of the age or maturity of potential petroleum deposits. Natural fats and oils vary widely in their physical properties, even though they are made up of identical or similar fatty acids. The reasons for this are (a) the structure of individual component triglycerides vary and (b) the proportion of component fatty

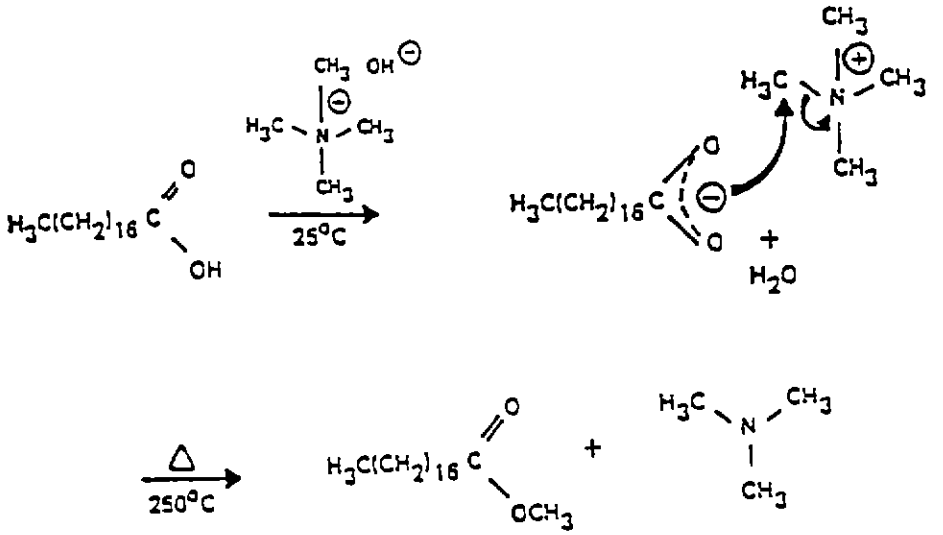
acids in each fat and oil varies over a wide range (Swern, 1979). Factors which are known to affect the composition of vegetable fats and oils are climatic (temperature and humidity), soil type, geography, maturity, health and other criteria such as proximity to certain molds, bacteria and enzymes.

There are several procedures for the determination of fatty acids in triglycerides by GC (Christie, 1990). The generally accepted standard method involves the saponification and acid catalysed methylation of the triglycerides. The methyl esters are analysed by GC Boron trifluoride is the most commonly used acid catalyst (Metcalfe *et al.*, 1966). One disadvantage of the multi-step procedure is the partial loss of lower molecular weight methyl esters by evaporative processes. These losses were avoided in an improvement which used iso-octane in the extraction step (Bannon *et al.*, 1982).

A base catalysed transesterification procedure using methanolic TMAH was also developed (Metcalfe and Wang 1981), in which the methyl esters were formed immediately in the ether layer. Any free fatty acids, collected in the separated glycerol layer, were converted to the TMAH salts which were alkylated pyrolytically to the methyl esters. Trimethylsulphonium hydroxide (TMSH) has also been used as a transesterification reagent (Butte 1983), and has the advantage that any by-products cause no interference and the reagent is relatively cheap. TMAH transesterification procedures have been applied to the analysis of animal tissue in which capillary column GC was used (Misir 1985), and have been used in the analysis of milk fats (Martinez-Castro *et al.*, 1986).

This "pyrolytic methylation" procedure, using a quaternary ammonium hydroxide, e.g. TMAH, to convert free acids to methyl esters, involves the injection of the free acid and the quaternary ammonium hydroxide into the injector of the GC. The quaternary ammonium salt of the free acid undergoes thermal decomposition in the injector to the methyl ester. The applications of the technique have been reviewed (Kossa *et al.*, 1979) and the experimental parameters studied (Abraham and Criddle, 1985). In the case of the reaction of TMAH with stearic acid, the mechanism of the thermal

decomposition of the TMAH salt involves nucleophilic attack on the tetramethylammonium cation by the carboxylate anion (Kossa *et al*, 1979) as follows -



The procedure has the advantage of a one-step esterification process and consequently avoids losses of the more volatile lower molecular weight methyl esters. One disadvantage of this method is that the saponification of triglycerides must be undertaken, prior to conversion to methyl derivatives. Further, PUFAs e.g. linoleic and linolenic acids, undergo base catalysed / thermal isomerisation to give conjugated polyenes, resulting in reduced yields of the polyunsaturated acids (Downing and Green, 1968). The reaction, which is an electrophilic substitution with an accompanying allylic rearrangement, is exploited in the production of conjugated fatty acid methyl esters (FAMES) in a microreactor apparatus using methanolic TMAH (Bitner *et al.*, 1971).

Attempts have been made to prevent this isomerisation by removing the excess TMAH prior to injection. This was achieved by neutralisation of the excess TMAH with acetic acid (Downing and Green 1968). Methyl propionate has also been used for this purpose (MacGee and Allen 1974) with trimethyl(trifluoro-m-tolyl)ammonium hydroxide (TMTFTH) as derivatising reagent. Methyl acetate was suggested as a substitute to improve the results (Williams and MacGee 1982), allowing a slightly

more basic derivatising reagent, trimethylphenylammonium hydroxide (TMPH), to be used for the saponification.

In Chapter 4, a range of materials including fatty acid triglycerides and waxes were examined using the THM reaction. It was possible to identify the fatty acid and alcohol components in waxes by reacting submicrogram quantities with aqueous TMAH without a prior hydrolysis step. The problem of base catalysed / thermal isomerisation was also experienced in the identification of PUFA methyl esters in linseed oil when this technique was used.

The purpose of the work described in this chapter was to investigate the wider scope of the reaction in order to convert triglycerides directly to fatty acid methyl esters. Attempts to eliminate base catalysed / thermal isomerisation problems were addressed. The THM-GC procedure was used to ascertain the components in traces of human body lipids from fingerprints, cosmetic lotions containing jojoba oil and triglycerides, and complex mixtures of triglycerides in woolgrease, the raw material for the manufacture of lanolin.

7.3 EXPERIMENTAL.

7.3.1 Method

Approximately 100 ng (1×10^{-4} μL) oil or fat was smeared onto the inner surface of a flattened, bent 770° C Curie point pyrolysis wire. Approximately 0.5 μL of 25% aqueous tetramethyl ammonium hydroxide (TMAH) was added to the oil by syringe and stirred with a fine tipped stainless needle to produce an even dispersion. The prepared wire was immediately located in the pyrolyser and duplicate experiments were carried out using the same procedure.

In experiments to monitor changes in FAME composition with curing, linseed oil was coated onto a glass slide as a thin film and allowed to dry in air at room temperature.

7.3.2 Reagents

a) 25% w/w aqueous solution of TMAH (Sigma Chemicals).

b) 0.2 N aqueous TMTFTH (Methprep I, Alltech Chemicals).

7.3.3 Instrumentation

The instrumental conditions were as described in Chapter 2 (Section 2.2) except for the experiments concerning woolwax, where the GC settings were initial temperature 100° C, increase at 4° C min^{-1} to 270° C.

A polar phase phase, 50% cyanopropyl methyl silicone (J&W, DB 23), 30 metre vitreous silica was used for fatty acid methyl ester determinations, where a significant proportion of the acid methyl esters were polyunsaturated.

7.3.4 Materials

Vegetable oils - Sigma Chemicals.

Woolgrease - AWD Holdings. Fremantle, Western Australia.

7.4 RESULTS AND DISCUSSION

Individual vegetable oils may be classed into groups which are rich in particular long chain carboxylic acids such as oleic, linoleic, linolenic, ricinoleic or lauric acid. The THM reaction of vegetable oils and other triglycerides produces a mixture of their component fatty acid methyl esters (FAMES). THM-GC of some of these oils indicates the differentiation that can be achieved on submicrogram quantities without prior sample preparation (Figure 7.1). A high polarity capillary column and 100°C oven programme starting temperature was used for the GC separations.

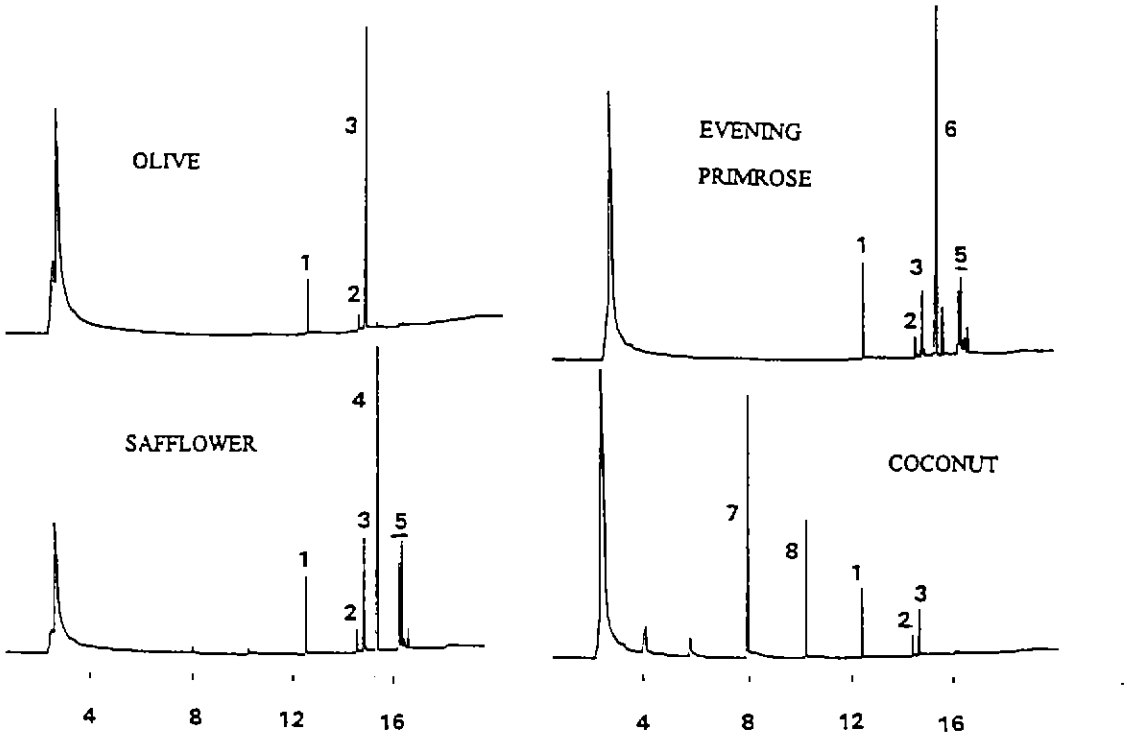


Figure 7.1. Chromatograms showing THM (pyrolysis derivatisation)-GC of olive oil (oleic rich), safflower oil (linoleic rich), evening primrose oil (γ-linolenic rich) and coconut oil (lauric rich) using aqueous TMAH and a 50% cyanopropyl - methyl silicone phase (J&W, DB 23) capillary column.

Key: 1=methyl palmitate, 2=methyl stearate, 3=methyl oleate, 4=methyl linoleate, 5=isomers of methyl linoleate, 6=methyl γ-linolenate, 7=methyl laurate and 8=methyl myristate.

Olive oil and other oleic rich oils were distinguished by a relatively high content of oleic acid methyl ester (peak 3). Palmitic and stearic acid methyl esters, (peaks 1 and 2), were also detected. Linoleic-rich safflower oil, and similarly soyabean, sunflower and dehydrated castor oils, could be recognised by the high proportion of linoleic acid methyl ester (peak 4). Evening primrose oil had a characteristically high γ-linolenic acid (6,9,12-octadecatrienoic acid) methyl ester (peak 6) content. Coconut oil fatty acids contain a high proportion of lauric (C12) and myristic (C14) acid methyl esters (peaks 7 and 8). Some base catalysed / thermal isomerisation of the polyunsaturated fatty acids in safflower and evening primrose oil (peak 5) was observed in the THM procedure.

A further fifteen vegetable oils were examined to determine the discrimination which could be made between these triglycerides. A mid-polarity 17% cyanopropylphenyl methyl silicone phase was used for the separations of the FAMES. The relative abundances of the FAMES, determined from peak heights and expressed as percentages, are tabulated (Table 7.1).

LIPID SOURCE	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C20:0	C22:0	C22:1	OTHER	CLASS
APRICOT KERNEL	-	-	18	<5	59	23	<5	<5	-	-	OL
CANOLA	-	-	<9	<9	91	9	-	-	-	-	OL
CASTOR	-	-	<10	<10	<10	<10	-	-	-	RIC 100	RIC
COCONUT	48	20	10	<5	5	-	-	-	-	C8.0=10 C10.0=10	LAU
COCOA BUTTER	-	-	28	36	36	-	-	-	-	-	ST/OL
CORN	-	-	16	<5	32	52	-	-	-	-	LIN
COTTONSEED	-	-	32	5	18	45	<5	-	-	-	LIN
MUSTARD SEED	-	-	5	<5	≈25	≈15	<5	-	50 ERU	C24:1 <5	ERU
OLIVE	-	-	20	6	62	12	<6	<6	-	-	OL
PALMKERNEL	45	18	14	23	<5	-	-	-	-	-	LAU
PEANUT	-	-	18	<6	59	24	<6	<6	-	-	OL
SAFFLOWER	-	-	16	5	26	53	-	-	-	-	LIN
SESAME SEED	-	-	24	21	34	21	-	-	-	-	OL
SOYABEAN	-	-	15	5	30	50	-	-	-	-	LIN
SUNFLOWER	-	-	11	5	28	56	-	-	-	-	LIN

Table 7.1. Relative abundances of FAMES in vegetable oils determined by pyrolysis derivatisation-gas chromatography from peak heights and expressed as percentages. Key: OL = oleic, RIC = ricinoleic, LAU = lauric, ST = stearic, LIN = linoleic and ERU = erucic. CX.Y: X denotes the carbon chain length, Y denotes the number of sites of unsaturation.

These results are in general agreement to those obtained previously, by GC of FAMES (Swern, 1979). Inspection of the data reveals that lipids may be readily classified and that determinations of the relative proportion of the total fatty acids of varying degrees of unsaturation may also be made.

Linolenic acid-containing vegetable oils, e.g. linseed and evening primrose oil, are preferably chromatographed on a 50% cyanopropyl phase column in order to obtain better resolution of the methyl esters of C18 acids.

The relative proportion of FAMES would be expected to depend on factors such as harvest season, and the maturity of the source plant or animal.

7.4.1 Base Catalysed and Thermally Induced Isomerisation

As discussed in Chapter 4, the THM-GC profiles of lipids containing low proportions of PUFAs compared favourably with the results from conventional transesterification procedures. However, additional compounds were produced in the pyrolysis derivatisation profile where the lipid contained fatty acids with two or more sites of unsaturation, In the case of the THM reaction of linoleic acid, (Z,Z)-9, 12-octadecadienoic acid, MS indicated that these compounds were isomers of the methyl ester of this compound. Linseed and tung oil triglycerides contain a high proportion of triply unsaturated fatty acids, such as linolenic acid, (Z,Z,Z)-9, 12, 15-octadecatrienoic acid. The THM reaction of linseed oil, for example, also gave products which are not detected in conventional transesterification reactions. It appeared that these compounds are produced by base catalysed and thermally induced isomerisation and that they were probably conjugated and *trans- trans-* isomers.

Base catalysed isomerisation may be caused by the effects of excess TMAH with the analyte at the THM reaction stage. Similar problems have been encountered in work on "pyrolytic methylation" of vegetable oils where acetic acid (Downing and Green, 1968), methyl propionate (MacGee and Allen, 1974) and methyl acetate (Williams and MacGee, 1984) were used to neutralise excess quaternary ammonium hydroxide derivatising reagents in an attempt to avoid this isomerisation of PUFAs.

In this present work, the isomerisation of PUFAs could not be eliminated by mixing these reagents with the reactants prior to pyrolysis. The lowering of the pyrolysis temperature did not resolve the the problem of thermal isomerisation.

These artefacts (shown in Figure 4.6) were not produced when TMAH was replaced by aqueous TMTFTH solution (MethPrep 2). This was demonstrated in an experiment to determine the changes in composition occurring in the autoxidative curing of linseed oil. A 50% cyanopropyl - methyl silicone phase (J&W, DB 23) capillary column was used for the separations (Figure 7.2).

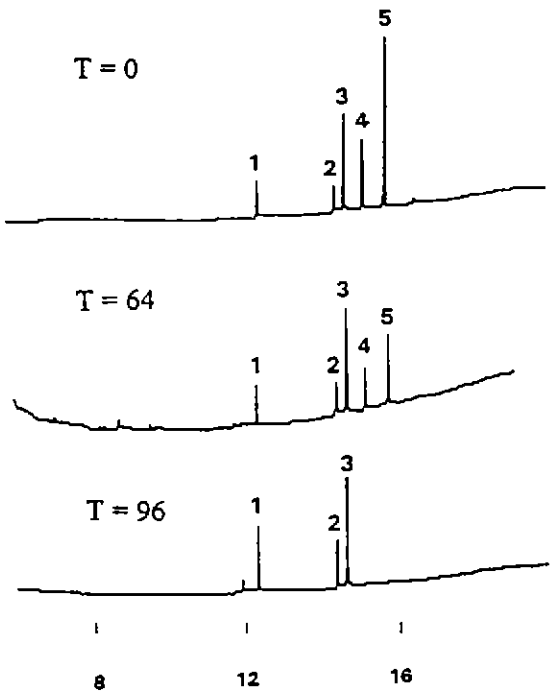


Figure 7.2. Partial-chromatograms (DB 23 capillary column) obtained from pyrolysis derivatisation GC using MethPrep 2 reagent to monitor the curing of linseed oil, cured for 0, 64 and 96 minutes, respectively.

Key: 1=methyl palmitate, 2=methyl stearate, 3=methyl oleate, 4=methyl linoleate and 5=methyl linolenate.

No additional peaks, arising from isomerisation of PUFAs were detected eluting after peak 5. The curing results indicated that there was a progressive loss of free linoleic and linolenic acids with time. After a period of 64 hours, methyl linoleate was reduced by half and methyl linolenate by two-thirds with respect to the saturated FAMES. After 96 minutes, the free PUFAs had been completely reacted by crosslinking, in the curing process.

During the course of these experiments, it became apparent that yields of FAMES were significantly lower than those obtained using 25% aqueous TMAH for the reaction. The lower yields were attributed to the reduced efficiency of hydrolysis by the MethPrep 2 reagent which is less basic than the 25% aqueous TMAH.

Base catalysed isomerisation was also significantly reduced in an experiment in which a lower concentration of aqueous TMAH was used in the THM reaction. A maximum yield of methyl linolenate and methyl linoleate, and minimum isomerisation product was achieved using an approximately 4% aqueous solution of TMAH.

7.4.2 Human Skin Lipids

Body fat secreted from the human skin through the sebaceous glands has a composition which depends on a number of individual and environmental factors. Studies on the effects of diet (Morello and Downing, 1976), gender (Yamamoto *et. al.*, 1990) and age of the individual (Stewart *et. al.*, 1989) have indicated that such effects produce variations in fatty acid composition. FAME composition may therefore provide an identifying signature for skin lipids in human individuals.

Figure 7.3. shows a comparison of the composition of human body skin fat obtained from the hand surface of two volunteers as determined by THM-GC.

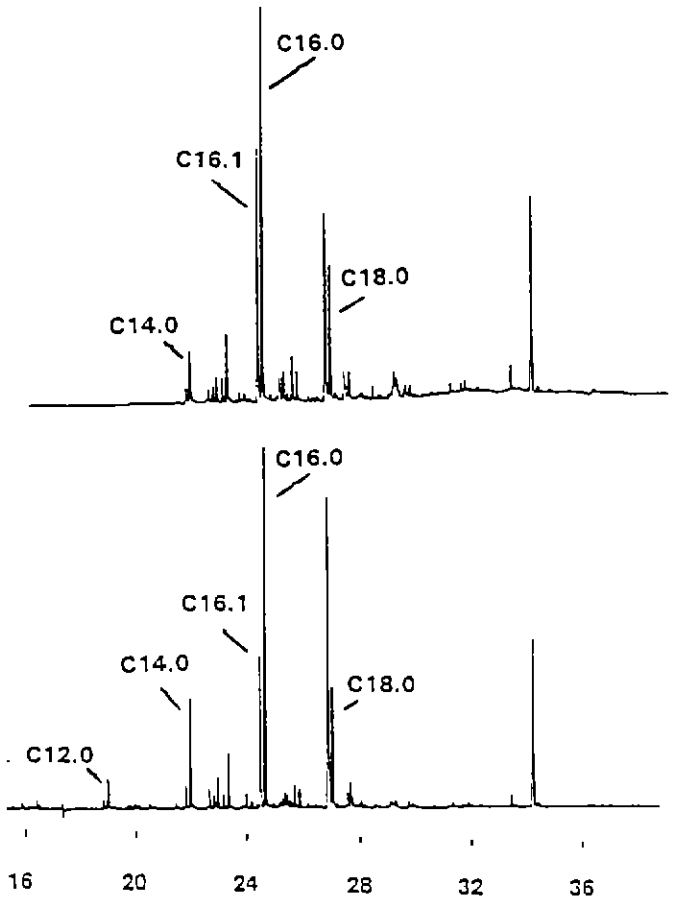


Figure 7.3. Partial chromatogram obtained by pyrolysis derivatisation (THM - GC) of human body skin fat from fingerprints taken from two individuals.

Key:- CX.0 refers to saturated FAMES with carbon chain length, X. CX.1 refers to monounsaturated FAMEs with carbon chain length X. The peak eluting at 34 minutes was identified as squalene.

The relative peak height reproducibility of FAMES in each examination was approximately +/-5%. The FAME peak height ratios may reflect the diets of the two individuals. In particular, the lower chromatogram represented the FAME composition from a volunteer who had lived in South East Asia for a period of two weeks.

This relatively sensitive method has the potential to provide a further technique for identification of individuals from human skin lipids in fingerprints left at the scene of a crime.

7.4.3 Cosmetic Products

Skin care body lotions and lipsticks, are formulated with many ingredients which include fatty acid triglycerides from vegetable oils and higher molecular weight oils and waxes of natural origin. The identification of these components may be achieved using the THM reaction. The castor oil component in one of sixty-five lipstick bases studied, and the jojoba oil component in one of forty-nine skin care lotions, are readily identified as shown in Figure 7.4

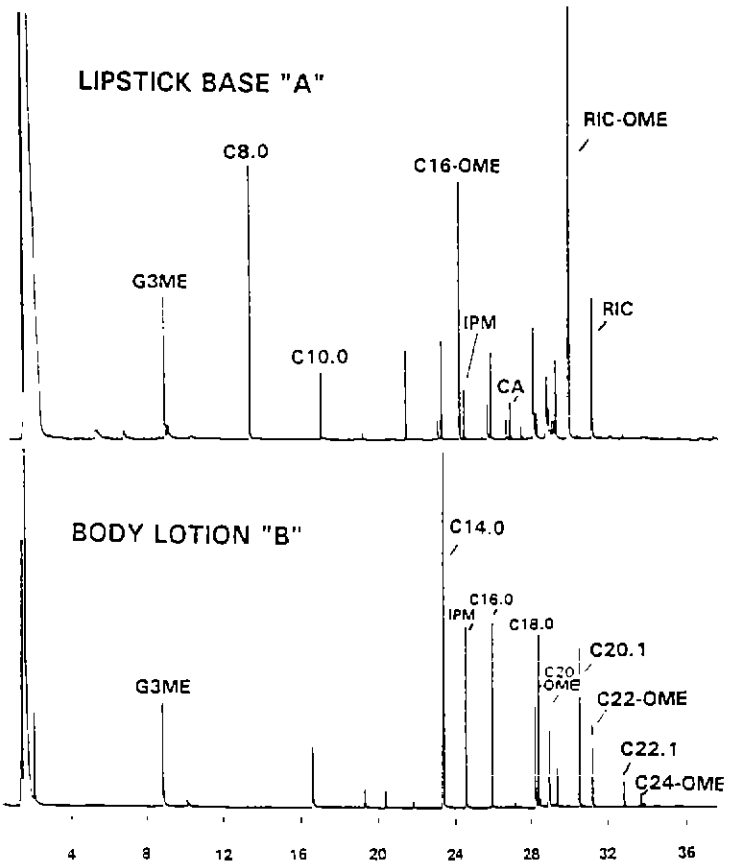


Figure 7.4. Chromatograms showing THM-GC of lipstick base A and body lotion B. Key:- G3ME= glycerol trimethylether, IPM=isopropyl myristate, CA=cetyl acetate. RIC-OME=ricinoleic acid methyl ester with a methylated hydroxy group, RIC=ricinoleic acid methyl ester. CX.0 refers to saturated FAMES with carbon chain length X. CX.1 refers to mono-unsaturated FAMEs with carbon chain length X. CX-OME refers to mono-unsaturated fatty alcohol methyl ethers.

Ricinoleic acid, detected as the major methyl ester component in the lipstick base, indicated that castor oil was a principal component. The lipstick also contained isopropyl myristate and cetyl acetate emollients. The octanoic and decanoic FAMES (C8.0 and C10.0) probably originate from emollient short chain fatty acid esters. The C20 to C24 monounsaturated FAMES and monounsaturated fatty alcohol methyl ethers in the body lotion were representative of the THM products of jojoba oil. Tetradecanoic FAME (C14.0) was a hydrolysis / methylation product of isopropyl myristate (IPM), whilst hexadecanoic and octadecanoic FAMES and glycerol trimethyl ether (G3ME) resulted from the THM reaction of the respective triglycerides.

The method requires sub-microgram quantities, and is hence appropriate for trace material forensic investigations. Structure determination of the complex mixtures in these proprietary products would otherwise require lengthy analytical procedures involving multiple chemical degradation and derivatisation steps.

7.4.4 Lanolin

Woolgrease is extracted from wool prior to processing for textile use. It posesses emollient properties and the refined product, lanolin, is used in some cosmetic and skin care products. The THM-GC pyrogram of woolgrease is shown in Figure 7 5.

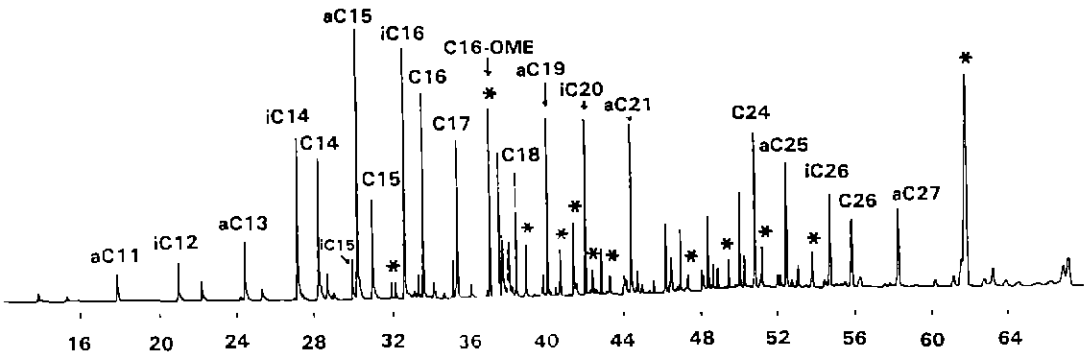


Figure 7.5. Partial-chromatogram obtained by THM-GC of woolgrease (DB1701 phase). aCX, iCX and CX refer to saturated anteiso branched FAMES, iso branched chain FAMES and straight chain FAMES with carbon chain length X, respectively.

C16-OME = methoxy substituted palmitic acid methyl ester. The asterisks indicate peaks corresponding to compounds not detected when woolgrease was hydrolysed and methyl derivatives were prepared using boron trifluoride in methanol.

The THM-GC profile of woolgrease is particularly interesting because the THM reaction products are complex and contain even and odd carbon number chains and branched chain iso- and anteiso- FAMES. Hydroxy substituted fatty acids are detected as the methyl ethers in the THM-GC determination, although they are not usually detected when the material is analysed by conventional methods. Differences in FAME composition of woolgreases from different sources may be anticipated, because the fatty acid composition of woolwax would be expected to depend on environmental factors and the diet of the animal.

7.5 SUMMARY

The THM pyrolysis derivatisation reaction of TMAH with a wide range of triglycerides allowed fatty acid identification at the microgram level with minimal sample handling requirements. The isomerisation problems with PUFAs in linseed oil were resolved by employing 0.2 M aqueous TMTFTH reagent or lowering the concentration of TMAH, although the yield of FAMES was reduced. The sensitivity of the THM-GC procedure was demonstrated by its application to human skin lipid fatty acid profiling, and its versatility by the capacity for the identification of a number of components in cosmetic products, including lanolin.

CHAPTER 8

CHARACTERISATION OF WOOD BY THERMALLY ASSISTED HYDROLYSIS AND METHYLATION GAS CHROMATOGRAPHY - MASS SPECTROMETRY

8.1 ABSTRACT

The pyrolysis derivatisation reaction, involving co-pyrolysis with TMAH, was used to characterise the basic chemical structure of heartwood lignocellulose from selected softwoods and hardwoods, including a number of *Eucalyptus* hardwood species. The process is considered to be a thermally-assisted hydrolysis and methylation (THM) reaction of the lignocellulose. The composition of lignin-derived guaiacyl and syringyl dimethoxy and trimethoxy benzenoid compounds could be used to distinguish softwoods from hardwoods, and was particularly useful for distinguishing gymnosperms from angiosperms. The relative proportion of the products formed was used to differentiate the woods in this study. Compounds not usually associated with lignocellulose were identified as extractives, and this forms the subject of the second part of this study in Chapter 9. The heartwood and the sapwood of two species of cypress were examined by the method in order to attempt to differentiate the species.

8.2 INTRODUCTION

The chemical structure of lignin, which together with cellulose, is the structural support medium for woody plants, would be expected to have a direct bearing on the physical and biological properties of timber. An important characteristic of timber is its resistance to disease and predators, which is a property related to chemical composition. Problems in the industrial pulping of timber and processing of timber for remanufacturing into plywood and associated materials are being resolved as a result of a greater understanding of the chemical composition of wood (Sjostrom, 1993). The identification and comparison of woody plants and processed wood is required in forensic investigations. Examples of this are assaults with wooden objects, sawn-off shotgun butt wood comparisons, burglary offences where entry has been gained by cutting wooden structures and comparisons of sawdust from safe ballast. The identification of timbers is often required in other fields, such as archaeology

The lignin component of wood is a natural polymer of great structural complexity (Figure 1.7). It has a polyhydroxy phenolic macromolecular structure composed of

phenylpropane units. The complexity of the structure partly arises from the way the C_1-C_3 propane carbon atoms attach to other centres. The phenylpropane units are based on *p*-coumaryl alcohol, *trans*-coniferyl alcohol and / or *trans*-sinapyl alcohol precursors (Figure 8.1) which have been subjected to enzyme-initiated dehydrogenative polymerisation in the formation of the lignin. The proportions of the three precursor alcohols differ between the gymnosperm and angiosperm lignins. Gymnosperm lignins are commonly derived from *trans*-coniferyl alcohol and a low proportion of *p*-coumaryl alcohol structural units. Dicotyledonous angiosperm lignins have structures based on *trans*-coniferyl alcohol, *trans*-sinapyl alcohol and a low proportion of *p*-coumaryl alcohol structural units, while in monocotyledonous angiosperms *trans*-coniferyl and *p*-coumaryl alcohol structures usually predominate (Kirk and Farrell, 1987; Stafford, 1988). All three alcohols are the building blocks of the lignin structure in grasses (Saiz-Jimenez and de Leeuw, 1985). Other constituents of woods are extractives which are a non-structural component of plants, whose chemical constituents include aliphatic and diterpenoid carboxylic acids.

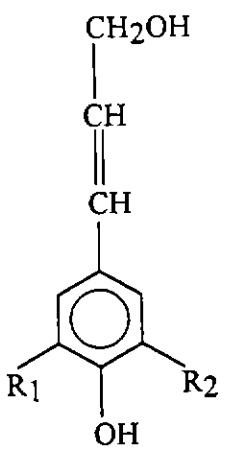


Figure 8.1. The structure of lignin alcohol precursors. R_1=R_2=H:- *p*-coumaryl alcohol; R_1=H, R_2=OCH_3:- *trans* coniferyl alcohol; R_1=R_2= OCH_3:- *trans* sinapyl alcohol.

Pyrolysis techniques have been widely used to examine the chemical structure of lignins and lignocellulose. In some more recent applications, pyrolysis gas chromatography (Py-GC) was used to characterise lignin isolated from softwoods, hardwoods and grasses (Saiz-Jimenez and de Leeuw, 1985). The technique also showed promise in the investigation of the effect of fungal attack on beechwood (Faix *et al.*, 1991). The method was sensitive and reproducible but not very specific. Pyrolysis mass spectrometry examination of the same type of wood gave an insight into the identity of higher molecular weight pyrolysis fragments, which was useful for an understanding of lignin composition (Pouwels and Boon, 1990).

The pyrolysis derivatisation reaction, using gas chromatography procedures for separation and detection, has assisted in the structural elucidation of synthetic polymers (Chapter 3), rosin based commercial resins (Chapter 6), natural resins (Challinor, 1990; Anderson and Winans, 1991), humic substances in soils (Hatcher and Clifford, 1994; Martin *et al.*, 1994, Martin *et al.*, 1995a), whole soils (Schulten and Sorge, 1995) and kerogens (Chapter 4, Kralert *et al.*, 1995). Py-MS of biological material mixed with TMAH was used to study phenolic acids (Mulder *et al.*, 1992).

In this Chapter, the heartwood of a selected number of conifer softwoods, European hardwoods and a small group of Australian *Eucalyptus* species, were investigated by the THM-GC method to determine whether it was possible to identify the genera. In the case of *Eucalypts* and two cypress woods, attempts were made to differentiate species. Mass spectrometry was used to identify the products. Sapwood in the cypress species was also studied. Biomarker compounds which are not derived from lignocellulose were investigated in an attempt to identify species, and to provide further discrimination. A further study of these biomarkers will be discussed in Chapter 9

8.3 EXPERIMENTAL

8.3.1 Method

Finely divided scrapings of heartwood and sapwood (approximately 50 μg) were added to approximately 0.5 μl 25% w/w aqueous TMAH solution deposited in the hollow of a flattened, bent 770°C. Curie-point pyrolysis wire.

8.3.2 Instrumentation

Conditions for pyrolysis gas chromatography and mass spectrometry were as described in Chapter 2 (Section 2.2).

8.3.3 Materials

Wood examined by the THM procedure was obtained from the Western Australian Maritime Museum, from Bunnings Ltd. (a local timber supplier) and the Department of Primary Industry, Queensland, Australia.

8.4 RESULTS AND DISCUSSION

A complex mixture of products was formed when the heartwood of selected examples of a softwood, a European hardwood and an Australian hardwood were subjected to the THM-GC procedure. The product profiles appeared to be distinctly different (Figure 8.2).

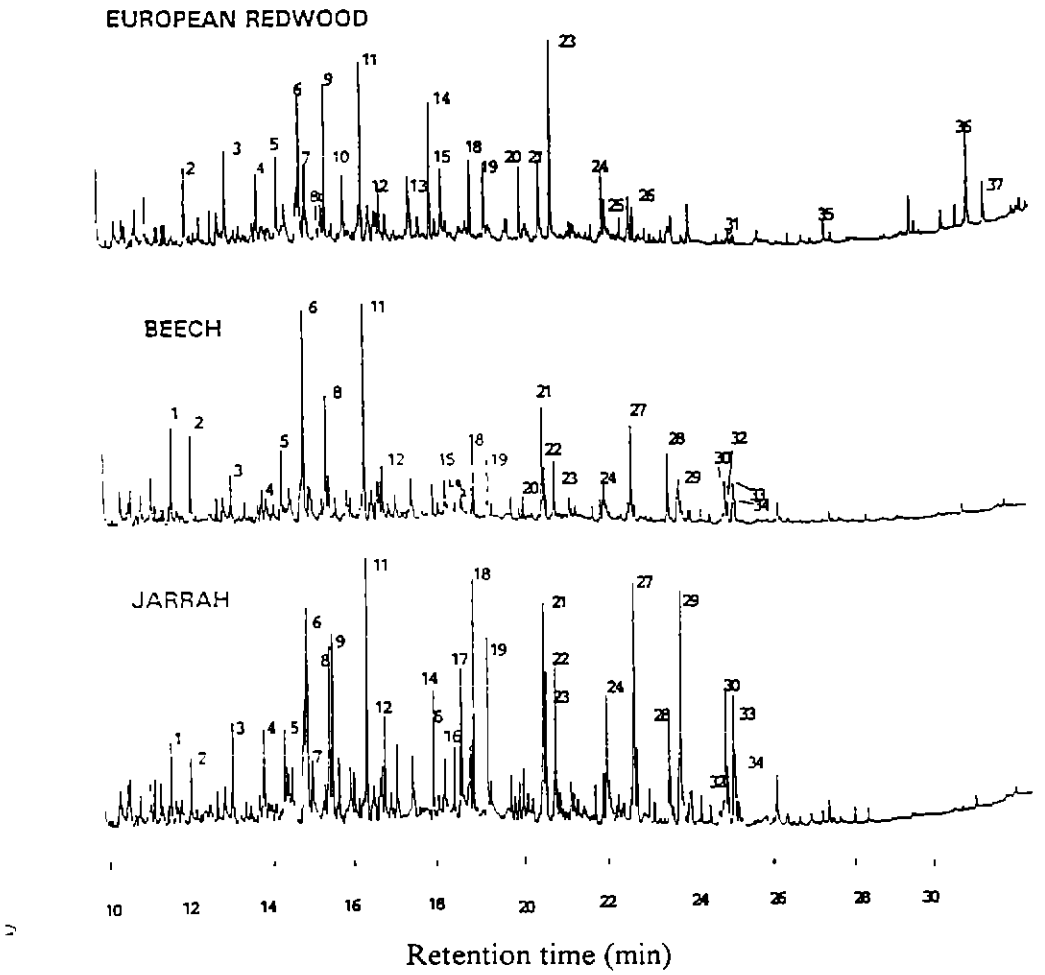


Figure 8.2. Chromatograms showing THM-GC analyses (retention time 10-32 minutes) of a softwood, European redwood timber (*Pinus sylvestris*), a European hardwood, beech (*Fagus sylvatica*), and an Australian hardwood, jarrah (*Eucalyptus marginata*). For peak identifications refer to Table 8.1.

The products of European redwood (*Pinus sylvestris*), a conifer sub-class of the gymnosperm family, were identified by the THM-GC procedure as a complex mixture

of dimethoxy substituted benzenoid compounds, substituted pyrans, aliphatic carboxylic acid methyl esters, rosin acid methyl esters and other oxygen containing compounds of carbohydrate origin. 3,4-Dimethoxybenzaldehyde was the major component in the THM-GC profile in the 10 - 32 minute retention time region. The products of the hardwood, beech (*Fagus sylvatica*), an angiosperm, were identified as a multicomponent mixture of dimethoxy- and trimethoxy- substituted benzenoid compounds and compounds of oligosaccharide origin. Substituted pyrans were the major components in the THM-GC profile. The *Eucalyptus* genus is another hardwood growing widely in Australia and exists as many species. The heartwood of jarrah (*Eucalyptus marginata)* had a similar profile to beech, but trimethoxy- and tetramethoxybenzenoids were more abundant.

The identities of the THM products, as determined by interpretation of their mass spectra, and their possible source are listed in Table 8.1 Some of these compounds are not fully methylated. This could be due to steric factors influencing the reaction of TMAH with the phenolic hydroxyl groups.

PEAK NUMBER	COUMPOUND	SOURCE
1	2-Hydroxydihydropyranone	P
2	3-Methyl-1,2-cyclopentanedione	C
3	2-Methoxyphenol	G
4	1,2-Dimethoxybenzene	G
5	2-Hydroxy-3-methyl-2-cyclopenten-1-one	C
6	3,4-Dihydro-2,5-dimethylpyran-2-carboxaldehyde	C
7	4-Methyl-2-methoxyphenol	G
8	2,3,4,5-Tetramethyltetrahydrofuran	-
9	3,4-Dimethoxytoluene	G
10	3,4-Dimethoxyphenylacetate	G
11	5-Hydroxy-2-(hydroxymethyl)-pyran-3-one	H

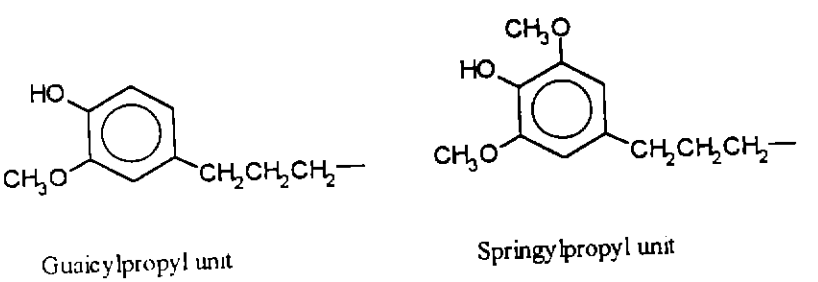
PEAK NUMBER	COMPOUND	SOURCE
12	Permethylated monosaccharide	C
13	Cyclohexylmethyl acetate	-
14	3,4-Dimethoxystyrene	G
15	4-Hydroxymethylfuran-2-carboxaldehyde	H
16	3,4-Dimethoxyphenol	S
17	3,4,5-Trimethoxytoluene	S
18	Permethylated monosaccharide	C
19	Permethylated monosaccharide	C
20	3-Hydroxy-4-methoxybenzaldehyde	G
21	Methyl-O-methylxylopyranoside	P
22	2-Methoxyethenyl-3,4-dimethoxybenzene	G
23	3,4-Dimethoxybenzaldehyde	G
24	Methyl-3,4-dimethoxybenzoate	G
25	(3,4-Dimethoxyphenyl) ethanone	G
26	1,6-Anhydro-β-D-glucopyranose isomer (levoglucosan)	C
27	3,4,5-Trimethoxybenzaldehyde	S
28	1,6-Anhydro-β-D-glucopyranose isomer (levoglucosan)	C
29	Methyl-3,4,5-trimethoxybenzoate	S
30	(3,4-Dimethoxyphenyl) dimethoxypropene isomer	G
31	Methyl hexadecanoate	E
32	(3,4-Dimethoxyphenyl) dimethoxypropene isomer	G
33	Unknown	-
34	Unknown	-

PEAK NUMBER	COMPOUND	SOURCE
35	C_{18} fatty acid methyl esters	E
36	Methyl 3,4-dimethoxyphenyl-2-methoxyacetate	G
37	Methyl dehydroabietate	E

Table 8.1. The identities of THM products in the redwood (*Pinus sylvestris*), beech (*Fagus sylvatica*) and jarrah (*Eucalyptus marginata*). For peak numbers refer to Figure 8.1.

Key:- G=guaiacylpropyl-derived, S=syringylpropyl-derived, H= hexose-derived, P= pentose-derived, C= carbohydrate non-specific, E=extractive.

The peak heights of cellulose-derived and lignin-derived products, as indicated in Figure 8.1 and Table 8.1, reflect the relative proportions of cellulose, hemicellulose and lignin components in the wood The lignin-derived 3,4-dimethoxybenzene substituted compounds found in the softwood conifer THM products are consistent with the reported lignin structures for softwood in that conifer (gymnosperm) heartwood contains exclusively guaiacylpropyl units (Figure 8.3). In contrast, the hardwoods contain lignin-derived 3,4-dimethoxy and 3,4,5-trimethoxybenzene substituted compounds in the THM products. These compounds appear to originate, not only from the guaiacylpropyl units but also from syringylpropyl units (Figure 8.3), which are the types of compounds expected to be derived from angiosperms (Wenzyl, 1970).



Guaicylpropyl unit

Springylpropyl unit

Figure 8.3 The chemical structures of guaiacylpropyl and syringylpropyl units.

8.4.1 Origin of Lignin Derived THM Products

It is now considered that polyhydroxyphenylpropane units are combined in the macromolecular lignin and that these units are connected through aryl ether or alkyl ether linkages and / or by carbon to carbon linkages (see structure in Figure 1.7, Section 1.4.3). High temperature hydrolysis and methylation mechanisms (Section 2.3) might be expected to produce many of the products observed in the THM reaction of the lignocellulose in the woods as observed in Figure 8.2.

It would appear that some of the products from the THM reaction of the woods are also formed by conventional pyrolysis except that the free hydroxy groups in guaiacyl and syringyl units are methylated. However, no carboxylic acids are detected in conventional Py-GC experiments (Saiz-Jimenez and de Leeuw, 1985). It has been postulated that carboxylic acids are decarboxylated in these high temperature conditions (Martin *et al.*, 1994). It is also claimed (Hardell and Nilvebrant, 1996) that the acid methyl esters formed in the THM reaction are oxidation products from the high temperature pyrolysis process, since carboxylic acids are rare in lignin. An alternative hypothesis is that both 3,4-dimethoxybenzoic acid and the syringyl-derived 3,4,5-trimethoxybenzoic acid, detected in the THM experiments as the methyl esters, may be produced as a result of a maturing process as the heartwood ages. Future work on timbers of different maturity will establish the validity of this hypothesis. This possibility could explain differences observed in THM profiles of wood of the same species from different sources.

8.4.2 Discrimination within Species.

Discrimination within softwoods can be made on the basis of their different THM profiles. The conifers, European redwood (*Pinus sylvestris*), Monterey pine (*Pinus radiata*), larch (*Larix decidua*), Douglas fir (*Psuedotsuga menziesii*) and Western red cedar (*Thuja plicata*) display reproducible THM-GC profiles over the whole chromatographic range (Figure 8.4).

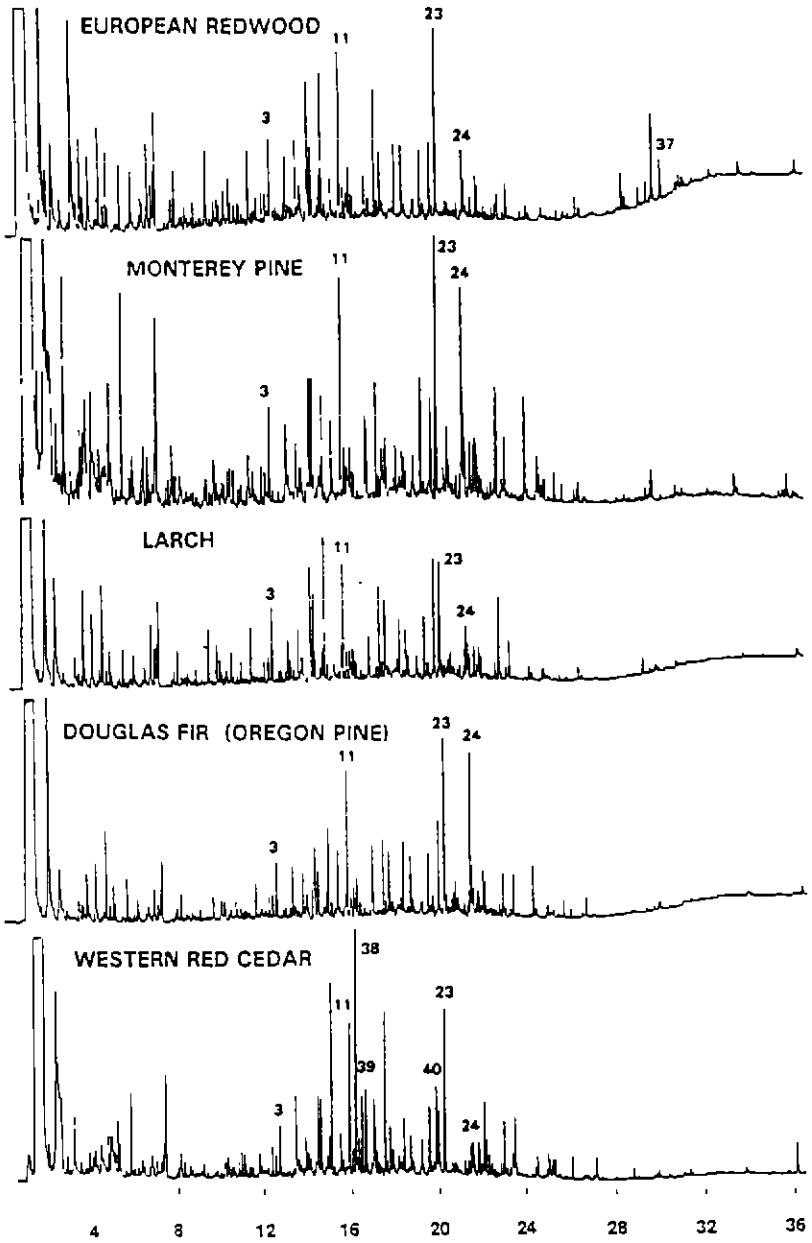


Figure 8.4. Chromatograms showing THM-GC analyses (retention times 0 - 36 minutes) of conifer woods, European redwood (*Pinus sylvestris*). Monterey pine (*Pinus radiata*), larch (*Larix decidua*), Douglas fir (*Psuedotsuga menziesii*) and Western red cedar (*Thuja plicata*).

Key: 3= 3-methoxyphenol, 11= 5-hydroxy-2-(hydroxymethyl)-pyran-3-one, 23= 3,4-dimethoxybenzaldehyde, 24= methyl 3,4-dimethoxybenzoate, 37= methyl dehydroabietate, 38= methyl 4-isopropylbenzoate, 39= dimethyltetrahydro-isobenzofurandione isomers, 40= γ-thujaplicin. The identity of the remaining

compounds in the 10-32 minute retention time region may be deduced by interpolation from peak numbers in Figure 8.2 and data in Table 8.1.

Peak height ratio differences distinguished the specimens of redwood, Monterey pine, larch and Douglas fir. Also, the THM profile of Western red cedar displayed distinctive biomarker compounds which were characteristic of this species. These compounds have been identified by MS as methyl 4-isopropylbenzoate (peak 38), dimethyl tetrahydro-isobenzofurandione isomers (peaks 39) and γ-thujaplicin (peak 40). The latter compound is a known extractive of this species (Hillis, 1962).

The profiles of the THM products of samples taken from various sites in a single specimen of Monterey pine were reproducible except for the relative proportions of the rosin acid methyl esters, and the proportion of these compounds varied within the wood. However, there was some variation in peak height ratios in this species within specimens acquired from different sources, particularly in the relative abundance of methyl 3,4- dimethoxybenzoate. This acid, detected as the methyl ester, may increase in proportion to the other THM products progressively with time as the heartwood is maturing, as discussed previously.

The THM-GC profiles of a group of hardwoods, namely beech (*Fagus sylvatica*), elm (*Ulmus procera*), European white oak (*Quercus robur*) and Burma teak (*Tectona grandis*) species displayed characteristic reproducible differences which could be used to potentially identify the individual species (Figure 8.5). Peak height ratios were again found to be the major factor in determining differences in the beech, oak and elm profiles. From a comparison of these profiles, an inspection of the THM-GC profile of Burma teak reveals additional products which were not present in the other genera. These compounds have not, as yet, been identified.

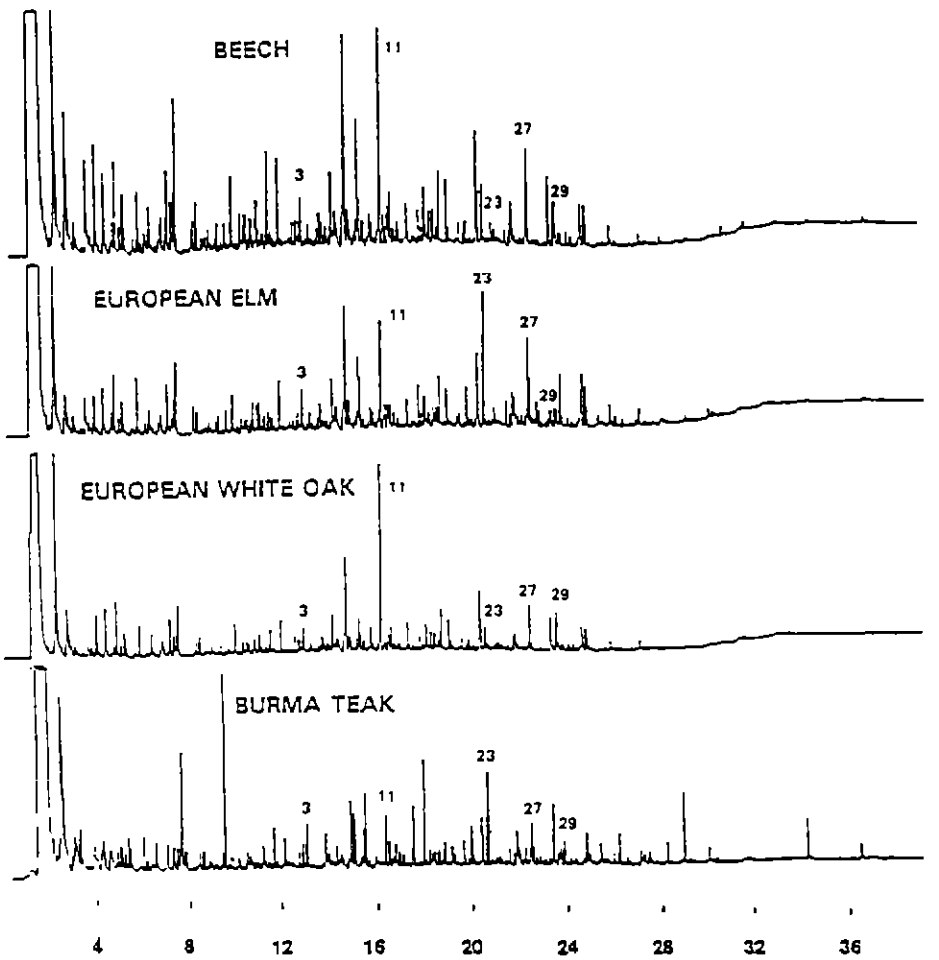


Figure 8.5. Chromatograms showing THM-GC analyses (retention times 0 - 36 minutes) of different species of hardwood, beech (*Fagus sylvatica*), elm (*Ulmus procera*), European white oak (*Quercus robur*) and Burma teak (*Tectona grandis*). Key: 3= 3-methoxyphenol, 11= 5-hydroxy-2-(hydroxymethyl)-pyran-3-one, 23= 3,4-dimethoxybenzaldehyde, 27= 3,4,5-trimethoxybenzaldehyde, 29= methyl-3,4,5-trimethoxybenzoate. The identity of the other compounds in the 10-32 minute retention time region may be deduced by interpolation from peak numbers in Figure 8.2 and data in Table 8.1.

The THM-GC profiles of the heartwood of four eucalypts, a hardwood genus common in Australia, jarrah (*E. marginata*), marri (*E. calophylla*), karri (*E. diversicolor*) and Tasmanian oak (*E. deligatensis*) are shown in Figure 8.6.

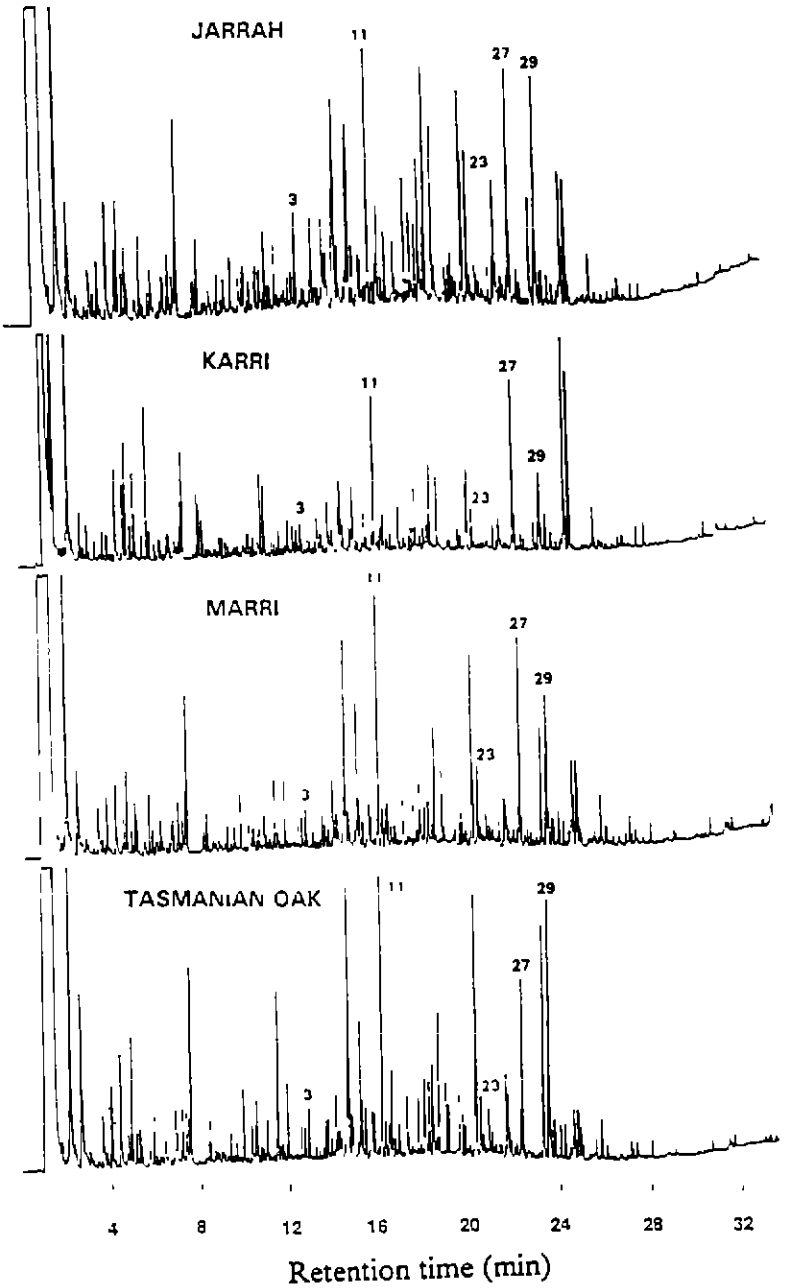


Figure 8 6. Chromatograms showing THM-GC analyses (retention times 0 - 36 minutes) of four eucalypts, jarrah (*E. marginata*), marri (*E. calophylla*), karri (*E. diversicolor*) and Tasmanian oak (*E. deligatensis*).

Key: 3= 3-methoxyphenol, 11= 5-hydroxy-2-(hydroxymethyl)-pyran-3-one, 23= 3,4-dimethoxybenzaldehyde, 27= 3,4,5-trimethoxybenzaldehyde, 29= methyl-3,4,5-

trimethoxybenzoate. The identity of the other compounds in the 10-32 minute retention time region may be deduced by interpolation from peak numbers in Figure 8.2 and data in Table 8.1.

The relative proportion of trimethoxybenzaldehyde to methyl trimethoxybenzoate in the THM-GC profiles varied according to the source of the heartwood. Methyl trimethoxybenzoate may be the most abundant compound in the THM-GC profile of jarrah and may be related to the age of the timber as previously discussed This provides another discriminatory factor in the differentiation within this species. It must be noted that this carboxylic acid, as well as most aromatic and aliphatic carboxylic acids, is not detected in conventional pyrolysis, due to decarboxylation.

The European hardwood and Australian *Eucalypt* THM profiles differ from those of the softwood conifers in the higher abundance of compounds having peaks of approximate retention time 23 to 25 minutes corresponding mainly to the syringyl-derived compounds. The main differences in these three groups occurred after retention time 10 minutes. Although the sequence of compounds appeared to be similar in many of these woods, peak height ratios varied from genus to genus in both the conifer, hardwoods and the eucalypt woods. Western red cedar and teak, had distinctive compounds present which clearly differentiated them from the other species. The hardwoods which were studied appear to be characterised by dominant pyrans (peaks 6 and 11) and the presence of a tetramethyltetrahydrofuran isomer (peak 8), in contrast to conifer THM-GC profiles which produce 3,4-dimethoxytoluene (peak 9) (Figure 8.2/Table 8.1). The *Eucalypt* species display a higher proportion of the syringyl-derived and tetramethoxy benzenoid compounds when compared to the other hardwood genera (Figure. 8.6).

Future studies will be directed towards an evaluation of variations in heartwood composition of the same species in specimens which have a different age, and are from different geographical locations.

8.4.3 A Case Study

The technique was applied to the characterisation of wood from the trunks of white cypress (*Callitris glaucophylla*) and black cypress (*Callitris endlicheri*), two trees grown in Queensland, Australia. These timbers are used as structural supports for houses. The purpose of the investigation was to differentiate the rot susceptible black cypress from the rot resistant white cypress species.

Shavings taken from different sites in a cross-section of trunks from each species were subjected to the THM-GC procedure. Profiles of wood taken from the centre of the heartwood are shown in Figure 8.7. Wood from other locations in the heartwood were also tested to determine variability in composition. The results indicated that the composition of the heartwood was homogenous within each species. White and black cypress could be distinguished on the basis of their THM-GC profiles.

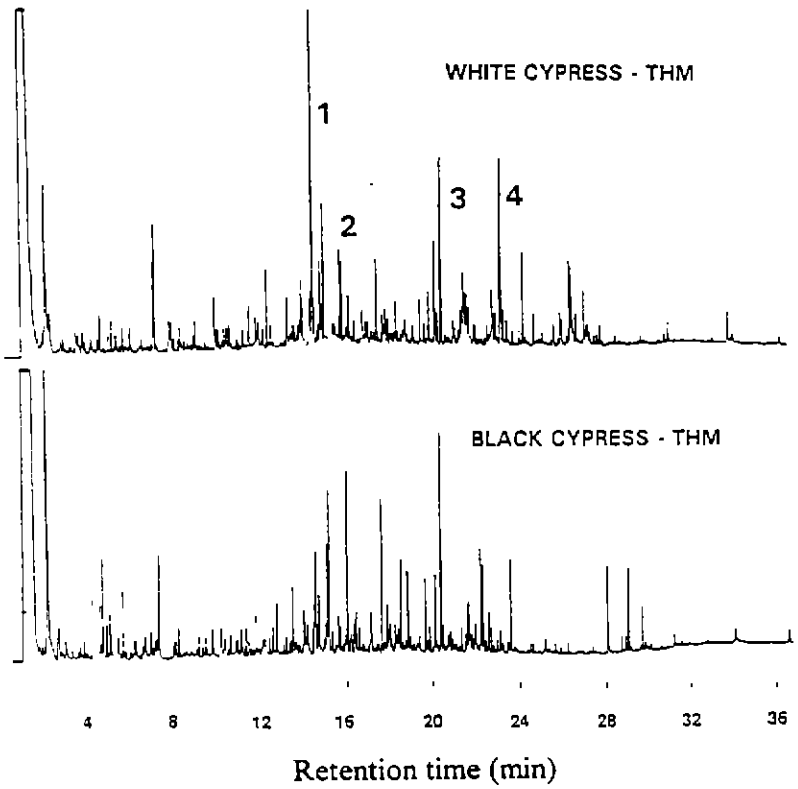
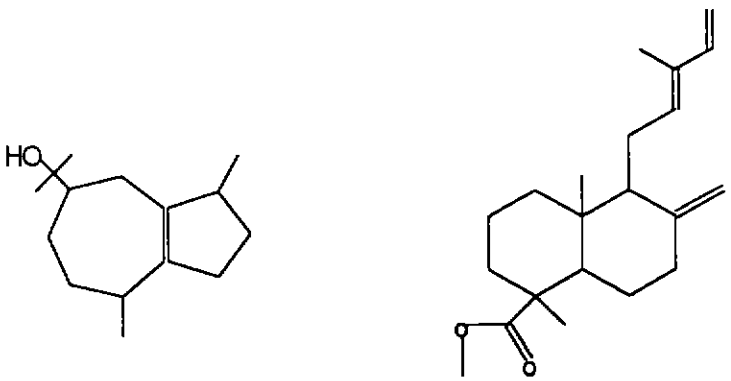


Figure 8.7. Chromatograms showing THM-GC analyses of white and black cypress wood taken from the centre of the heartwood.

Key: 1= methyl 3,7-dimethyl-6-octenoate, 2= methyl 3,7-dimethyl, 2, 6-octadienoate, 3= 3,8-dimethyl-5-(α-hydroxyisopropyl)-octahydroazulene, 4= a substituted

decahydronaphthalene carboxylic acid methyl ester having the structure shown in Figure 8.8.

The major differences in the heartwood composition of the two species were the high proportion of compounds 1 and 2 in white cypress, identified as methyl 3,7-dimethyl-6-octenoate (methyl ester of citronellic acid) and methyl 3,7-dimethyl-2,6-octadienoate. It was interesting to note that these acids were detected in methyl alcohol extracts of this species in a separate experiment. 3,8-Dimethyl-5-(α-hydroxyisopropyl)-octahydrohydroazulene, commonly known as Guaiol, (peak 3) was also a significant product in white cypress (structure shown in Figure 8.8). Peak 4 was tentatively identified as a substituted decahydronaphthalene carboxylic acid methyl ester having the structure shown in Figure 8.8. The structure and mass spectrum are consistent with a rosin acid precursor. These compounds were not detected in black cypress.



Guaiol

Figure 8.8. Chemical structures of Guaiol (peak 3) and a substituted decahydronaphthalene carboxylic acid methyl ester (peak 4).

The THM products from black cypress were predominantly 3,4-dimethoxybenzene substituted compounds which are consistently found in THM profiles of coniferous (Gymnosperm) woods.

The THM-GC profiles of sapwood showed variations within a sample, indicating that the sapwood was not homogeneous. The variations were in peak height ratios. Examples of the profiles of white and black cypress sapwood are shown in Figure 8.9.

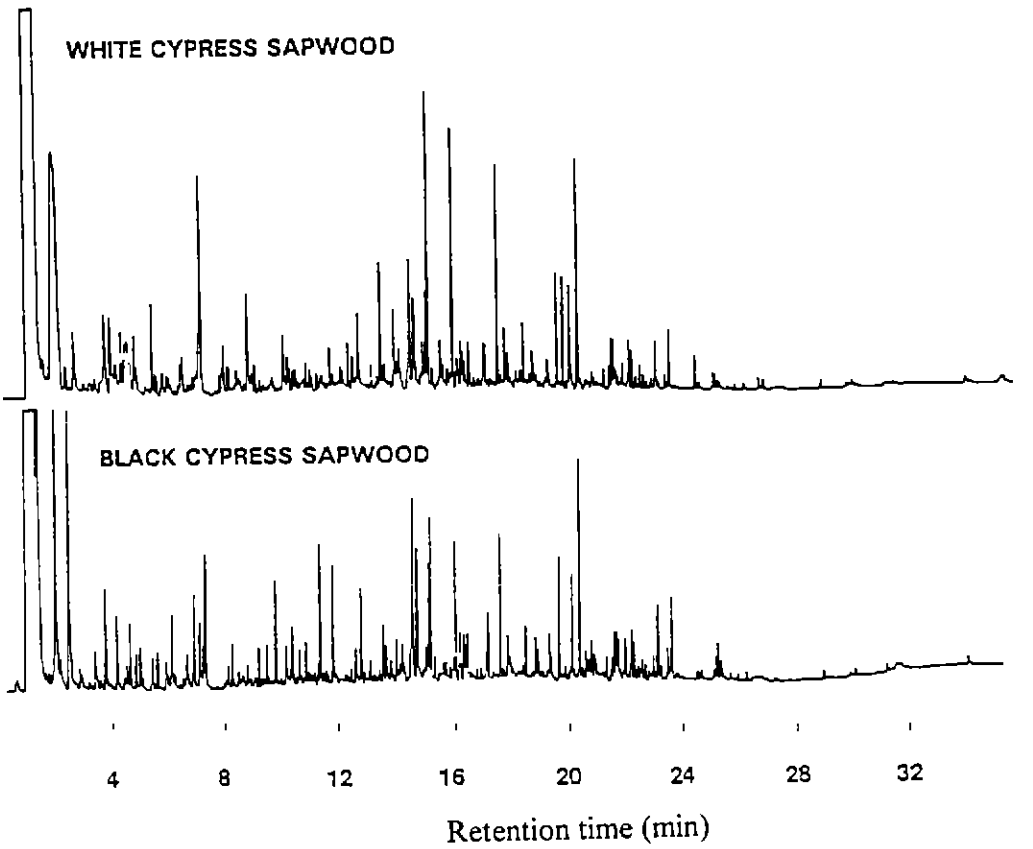


Figure 8.9. Examples of THM-GC chromatograms of white and black cypress wood taken from the sapwood.

It was concluded that it was possible to differentiate these two species on the basis of the results obtained by the THM-GC analysis of these two specimens. Further work is required to establish whether these differences are due to seasonal compositional variations.

8.5 SUMMARY

Conifer, European hardwoods and Australian *Eucalypt* species examined in this study can be differentiated on the basis of their THM-GC profiles. The detection of solely 3,4-dimethoxy benzenoid compounds indicates guaiacyl-containing lignin, identifying

the wood as a gymnosperm subclass. The detection of diterpenoid acids provides further confirmation of conifer species. The presence of both 3,4-dimethoxy benzenoid (guaiacyl) and 3,4,5-trimethoxy benzenoid (syringyl) compounds indicates that the wood has angiosperm origin. Certain wood species, notably, Western red cedar (*Thuja plicata*), teak (*Tectona grandis*) and white cypress (*Callitris glauca*) have very distinctive THM profiles with biomarker compounds superimposed on the normal lignocellulose profile.

A further study of the dependence of composition on the age, geographical location and growing climate of individual species is required.

CHAPTER 9

CHARACTERISATION OF WOOD EXTRACTIVES BY PYROLYSIS GAS CHROMATOGRAPHY - MASS SPECTROMETRY OF QUATERNARY AMMONIUM HYDROXIDE EXTRACTS

9.1 ABSTRACT

The aqueous TMAH extracts of the heartwoods of a range of conifer softwoods and various hardwoods were flash heated and analysed by gas chromatography. Unique chromatographic profiles of the pyrolysed extracts were obtained which were indicative of the species. Some of the extracted compounds were characteristic biomarkers and, hence, potentially valuable in chemotaxonomy investigations. In most cases, gymnosperm origin was indicated by the detection of guaiacyl derived compounds and angiosperm origin was reflected by the presence of both guaiacyl and syringyl derived compounds.

Profiles of the pyrolysed extracts were generally less complex and more species specific than those obtained by THM-GC, reported in the previous Chapter. The effects of variations in pyrolysis temperature on the pyrolysis profiles were also studied. The origin of the extractives of two species was investigated by methylating methyl alcohol extracts to determine whether the extracted compounds were free or bound to lignocellulose. Pyrolysis of a tetraethylammonium hydroxide (TEAH) extract was used to determine the sites of pre-existing methylation in a major extractive

9.2 INTRODUCTION

Wood extractives are extraneous substances located in various sites of the wood structure. Phenolic extractives are found in the heartwood and bark, and waxes and fats occupy the ray parenchyma cells. Some of these extractives are considered to be responsible for the resistance of wood to fungal attack and wood boring insects (Kirk-Othmer, 1984). Extractives from Brazilian trees provide a wide range of compounds of biological interest (Gottlieb and Mors, 1980). The presence of various extractives can affect physical strength and interfere in the manufacturing of plywood and other products (Hillis, 1972), and have detrimental effects in wood pulping and paper making processes (Sjostrom, 1993). They can also affect the curing of adhesives on wood and stain surface coatings (Hillis, 1962). There is a need for the development of

extractives. Such a method would be advantageous in chemotaxonomy and forensic science, and investigations into the isolation of useful chemicals from plant material for pharmaceutical and industrial applications.

In the previous Chapter, it was reported that it was possible to differentiate individual wood genera on the basis of their chromatographic profiles, as determined by THM-GC. 3,4-Dimethoxy- and 3,4,5-trimethoxybenzene substituted compounds and cellulose derived furans and pyrans were the major products identified from the wood. The detection of solely dimethoxy compounds, or a combination of dimethoxy- and trimethoxybenzenoid compounds, could be used to identify the wood as gymnosperm or angiosperm, respectively. THM-GC-MS identification of the products from many of these woods did not reveal any significant components derived from structures other than lignocellulose. However, three of the 15 species studied, Western red cedar, teak and white cypress, gave profiles which included compounds such as methyl 4-isopropylbenzoate, dimethyltetrahydro-isobenzofurandione isomers, γ-thujaplicin in Western red cedar, and methyl 3,7-dimethyl-6-octenoate, methyl 3,7-dimethyl, 2, 6-octadienoate, 3,8-dimethyl-5-(α-hydroxyisopropyl)-octahydroazulene, and a substituted decahydronaphthalene carboxylic acid methyl ester in white cypress, which could be used for differentiation. These compounds were not derived from lignocellulose and could be biomarkers for those species.

A desirable outcome of any chemical classification scheme for plants would be the identification of a set of characteristic chemical compounds which were common to a particular genus, one or more of which were characteristic of an individual species within a genus. Unfortunately, the composition of diagnostic compounds in many woods depends on location, growing season and other factors. Individual compounds can also be common to several different species (Hillis, 1962).

Basic conditions have been found necessary for the optimum recovery of extractives from woods. Many of the compounds are terpenoids, carboxylic acids or phenolics (Sjostrom, 1993), and often require multistep procedures for identification.

A method for characterising extractives in softwood resins using high performance liquid chromatography (Suckling *et al.*, 1990) and a method for measuring the total amounts of fatty acids and their esters, resin acids and triglycerides in softwood resin and pitch samples by ^{13}C NMR (Suckling and Ede, 1990) have been reported. A gas chromatographic method for thujaplicin extractives in Western red cedar using diazomethane for methylation has been described (Nault, 1987). Size exclusion chromatography has provided a semi-quantitative approach to wood extractives in deposits in pulp and paper mills (Sjostrom and Holmbom, 1987).

Flavonoids in gymnosperm and angiosperm woods in *Pinus* species have been extensively investigated from a chemotaxonomical standpoint (Harborne, 1989). The biflavonoids and proanthocyanidin condensed tannins have been popular taxonomical markers (Porter, 1989). Condensed tannins have been extracted from plant material with a variety of polar solvents. The constitution of the polymers have been deduced by ^{13}C NMR and chemical degradation procedures.

In this Chapter, a procedure utilising pyrolysis of aqueous TMAH extracts of heartwood to compare and identify wood species is described. The heartwoods from fourteen conifer softwood and fourteen hardwoods, including eucalyptus and tropical species have been examined. The results were compared to those obtained by directly injecting the TMAH and methanol extracts into the heated injector of the gas chromatograph.

9.3 EXPERIMENTAL

9.3.1 Method

TMAH and TEAH extractives

Approximately 100mg of finely divided wood shavings were ultrasonically mixed with a minimum quantity of 1% w/w aqueous TMAH or TEAH in a glass vial for a period of 15 minutes. Approximately 5µL of the supernatant extract was evaporated onto a flattened, bent, Curie point pyrolysis wire with the aid of a stream of warm air.

Stability of extracts

TMAH extracts were stable over a significant period of time, e.g. 6 months, and gave similar pyrolysis profiles to those obtained from the original extract. Evaporated extracts could be reconstituted by addition of water or aqueous TMAH solution.

Methanol extractives

Similar quantities of finely divided wood were extracted with redistilled methyl alcohol (A.R.) in the same procedure described above. Aliquots were analysed by GC as described below.

Pyrolytic methylation

Components of the methanol extract were also methylated by co-injection of 1μL extract with 25% w/w aqueous TMAH. Aqueous TMAH extracts were injected directly into the injection port of the GC. The injection port temperature was 300 ^{o}C.

9.3.2 Instrumentation

Conditions for Py-GC and MS were as described in Chapter 2 (Section 2.2).

9.4 RESULTS AND DISCUSSION

9.4.1 Conifer Softwood Extractives

The TMAH extractives of Monterey pine (*Pinus radiata*), larch (*Larix decidua*), Douglas fir (*Pseudotsuga menziesii*) and Western red cedar (*Thuja plicata*) gave distinctly different profiles when flash heated and analysed by gas chromatography (Figure 9.1). Monterey pine gave significant peaks for 3,4-dimethoxybenzaldehyde (peak 1), methyl 3,4-dimethoxybenzoate (peak 2), methyl hexadecanoate (peak 3), methyl octadecadienoate (peak 4) and methyl dehydroabietate (peak 5), having a chemical structure shown in Figure 9.2. Larch gave a higher proportion of lower molecular weight extractives which could have polysaccharide origin, although the major significant extractive was methyl 3-methoxybenzoate (peak 6). The major extractives in Douglas fir were a methyl dimethylbenzene-butanoate isomer (peak 7), methyl 4-(1,4-dimethyl-3-oxohexyl)-cyclohexane-1-carboxylate (peak 8) and methyl 4-(1,4-dimethyl-3-oxohexyl)-cyclohexene-1-carboxylate (peak 9). Western red cedar

had a major TMAH extractive, methyl 4-(1-methylethyl)-benzoate (peak 10), a number of dimethyltetrahydro-isobenzofurandione isomers (peak 11), having a chemical structure shown in Figure 9.2, and 2-hydroxy-5-(1-methylethyl)-2,4,6-cycloheptatrien-1-one (peak 12). The latter compound is commonly referred to as the tropolone, γ-thujaplicin, known to be present as a fungicidal heartwood constituent of this species (Hillis, 1962). These compounds were not likely to be derived from lignocellulose and were thus considered to be extractive biomarkers

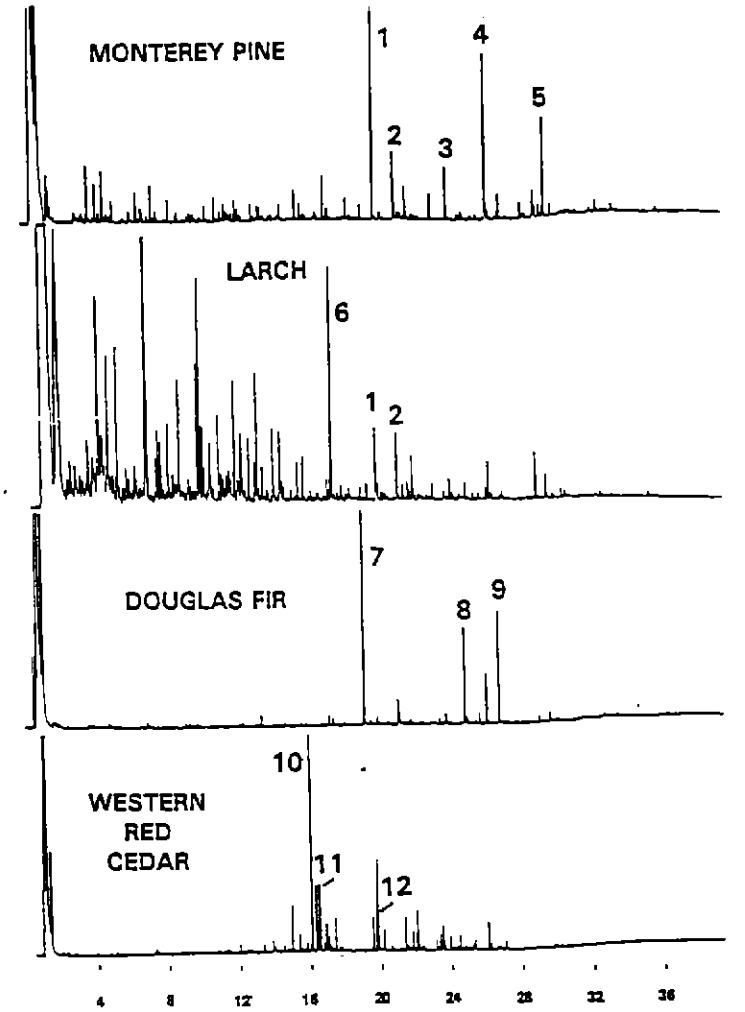


Figure 9.1. Chromatograms showing Py-GC of the TMAH extractives of conifer softwoods, Monterey pine (*Pinus radiata*), larch (*Larix decidua*), Douglas fir (*Pseudotsuga menziesii*) and Western red cedar (*Thuja plicata*).

Key: 1 = 3,4-dimethoxybenzaldehyde, 2 = methyl 3,4-dimethoxybenzoate, 3 = methyl hexadecanoate, 4 = methyl octadecadienoate, 5 = methyl dehydroabietate, 6 = methyl

3-methoxybenzoate, 7 = methyl dimethylbenzene-butanoate isomer, 8 = methyl 4-(1,4-dimethyl-3-oxohexyl)-cyclohexane-1-carboxylate, 9 = methyl 4-(1,4-dimethyl-3-oxohexyl)-cyclohexene-1-carboxylate, 10 = methyl 4-(1-methylethyl)-benzoate, 11 = dimethyltetrahydro-isobenzofurandione isomers, 12 = 2-hydroxy-5-(1-methylethyl)-2,4,6-cycloheptatrien-1-one

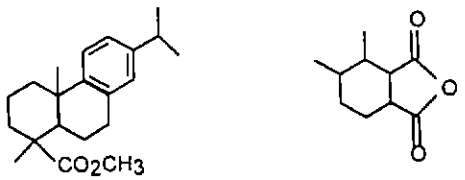


Figure 9.2 Chemical structure of methyl dehydroabietate (peak 5) and a dimethyltetrahydro-isobenzofurandione (peak 11).

Clearly, the Py-GC profiles of the TMAH extractives were less complex than those obtained by THM-GC of these woods, reported in Chapter 8, because the TMAH extract products would not be expected to contain any significant contribution from lignocellulose. The chemical composition of the extractive compounds provided distinctive biomarker patterns which could be used to potentially identify the genus or species. The presence of dimethoxybenzenoid compounds, particularly 3,4-dimethoxybenzaldehyde and methyl 3,4-dimethoxybenzoate, and the absence of 3,4,5-trimethoxybenzaldehyde and methyl 3,4,5-trimethoxybenzoate, confirmed that the Monterey pine and larch woods were gymnosperms. The dimethoxybenzenoid compounds were not as significant in the TMAH extracts of Douglas fir and Western red cedar.

By comparison to the results obtained by THM-GC of these woods, as reported in Chapter 8, where it was necessary to use peak height ratio differences to differentiate between four of these, namely European redwood, Monterey pine, larch and Douglas fir, analysis of the wood extracts gave better discrimination of the genera. It should be noted that the THM-GC profile of Western red cedar, included additional products

which were distinctive. These addditional products were also detected in the TMAH extract of Western red cedar.

The chromatograms obtained by pyrolysis of the TMAH extracts of eight additional *Pinus* species were compared to the results obtained from Monterey pine (*P. radiata*). The semiquantitative distribution of the major 1% TMAH extractives are shown in Table 9.1. The *Pinus* genus, with the exception of two species examined, may therefore be recognised by the general extractive profile which usually has 3,4-dimethoxybenzaldehyde as the major component and significant proportions of rosin acid acid methyl esters, particularly dehydroabietic acid methyl ester Individual species can be distinguished by the differences in the relative proportions of the above extractives in the specimens examined.

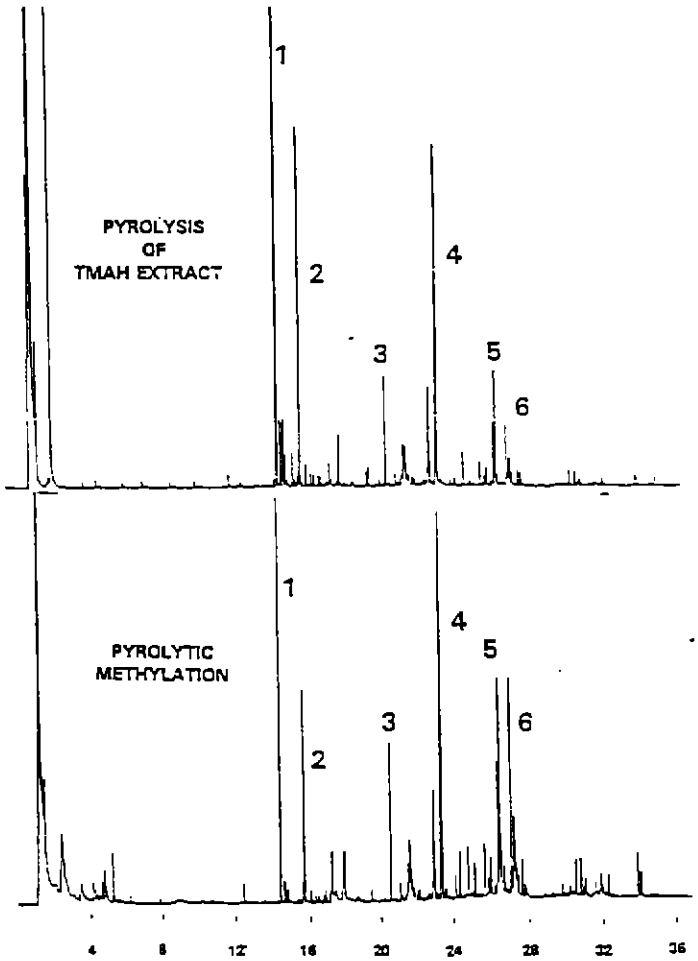
TMAH EXTRACTIVE	*P radiata* Monterey	*P sylvestris* Redwood	*P echinata* Shortleaf	*P strobus* White	*P resinosa* Red	*P elliotii* Slash	*P ponderosa* Ponderosa	*P contorta* Lodgepole	*P taeda* Loblolly
MB		+	-	++	-	-	-	++++	+
DMBA	++++	++++	++++	++	++++	++++	<+	++	++++
DMBAME	++	++++	++++	++	>+	+	<<+	++	++
C16	+	+	+	+	+	++	<+	+	++
C18	++	+	+	++	+	++	++	++	+
DMPMMA	-	++++	-	++++	-	-	++++	+	-
DHABME	++	++++	++++	+	+	++	+	+++	++

Table 9.1. Distribution of 1% TMAH extractives in nine *Pinus* species.

Key:- MB = methyl benzoate, DMBA = 3,4-dimethoxybenzadehyde, DMBAME = methyl 3,4-dimethoxybenzoate, C16 = methyl hexadecanoate; C18 = methyl octadecanoate (or unsaturated homologues), DMPMMA = methyl 3,4-dimethoxyphenyl-2-methoxyacetate, DHABME = methyl dehydroabietate. Plus signs indicate relative approximate proportions of extractive compounds and minus indicates compounds not detected.

9.4.2 Pyrolytic Methylation of TMAH Extracts

The TMAH extracts of white cypress (*Callitris glaucophylla*) heartwood, a species previously examined by the THM-GC method, were analysed by injecting the extract into the hot injection port (300°C) of the gas chromatograph. Any TMAH salts in the extract were converted pyrolytically to methyl derivatives by the technique as discussed in the review by Kossa *et al.*, (1979). The results were compared to those obtained by Py-GC of the TMAH extracts at a pyrolysis temperature of 770°C. The results show that the profiles closely correspond (Figure 9.3).



Retention time (min)

Figure 9.3. Chromatograms showing Py-GC and pyrolytic methylation of a TMAH extract of white cypress (*Callitris glaucophylla*).

Key:- 1 = methyl 3,7-dimethyl 6-octenoate (methyl ester of citronellic acid), 2 = methyl 3,7-dimethyl-2,6-octadienoate, 3 = 3,8-dimethyl-5-(α-hydroxyisopropyl)-octahydroazulene, (Guaiol). Tentative identifications of peaks 4, 5 and 6 were: a

substituted methyl decahydronaphthalene carboxylate, trimethylcyclohexahydro-decafuranone, and dimethyl-methylene-cyclohexahydrodecafuranone, respectively.

This "pyrolytic methylation" procedure appears to have the advantage that the need for a pyrolyser unit is eliminated. A disadvantage is that concentrated extracts are required to achieve sufficient sensitivity for many wood species where the extractive content is low.

9.4.3 Effect of Pyrolysis Temperature on the Extract

It was also observed, in separate experiments, that when 1% aqueous TMAH extracts of Douglas fir, Monterey pine and Western red cedar were subjected to Curie point pyrolysis at 358°C, 510°C and 980°C, the profiles corresponded closely with those obtained at pyrolysis temperatures of 770°C. At the lowest temperature (358°C), residues of the extract were observed on the pyrolysis wires indicating possible incomplete conversion. This observation suggests that such residues could be deposited in the injection port and the first few metres of the analytical column with detrimental results if such an extract is injected directly (as in the pyrolytic methylation procedure where the injection port temperature is of the order of 300°C). Also, water and unreacted TMAH will be deposited onto the column phase causing degradation. Water may condense on the cooler parts of the pyrolysis chamber insert, where the flash heating takes place in the pyrolyser, effectively removing it from the gas chromatograph system. Higher pyrolysis temperatures also ensure that excess TMAH is degraded to trimethylamine and methanol. A pyrolysis temperature of 770°C, therefore, is preferred for flash heating of TMAH extracts.

These potential problems would be overcome if a methanolic solution of TMAH was used for base catalysed transesterification of wood extractives, and the methyl derivatives were extracted into dichloromethane, as in the *in vitro* digestion method for degraded Douglas fir wood lignin as described by McKinney *et al.* (1995).

9.4.4 Hardwood Extractives

When the heartwood of a group of hardwoods of different genera, beech (*Fagus sylvatica*), elm (*Ulmus procera*), European white oak (*Quercus robur*) and Burma teak (*Tectona grandis*) were examined by pyrolysis of the TMAH extractives the following profiles were obtained (Figure 9.4). The compound tentatively identified as 5-hydroxy, 2-hydroxymethyl pyranone (peak 1), which was a major THM product in many species of hardwood, was present in beech and oak, whilst methyl benzene 3-propanoate (peak 6) was the most abundant compound in the elm specimen.

The TMAH extractive of teak had a higher abundance of significant biomarker compounds in the extractives. These compounds were identified as 5-bornene (a bicyclic monoterpenoid), 1,7,7-trimethyl bicyclo [2.2.1]hept-2-ene (peak 7), 3,4-dimethoxystyrene (peak 8), methyl 3-(3,4-dimethoxyphenyl) 2-propenoate (peak 9). 2-Phenylindane 1,3-dione (phenindone) (peak 10), a compound having structural analogy to, and the same molecular weight as, tectoquinone (2-methyl, 9,10-anthracenedione) which is a known extractive of teak (Hillis 1962), methyl 3-(3,4,5-trimethoxyphenyl) 2-propenoate (peak 11) and squalene (peak 12) were detected.

Scrutiny of the pyrolysis profiles of these extractives shows that 3,4-dimethoxybenzaldehyde (peak 2), methyl 3,4-dimethoxybenzoate (peak 3), 3,4,5-trimethoxybenzaldehyde (peak 4) and methyl 3,4,5-trimethoxybenzoate (peak 5) are found in significant proportion in beech, elm and oak although they were less significant in teak. The presence of these dimethoxy and trimethoxy compounds facilitated their identification as angiosperms. The profile of teak was the only wood which had a distinctly different profile when these hardwoods were examined by THM-GC.

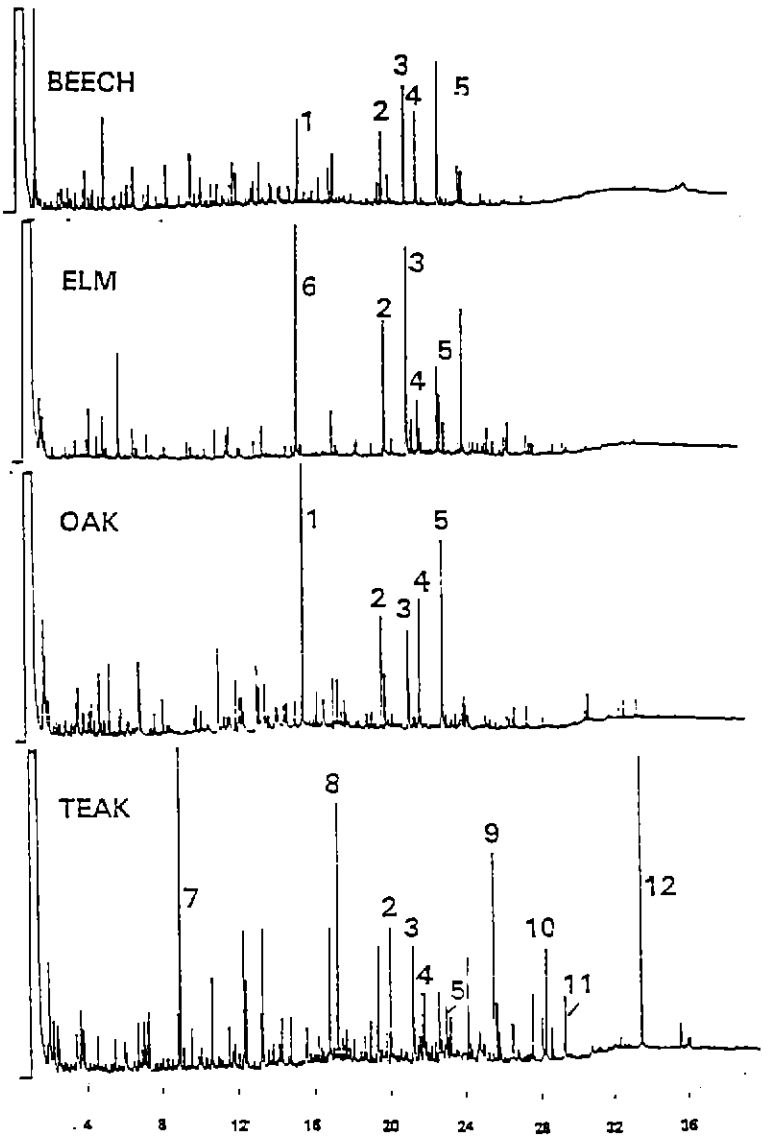


Figure 9.4. Chromatograms showing Py-GC of TMAH extracts of beech (*Fagus sylvatica*), elm (*Ulmus procera*), European white oak (*Quercus robur*) and Burma teak (*Tectona grandis*).

Key:- 1= 5-hydroxy, 2-hydroxymethyl pyranone (tentative identification), 2= 3,4-dimethoxybenzaldehyde, 3= methyl 3,4-dimethoxybenzoate, 4= 3,4,5-trimethoxy-benzaldehyde, 5= methyl 3,4,5-trimethoxybenzoate, 6= methyl benzene 3-propanoate, 7 = 1,7,7-trimethyl bicyclo [2.2.1]hept-2-ene, 8= 3,4-dimethoxystyrene, 9= methyl 3-

(3,4-dimethoxyphenyl) 2-propenoate, 10= 2-Phenylindane 1,3-dione (phenindone) 11= methyl 3-(3,4,5-trimethoxyphenyl) 2-propenoate, 12= squalene.

9.4.5 Intra-genus Differences in Extractives Composition

A group of tropical hardwoods of the *Shorea* genus were examined to ascertain if this genus had a common general extractive profile, while providing differentiation between species. Seven of the nine species had common features in their profiles. The other two species had distinctly different profiles (Figure 9.5) Significant extractives, common to seven of the nine species, were methoxy-benzene (peak 1), 4-methoxy-toluene (peak 2) and a compound (peak 3) having a base peak m/z 188. Therefore, there are common features in the profiles which can be used to identify the majority of the species examined. However, interspecies variations were present, particularly in red balau and red meranti species. These tropical woods were also identified as angiosperms by the presence of dimethoxy- and trimethoxy-substituted aromatic compounds. Methoxy-substituted benzaldehyde and benzoic acid derivatives were particularly useful for these identifications as was observed in the previous hardwood extractive profiles.

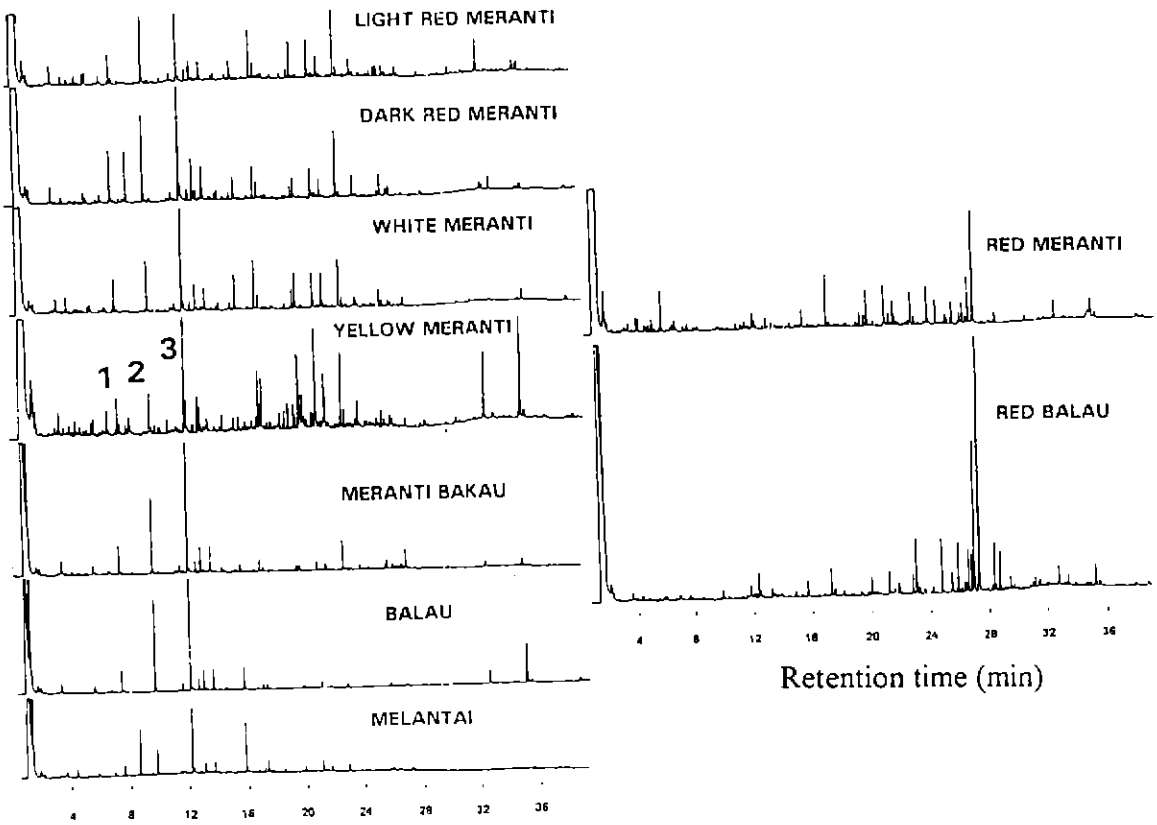


Figure 9.5. Chromatograms showing Py-GC of TMAH extracts of nine species of the *Shorea* genus.

Key:- 1= methoxy-benzene, 2= 4-methoxy-toluene, 3= an unknown compound having a base peak m/z 188

9.4.6 Within-species Differences in Extractives Composition

Jarrah (*Eucalyptus marginata*) is a commonly used construction timber in Western Australia. The pyrolysis profiles of TMAH extracts of several specimens obtained from different sources were compared (Figure 9.6). The major differences in the pyrograms are reflected in the relative proportions of dimethoxy- and trimethoxy-benzoic acid methyl esters to the other extractives. The proportions of these particular extractives may be related to environmental factors, such as growing locality, age and soil conditions.

Significant extractives comprise the compound with base peak of m/z 188 (peak 1), detected in many *Shorea* species, 3,4-dimethoxytoluene (peak 2), acetyl methoxystyrene (peak 3), 3,4-dimethoxystyrene (peak 4), 2,6-dimethoxyphenol (syringol) (peak 5), 3,4-dimethoxybenzaldehyde (peak 6), methyl 3,4-dimethoxybenzoate (peak 7), 3,4,5-trimethoxybenzaldehyde (peak 8) and methyl 3,4,5- trimethoxybenzoate (peak 9).

The procedure may be used to differentiate a particular timber from other timbers of the same species through a comparison of the relative peak height ratios in the profiles. These relative peak height ratios were sufficiently reproducible to permit discrimination of the jarrah specimens, but further experiments are required in order to validate these observations. Unique biomarkers which may assist in the unequivocal identification of this *Eucalyptus* species, have not been found at this stage.

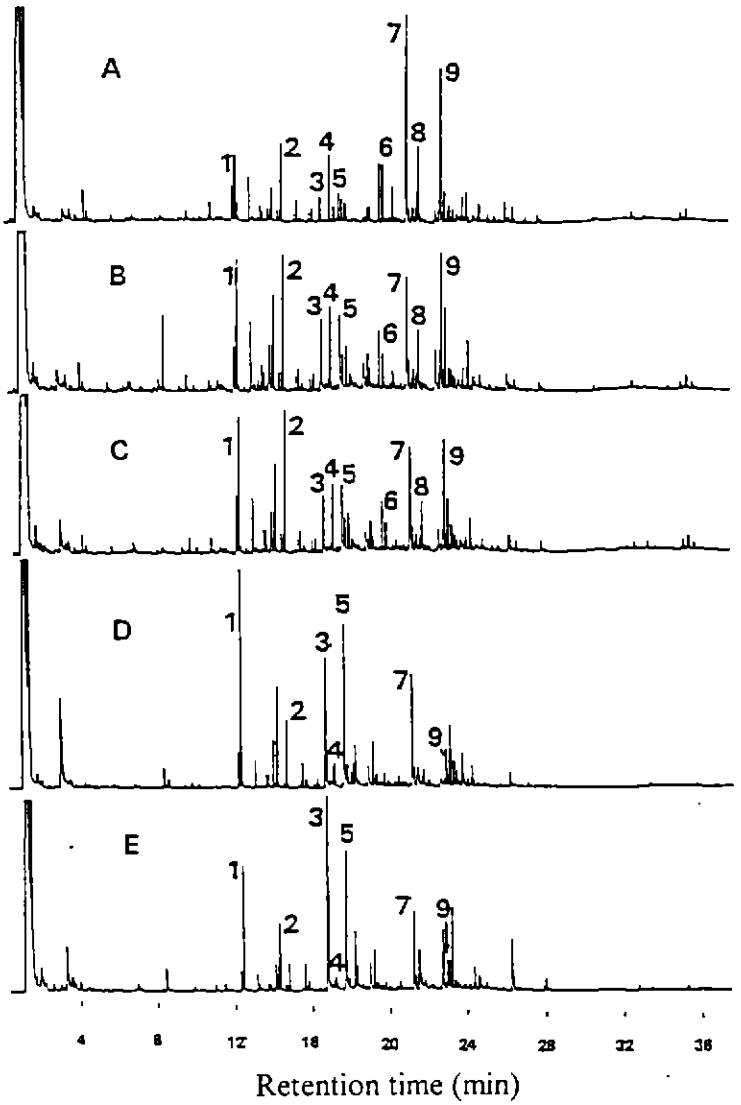


Figure 9.6. Chromatograms showing Py-GC of TMAH extracts of five jarrah specimens from different sources.

Key:- 1 = unknown compound with base peak of m/z 188, 2 = 3,4-dimethoxytoluene, 3 = acetyl methoxystyrene, 4 = 3,4-dimethoxystyrene, 5 = 2,6-dimethoxyphenol (syringol), 6 = 3,4-dimethoxybenzaldehyde, 7 = methyl 3,4-dimethoxybenzoate, 8 = 3,4,5-trimethoxybenzaldehyde, 9 = methyl 3,4,5- trimethoxybenzoate.

9.4.7 Origin of Extractives

The compounds extracted by TMAH may result from hydrolytic scission of the lignin polymer or lower molecular weight lignans. Alternatively, the compounds which are detected may be present in the free state rather than combined with other functionalities. These compounds would readily be extracted by organic solvents to give similar results as obtained by the classical methods of isolation of extractives.

The evolution of natural products, including these oligomeric and monomeric compounds is described by Gottlieb (1989).

For example, a methanol extract of teak was found to contain mainly tectoquinone and squalene, which were among the products detected in the pyrolysis profile of the TMAH extract. Co-injection of this methanol extract of teak with aqueous TMAH solution (pyrolytic methylation (Kossa *et al.*, 1979)), produced the dimethoxy- and trimethoxybenzenoid compounds, found in the TMAH extracts. These compounds were probably lost by column adsorption or thermal degradation e.g. decarboxylation, when the non-derivatised methanol extract was chromatographed. The methanol extracted compounds and, consequently, the TMAH extractives, were present in the wood in the unbound form and are not the products of alkaline degradation of lignocellulose or lower molecular lignans.

Similarly, no significant methanol extractives were detected in the GC profiles of the jarrah specimens. Pyrolytic methylation of the same extract gave methyl 3,4,5-trimethoxybenzoate as a major extractive with a profile similar to that obtained by pyrolysis of the TMAH extract. The results again indicated that the major extractives, as determined by pyrolysis of the TMAH extractives, were present in the uncombined form.

Methyl 3,4,5-trimethoxybenzoate could originate from 3,4,5-trihydroxybenzoic acid (gallic acid) or from 4-hydroxy 3,5-dimethoxybenzoic acid (syringic acid) by complete methylation. When TEAH was used as the extraction reagent, the major product (retention time 25 minutes) was identified as ethyl 4-ethoxy- 3,5-dimethoxybenzoate (Figure 9.7).

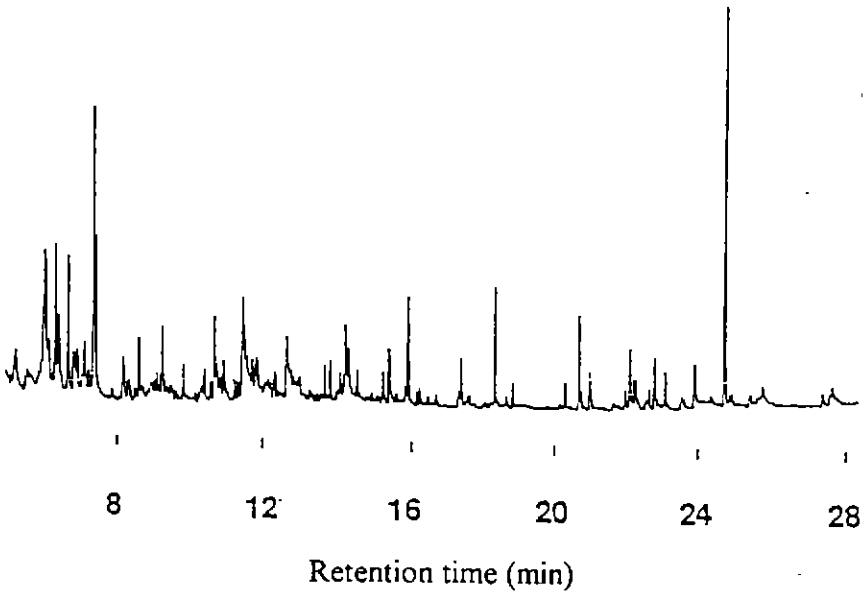


Figure 9.7. Chromatogram showing Py-GC of a TEAH extract of jarrah.

Since ethylation takes place at the free hydroxyl groups, the parent compound extracted is syringic acid (4-hydroxy- 3,5-dimethoxybenzoic acid). Therefore, the major extractive in this specimen of jarrah was present as syringic acid and not as gallic acid. The likely origin of this compound is as a precursor of hardwood lignocellulose, which contains syringyl - propane units. This hydrolysis and ethylation reaction could be considered as a parallel approach to that used for hydrolysis and butylation of acetate, methacrylate and cyanoacrylate polymers where TBAH was employed (Chapter 3).

Thus, the majority of teak and jarrah TMAH extractives do not necessarily require basic conditions to be released from the lignocellulose polymer. However, they must be converted to their alkyl derivatives in order to assist identification by GC. Their structure was consistent with the guaiacyl / syringyl structure of lignin.

9.5 SUMMARY

The pyrolysis of TMAH extractives provides a more specific characterisation method for wood than pyrolysis derivatisation of whole wood (Chapter 8), because base

soluble extractives are isolated as alkyl derivatives. Most of these extractives are usually too polar or thermally labile to be detected.

Some of the wood species have specific biomarkers present, whilst almost all of the extracts contain guaiacyl and/or syringyl indicators for softwood or hardwood botanical classification.

These studies indicate that similarities exist in the TMAH extractive composition in species within the *Pinus* genus and within the *Shorea* genus. Variations found in TMAH extractive composition within the *Eucalyptus marginata* species may be due to maturity and environmental factors.

CHAPTER 10

CONCLUSIONS

In this work, a rapid, high temperature chemical degradation reaction technique has been developed and applied to the chemical structure determination of a wide range of mainly polymeric organic materials. Submicrogram quantities of the organic materials are flash heated in a pyrolyser with an aqueous solution of a quaternary alkylammonium hydroxide. The products are identified on-line by GC-MS.

The mechanism of the reaction is a concerted hydrolysis and alkylation process, involving thermal degradation of the quaternary alkyl ammonium salts of the hydrolysed products to form alkyl derivatives. TMAH is the most useful derivatising reagent of the quaternary ammonium hydroxides for the production of the methyl derivatives. The reaction is termed "thermally assisted hydrolysis and methylation" (THM). Higher alkyl homologues are employed where methyl derivatives are not appropriate, particularly in the characterisation of natural products, where it may be necessary to determine sites of pre-existing methylation.

Aromatic and aliphatic polyesters, alkyd resins, lipid triglycerides and vegetable waxes react to produce methyl esters of the carboxylic acids and methyl ethers of the alcohol or polyol. THM-GC of phenolic polymers, including wood lignin and extractives gives methyl derivatives including those of aromatic carboxylic acids and polybasic aliphatic carboxylic acids. These acids are not normally detected by conventional Py-GC due to effects such as decarboxylation and analytical column adsorption. Proteins undergo hydrolysis and methylation of the carboxylic acid and amino groups in the respective amino acids. Pyrolysis of TMAH extractives of wood, soils and sediments are useful for chemotaxonomy and chemical structure identification of source material.

The procedure has advantages when compared to chemical degradation methods of determining chemical structure of organic materials because the THM method is suitable for submicrogram quantities and does not require lengthy manipulation steps. THM-GC-MS gives more information about the chemical structure of a polymer containing hydrolysable linkages than Py-GC and may be used in conjunction with the Py-GC technique to assess such chemical characteristics of the polymer.

CHAPTER 11

FUTURE DIRECTIONS

The THM reaction has been shown to be dependant on the susceptibility of ester, ether and other hydrolysable groups in the macromolecular chain to attack by base. The products of hydrolysis are conveniently converted to alkyl derivatives by the thermal fragmentation of the tetraalkylammonium salts.

Other high temperature reactions which could be developed to facilitate the structural characterisation of macromolecules using the pyrolysis technique are possible:-

- Triglycerides were found to be converted to hydrocarbons when they were intimately mixed with aqueous potassium hydroxide and flash heated. This occurs through hydrolysis and thermal decomposition of the carboxylate salt by a Kolbe reaction, and may have potential uses as a pyrolytic technique. The process of heating coconut oil with alkali has been recently reported as a method for producing a fuel said to have a composition similar to diesolene (media reports 1995).
- High temperature acid hydrolysis reactions would be potentially suitable for hydrolytic cleavage of polyamides in synthetic polymers and natural products such as proteins. Aqueous HCl, as an acid source, is easily lost in a flash pyrolysis process due to evaporation. Despite this drawback, some success has been attained by the acid hydrolysis of cellulose materials, such as cotton and viscose rayon fibres (Challinor 1990b), which may be identified by the detection of 2-furancarboxaldehyde. Recently, oxalic acid has been used successfully in the Py-GC based high temperature acid hydrolysis of chitin for the determination of the degree of acetylation (Sato, *et al.*, 1998).

The structure determination of polyamides by acid hydrolysis has been reported by Onishi *et al.* (1994) *In vitro* hydrothermal decomposition with *in situ* methylation of the resultant dicarboxylic acids and acetylation of the diamines on 100 μg of the polyamides was achieved. The polyamide was heated with HCl / methanol / acetic acid mixture in a sealed tube at 235^{o}C. in a furnace pyrolyser oven for 5 minutes, then the tube was broken and the products thermally desorbed into a gas chromatograph. More suitable hydrolysis and derivatising reagents for the flash pyrolysis process of polyamides would achieve a single step reaction.

- The oxidation of double bonds in unsaturated organic compounds by aqueous potassium permanganate with cleavage of the molecules is a classical method for the determination of sites of unsaturation. By choosing a suitable reagent it may be possible to achieve a rapid single step determination of sites of unsaturation in organic compounds by employing flash heating conditions.

- Phenols are converted to the parent hydrocarbon when mixed with finely divided zinc at a temperature of approximately 450°C. In preliminary experiments it was found that 1-naphthol was converted, in part, to naphthalene when flash heated using a 770°C Curie point wire under the same conditions used in the THM reaction. The reaction did not proceed at 358°C (Challinor, unpublished work).

- Organic matter in soils and sediments contains lignin derived phenolic components with some carboxylic acid functionality, carbohydrates and fatty acid components. The separated humic and fulvic acid fractions have recently been examined using the THM-GC method (Martin *et al.*, 1994; Martin *et al.*, 1995a). Whole soil organic matter was examined by an off-line methylation reaction with TMAH at elevated temperature, followed by Curie point Py-GC and pyrolysis field ionisation mass spectrometry (Schulten and Sorge, 1995, Schulten *et al.*, 1996). Whole soil which contains a low proportion of organic matter may also be determined by the same method used for wood extractives (Chapter 9). The method has the advantage that larger volumes of material may be treated. The results at this stage appear promising and could assist in the determination of the cause of hydrophobicity in agricultural soils and would also have forensic applications.

- Organic pigments in paint binder matrices are rarely identifiable by techniques such as FTIR and Py-GC. . In isolated cases, fragments of diazo compounds have been detected by Py-GC (Challinor, unpublished work). It may be possible to design "softer" chemolytic reactions to identify specific pigment and dye classes, such as anthraquinones, phthalocyanines, indanthrones and diazo dyes.

- Trimethylsulphonium hydroxide has been used as a derivatising reagent for fatty acids in triglycerides (Butte, 1983). This reagent should be considered as a

replacement for TMAH in the THM reaction. Proteolytic enzymes may also have a useful function as a pre-reaction step before high temperature derivatisation.

- Ester groups in polymer side chains may be determined by specific reactions. It has been shown (Chapter 3) that polyvinyl acetate and polycyanoacrylates can be reacted with TBAH to give butyl acetate and cyanoacrylate, respectively. Polyacrylate resins could possibly be reacted with lower molecular weight organic acids after side chain hydrolysis, to give corresponding derivatives.
- Further work on wood TMAH extractives to expand the database is justified with the aim of providing a rapid, simple means of chemotaxonomy.
- A number of comments have been made in the thesis regarding future investigations which may be undertaken in specific areas:

(i) Some of the minor products from the THM reaction of white beeswax were not identified (Chapter 4, Section 4.4.2).

(ii) Identification of the THM products of the proteins, collagen, keratin, albumin wild silk and Merino wool require work on the interpretation of their mass spectra (Chapter 4, Section 4.4.4).

(iii) Molecular fragments from the THM reaction of the drug, haloperidol were not detected under the GC conditions used (Chapter 4, Section 4.4.5)

(iv) The variable yields of dimethyl orthophthalate in the THM reaction of alkyd resins should be studied (Chapter 5, Section 5.4.2).

(v) Confirmation of the identity of methyl ricinoleate in hydrogenated castor oil - modified alkyd resins is required (Chapter 5, Section 5.4.3).

(vi) Gas chromatographic resolution of RAMES (peaks 3 and 4) was not achieved under the conditions used in the analysis (Chapter 6, Section 6.4.3

(vii) Interpretation of some of the mass spectra of phenol-formaldehyde resin THM products is required (Chapter 6, Section 6.4.4).

(viii) A study of the effects of maturity on the THM products of heartwood is justified (Chapter 8, Section 8.4.1).

(ix) The THM products of Burma teak were not identified. Work in this area is required (Chapter 8, Section 8.4.2).

(x) THM-GC-MS studies on the variations in heartwood composition of the same species which have different age and geographical location would constitute a useful project (Chapter 8, Section 8.4.2).

(xi) The dependance of composition on the age, geographical location and growing climate of heartwoods would be a useful study (Chapter 8, Section 8.5).

(xii) Validation of the discrimination of different jarrah specimens by relative peak height ratios of the THM extracts is required (Chapter 9, Section 9.4.2).

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