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Organic Mass-Spectrometry of Art Materials: Work in Progress

John Mills and Raymond White

Introduction

The problems inherent in the detection and identification of the natural organic materials used in art objects such as paint media, varnishes, and so on have been outlined by us in a previous article [1]. Effective analysis usually involves separating these complex mixtures into their constituent chemical components, the pattern of these being characteristic for the natural material as a whole. One of the most efficient ways of doing this on the necessarily very small samples is to employ gas-liquid chromatography but the components so separated can only be identified by comparison with the gas-chromatographic behaviour of known standards. Selection of these standards must involve an initial guess as to what the compounds are likely to be, for unfortunately gas-chromatography conveys very little intrinsic information regarding chemical structure. With uncommon samples such guesswork often fails and in any case identification simply on the basis of retention times can be unreliable especially when many peaks are present. We had long felt the need for a more fundamental method for identification of unknowns. The technique of mass-spectrometry provides such a method and the Scientific Department acquired a Kratos MS25 gas-chromatography – mass-spectrometry system with associated data-handling equipment at the end of 1979 (Fig.1). The aim of this article is briefly to explain the fundamentals of mass-spectrometry to those unfamiliar with it and to indicate some ways in which it is being used to investigate the chemistry of dried oils, to develop new analytical approaches, and to solve straight analytical problems.

Mass-spectrometry [2]

The mass-spectrum

In essence a mass-spectrum consists of the pattern of fragment ions which results when a molecule is ionized at a certain energy level in a mass-spectrometer. This ionization is effected in the

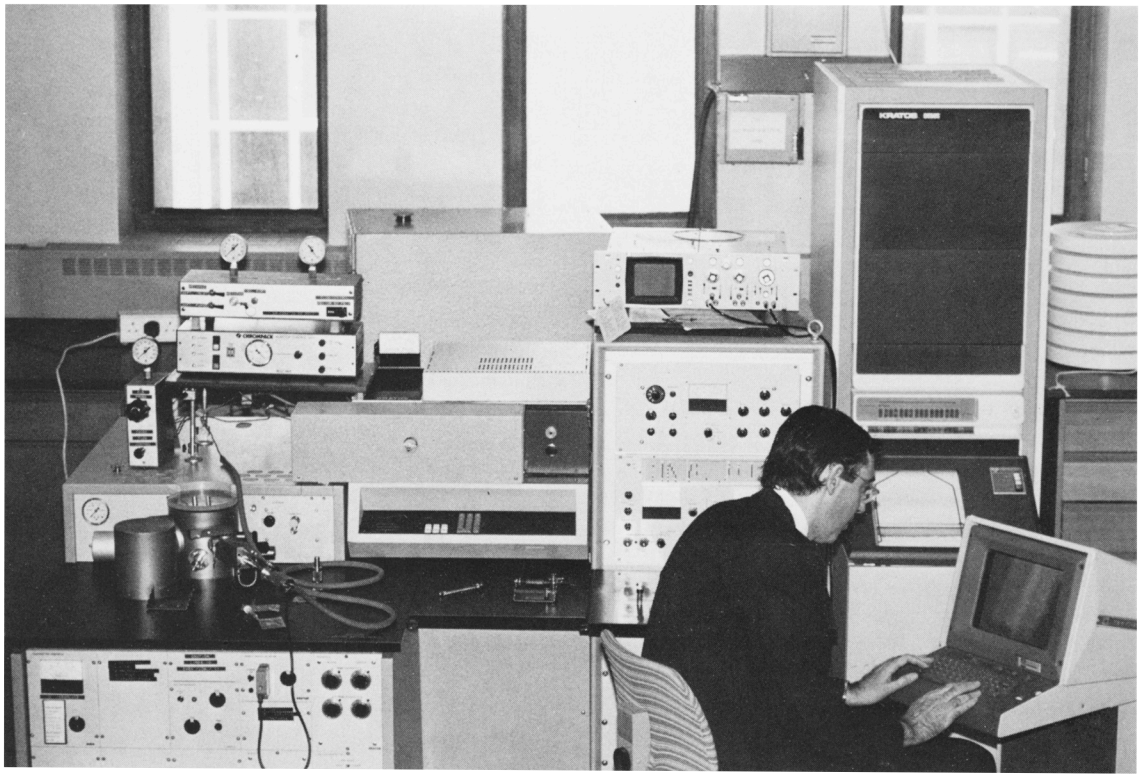

Figure 1 The Kratos MS25 gas-chromatography – mass-spectrometry apparatus installed in the National Gallery Scientific Department. In the centre of the main unit is the gas-chromatograph with its small control keyboard. To the left and above it is the Chrompack injection system with controls for carrier-gas supplies. The cylindrical structure in front of this contains the mass-spectrometer source while the analyser unit is concealed below and behind this. The spectrometer electronics unit lies behind the operator who is seated at the Tektronix visual display unit. Behind this in turn are the Versatec printer/plotter and the Data General Nova 3 computer and disk drive unit. Some cartridge disks for data storage are piled to the right of this.

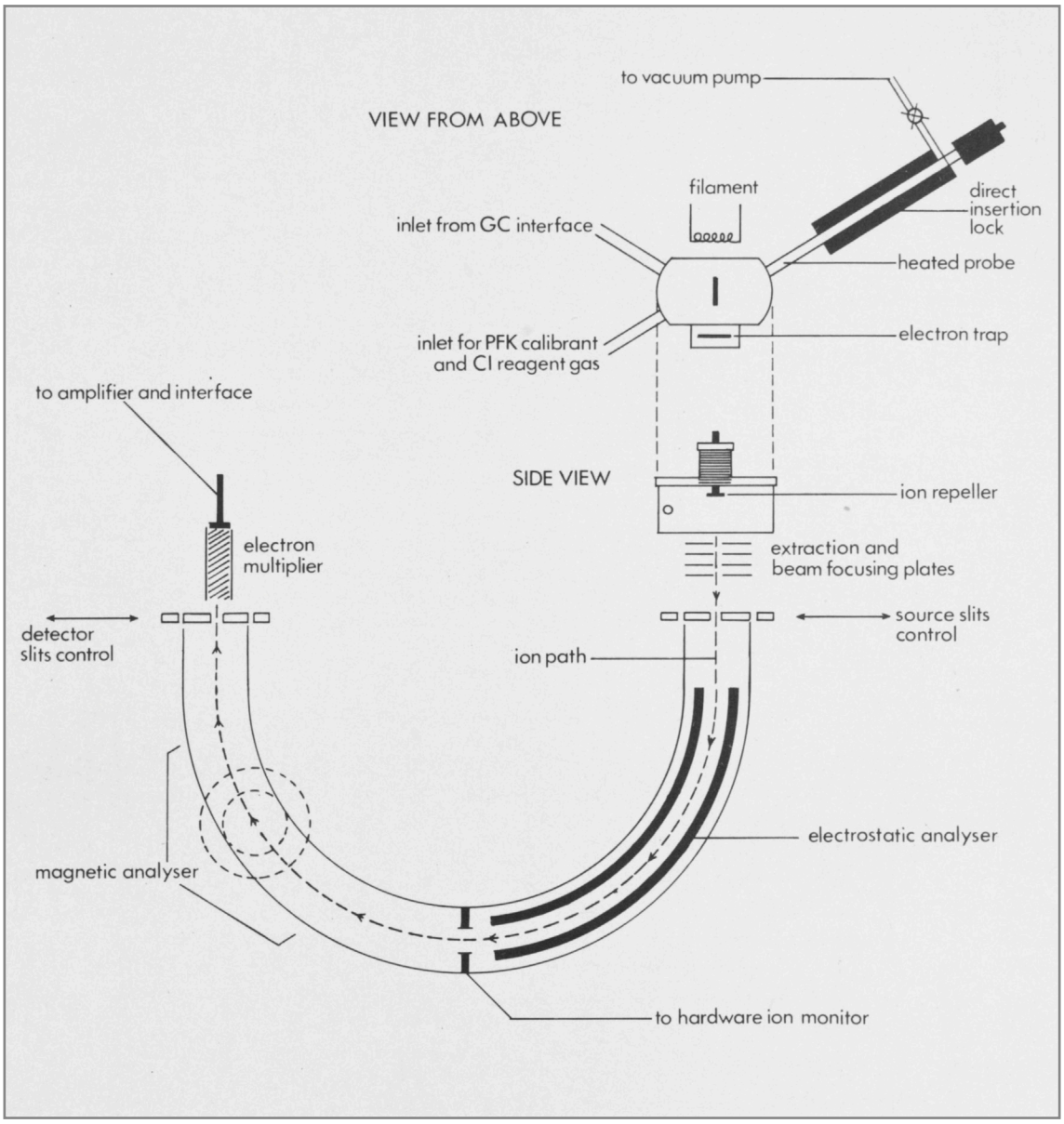

Figure 2
Diagrammatic representation of a mass-spectrometer.

vaporized state at low pressure by bombarding the compound with electrons or an ionized gas. The positive ions which result are accelerated into a magnetic field and dispersed thereby into a spectrum according to their mass-to-charge *(m/e)* ratio. Since essentially only singly-charged ions are produced this reduces to a spectrum of mass alone. Determination of the relative intensities of these fragment masses gives a *fragmentation pattern* or *fragmentogram* which is usually sufficiently distinctive to identify the original molecule.

The spectrometer

The spectrometer is shown diagrammatically in Fig.2. It consists of three regions: the source with its associated sample inlet system, the analyser region, and the electron-multiplier detector. A system of backing and diffusion pumps maintains a low pressure within the spectrometer. The vaporized sample, consisting of uncharged molecules, diffuses into the ionization chamber of the source where it is bombarded by a highly collimated electron beam which is passing between a heated tungsten filament, maintained at a negative potential, and the anode or trap on the other side of the chamber. The electron beam energy can be varied from 10 to 100 electron volts by varying the potential difference between the cathode emitter and the trap. At a certain electron energy sufficient energy will be imparted to a molecule to cause one of its electrons to be ejected leaving a molecular ion. If the beam energy is raised by about 10 eV above this, then fragmentation as well as ionization of the molecule will result. The positive ion fragments so formed are directed out of the chamber through a slit by means of a repeller electrode maintained at a positive potential, and the ion beam is then focused and accelerated by a potential of several kilovolts into the analyser region where separation by mass occurs. A double focusing system is employed, the beam elements being brought to a point image with the electrostatic analyser and then scanned across the detector slit by varying the field of the magnetic analyser from high to low field strength, thus bringing each ion mass in turn to focus on the

detector. The electron multiplier detector works on a cascade system and can give gains of the order of 10^6, so compensating for the low efficiency of ion production in the source and losses by collision in the analyser section.

Ionization modes

The foregoing description covers ionization using the simple electron impact technique. The highest mass in the spectrum so obtained, that of the molecular ion, gives the molecular weight of the compound which is probably the single most important item of information yielded to the chemist by the method. However it may be that in some cases where the molecule is particularly vulnerable to fragmentation by virtue of its structural features that the molecular ion may be absent or so weak as to preclude its resolution from the background. This is so with highly branched aliphatic hydrocarbons, for example, and often with hydroxyl compounds which easily lose the elements of water. It may sometimes be possible to minimize bond disruption by reducing the electron beam energy to a low electron voltage but this often introduces problems in source stabilization or too greatly reduces ionization efficiency.

To overcome excessive fragmentation a technique has been developed known as chemical ionization. This relies on introducing a so-called reagent gas (examples are methane, butane and ammonia) into the source and ionizing this. As a result of collisions, excited reagent gas species then pass on sufficient energy to the substrate molecules to ionize them in turn, but without sufficient energy to cause much fragmentation. Strictly speaking the ion which results is not a molecular ion but rather a pseudomolecular ion resulting from addition of ions from the reagent gas. Thus for example ammonia yields ions of molecular weight plus one and molecular weight plus eighteen, corresponding to molecular weight plus hydrogen ion and ammonium ion respectively. Relative proportions of each depend on the basicity of the compound with respect to ammonia. We have found ammonia to have considerable advantages over methane or butane in that these two gases quickly contaminate the source.

Sample introduction

Samples may be introduced into the source in either of two ways. The simpler of these is by means of the heated probe which permits direct vaporization of (or from) the sample into the ionization chamber. The sample (typically 1 μg or less of pure compound) is placed in a short capillary tube sealed at one end and this is inserted into the tip of the probe. The probe itself can be electrically heated, is equipped with a temperature measuring device, and also has cold air cooling to forestall premature evaporation of the sample once the probe is inserted into the low-pressure source through the evacuated insertion port. The sample is then vaporized by controlled heating while repeatedly scanning through the mass range of interest. This technique is most suitable for pure compounds since, obviously, mixtures will give mixed spectra though advantage can be taken of the partial fractionation effected by the heating schedule of the probe. Also, characteristic masses for particular individual compounds can be sought for in the spectra of mixtures, as will be demonstrated in an example described below.

As implied earlier the full analytical potential of the mass-spectrometer is realized when the input to the source has already been separated into individual components by means of gas-chromatography. In our system quartz capillary chromatography columns are used and connected to the source through an interface. Since the ion source must operate at low pressures of the order of 10^{-6} torr the inflow of gas from the chromatograph must not exceed 1 ml per minute or the diffusion pumps will not be able to maintain the low pressure. In fact the flow-rate for the capillary column is *c.* 0.5 – 1 ml per minute and the column could be connected to the source via the direct line. In practice however it has been found better to make up the gas flow with more helium to about 30 ml per minute and pass this to the glass jet separator whose function is to effect the change in operating pressure (*c.* 1 bar in the column, 10^{-6} torr in the source) and to separate and pump away most of the helium. To prevent any condensation of organic material from the gas stream, the lines, jet separator, and source are all heated.

While the gas-chromatogram is being run, scanning through the mass range of interest is carried out every few seconds. Progress is observed by the simultaneous display of the chromatogram, on a visual display unit (VDU), constructed out of single points per scan, the points representing a summation of the individual ion intensities for the scan to give a total ion current.

Output and data-handling

The amount of information accumulated by the spectrometer during a capillary gas-chromatography run is prodigious. Scanning rates are typically 0.5 or 1 second per mass decade and two-thousand or more scans may be made during a run. To process the data and convert it into useful form a computer is essential. Nothing has been said so far as to how the ion masses are actually measured: in fact this is done by comparison with the spectrum of a standard (perfluorokerosene, PFK) whose masses and their intensities are accurately known. The data actually recorded from the spectrometer consist of digitized samples of the signal from the detector in the form of voltage/elapsed-time-from-start-of-scan pairs. The time-to-mass conversion, based on the standard, is carried out by the computer and the mass/intensity data is then written onto a disk storage unit consisting of a 5 megabyte fixed disk, holding all program software, and a 5 megabyte removable disk to record the mass-spectral data.

Once the above data are recorded on disk they can be utilized in many ways as will be demonstrated by the examples to follow. There is no need to detail all the available facilities here but they include reconstruction of the chromatogram either as total ion

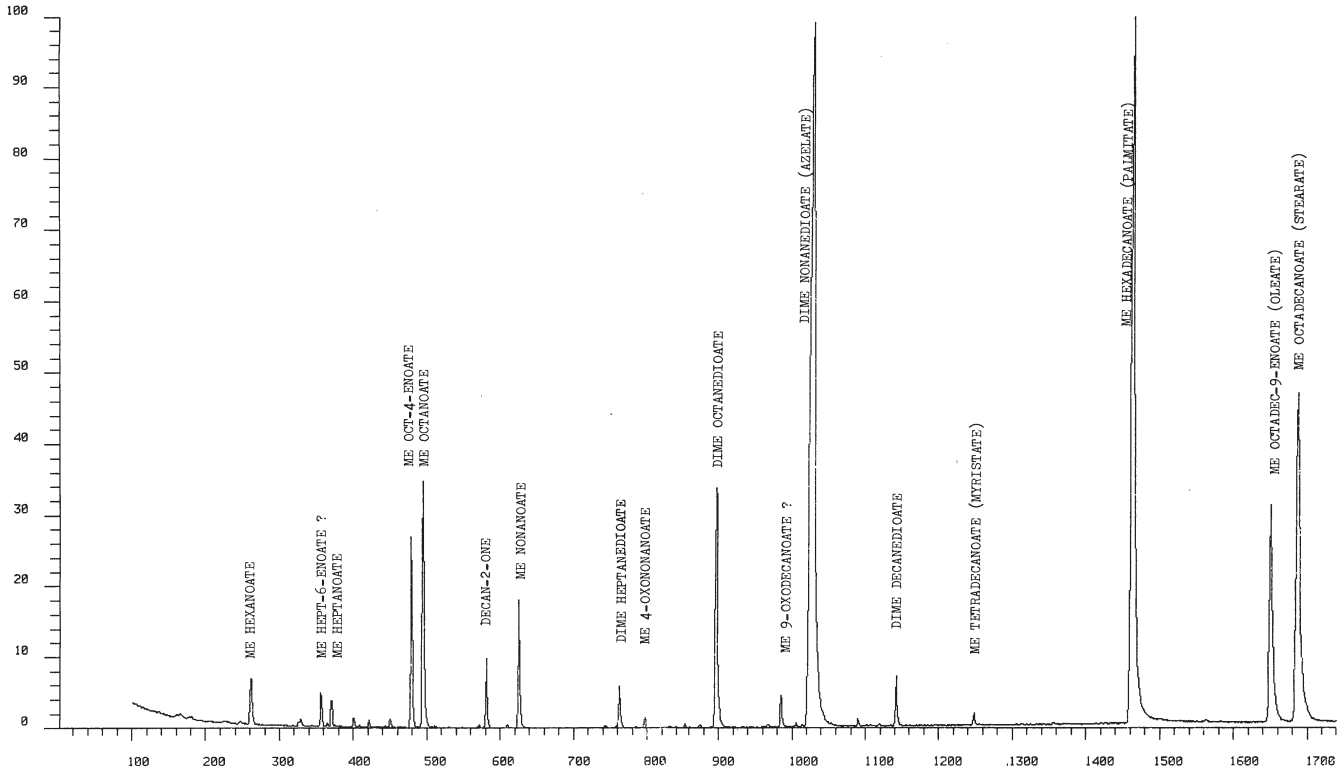

Figure 3 *(Above)* Chromatogram yielded by a 2.5-year-old paint film of raw linseed oil and smalt after saponification followed by methylation of the acids. The scans have been background-subtracted and the chromatogram has been constructed by summing the total ions of each scan. The peaks have been normalized to palmitate peak = 100%. The separation was effected on a 50 m quartz capillary column coated with SE30, temperature programmed from 90° (3 minutes) at 4°/min to 210°. Scanning was carried out at a rate of 0.5 second/mass decade.

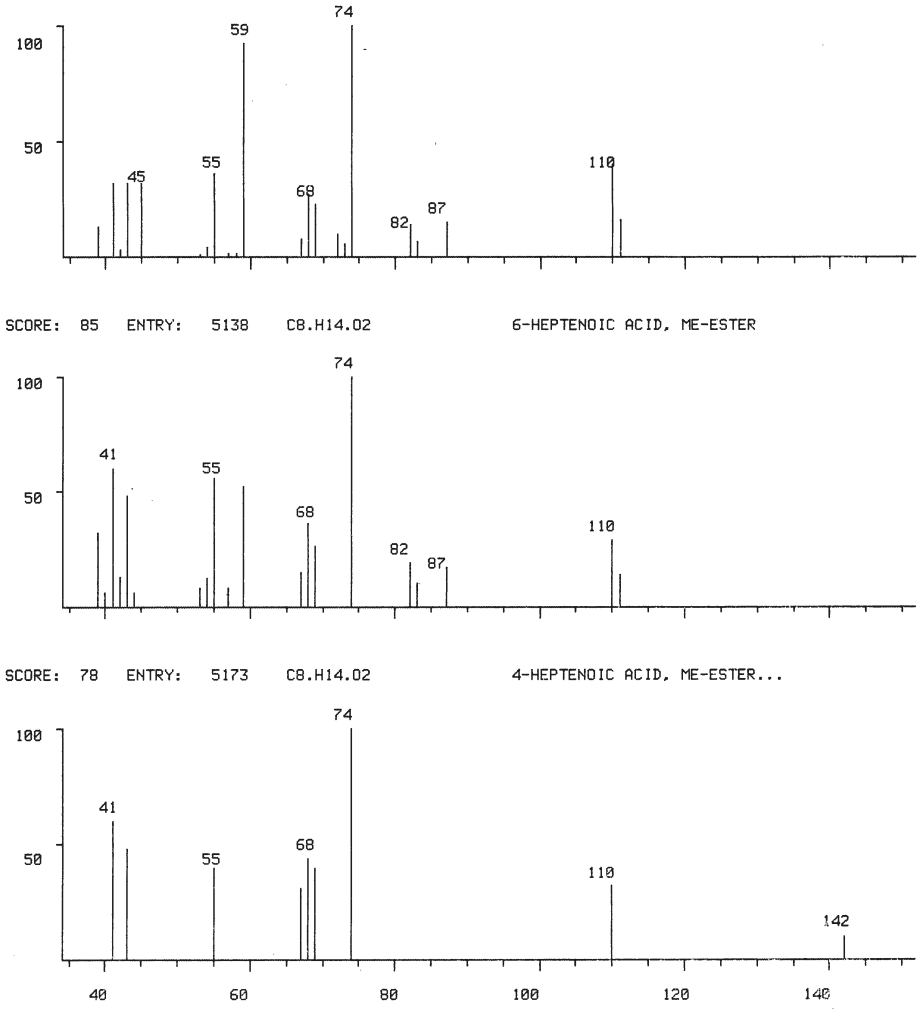

Figure 4 *(Right)* The mass-spectrum of scan 356 of Fig. 3 compared with the library spectra of the top two matches found by the library search routine.

or selected ion plots; reportage of individual mass-spectra either as listings or bar-charts; a library search procedure for matching unknown to library spectra stored in condensed form on disk; a subtraction facility which permits 'cleaning-up' of spectra by removing contributions due to lock masses (that is, oxygen and nitrogen from air), stationary phase bleed from the column, and background source contamination. In addition, by normalizing to carefully selected peaks, this algorithm can be used to resolve the spectra of two partially overlapping chromatographic peaks. This particularly powerful facility is well illustrated below.

Dried oil study

It is now almost twenty years since gas-chromatography was first applied to the study of the composition of dried drying oils [3], and the general picture of the fatty and dicarboxylic acid content has been confirmed and added to by several groups of workers [4 – 7]. However, among the unsolved problems of paint medium analysis there remains that of differentiating, in the dried state, between raw drying oils and prepolymerized (stand) oils. Prepolymerization of drying oils by heat or light in the relative absence of air involves the formation of carbon – carbon linkages between the unsaturated fatty acids of the triglycerides, rather than the oxy- or peroxy- linkages which result predominantly from the normal drying in the presence of air. The resulting differences of structure will thus be reflected in the composition of the polymer network, and of the polymerized fatty acids liberated when the dried oil is saponified, rather than in the composition of the main fatty and dicarboxylic acids sufficiently volatile to be observed by gas-chromatography. It is possible however that some differences might be found in the pattern of minor degradation products which occur alongside these, either qualitatively or in their relative proportions, and as a first step towards studying this it seemed desirable to identify as many as possible of the minor peaks observed by GLC.

In December 1978 we had prepared a number of paint films for experimental purposes involving a selection of pigments (lead white, light red, smalt, verdigris, vermilion) and both simple and mixed media, including both raw and stand linseed oil. The composition of these, after saponification and methylation, was checked after about thirty months. No qualitative differences were found but there were quantitative differences in the minor components both between the raw and the stand oil films and also between films of the same oil with different pigments. It was found possible to identify most of the minor peaks using the library search facility of the data system and they are so identified for the raw linseed oil/smalt film shown in Fig.3. The compounds found, mostly short-chain acids, are such as would be expected. With the unsaturated short-chain acids the identifications are not entirely unambiguous since the search comes up with two or three double bond isomers as possible matches, and we had no reference sample on which to run our own spectra for more precise checking. Library spectra are incomplete in that, to conserve space on disk, only up to twenty of the most intense mass peaks are stored in coded form. Exact matches cannot be expected. Fig.4 shows the spectrum of scan 356 in comparison with the search routine's top two matches. In this instance no molecular ion (142) was present in the spectrum.

In an examination of the unmethylated saponified material from the paint films several short-chain ketones and aldehydes were also identified. These were very minor components however and do not show up as significant peaks on the chromatogram of the methylated material. The proportions of the minor components found were, as stated already, dependent upon the pigments present as well as the nature of the oil. Without a full-scale multivariate analysis of the results it seems unlikely that the differences due to the latter alone could be separated out. This has not been pursued so far since it seems unpromising and troublesome as an analytical method. Moreover the short-chain compounds, being significantly volatile, are probably mostly lost from the film in the course of time.

Rather consistent differences between the stand oil and the raw oil paint films, however, were noticed in the relative proportions of the three principal dicarboxylic acid esters: dimethyl octanedioate, dimethyl nonanedioate (azelate), and decanedioate. The raw linseed oil films had consistently higher ratios of nonanedioate to either of the other two esters than did the stand oil films. Thus the average ratios of the heights of these peaks for the five differently pigmented films were nonanedioate/octanedioate: raw oil 7, stand oil 2; nonanedioate/decanedioate: raw oil 25, stand oil 4. Whether these findings will prove to be consistent for other oil samples or to have any significance in practice remains to be seen.

Cholesterol in egg fats

A difficult problem in the analysis of paint media is the detection of egg in the presence of drying oil. The presence of egg in oil is sometimes suggested by gas-chromatography when the proportion of azelate is lower than would be expected from oil alone but this needs confirmation since the amount of azelate is quite variable anyway due to a number of unquantifiable factors. Staining techniques on cross-sections come in useful here but still sometimes give confusing results. Positive detection of egg protein, perhaps by amino acid analysis, would be the definitive method of detection but this is not usually feasible since it requires a second and usually impossibly large sample. Some years ago it was suggested [8] that different oils and egg yolk could be distinguished and identified by the thin-layer chromatography of their non-saponifiable components, which consist mainly of sterols. A paper from this laboratory [9] showed later that these claims went too far but that nonetheless significant amounts of sterols could survive in paint films under certain conditions and be detected by gas-chromatography. It seemed worthwhile to look into the possibility of detecting cholesterol in paint films by

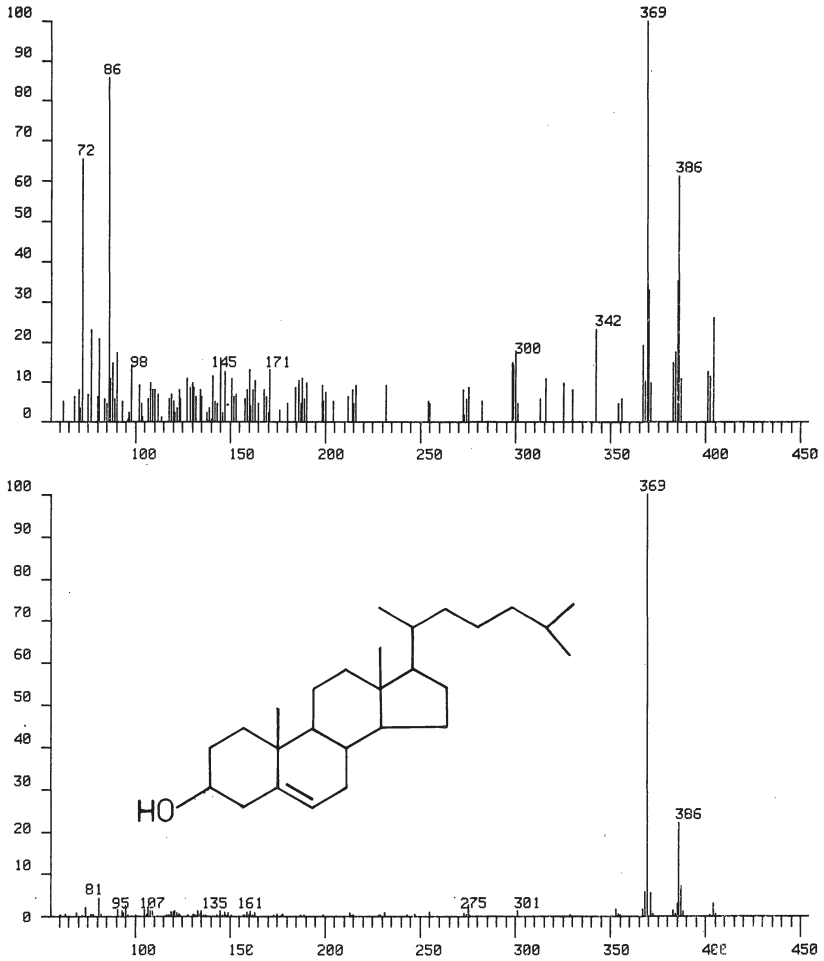

Figure 5 Top: background-subtracted scan no. 71 obtained from a 3-year-old verdigris/egg yolk film using the heated direct probe. Chemical ionization with ammonia as reagent gas. Below: spectrum of cholesterol under similar conditions.

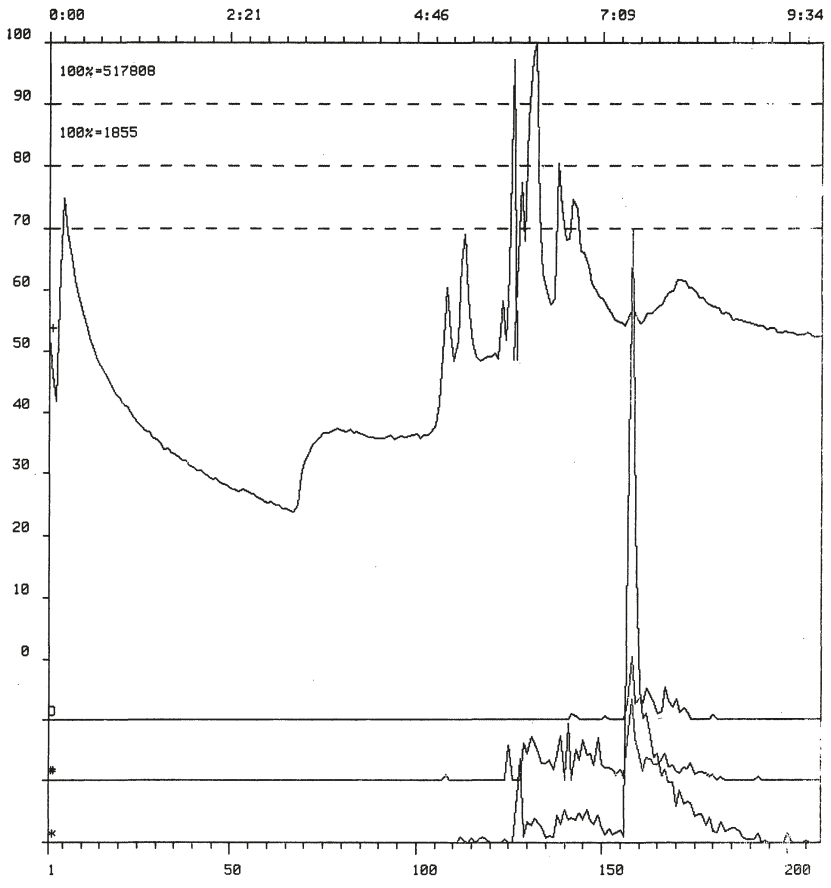

Figure 6 Chromatogram given by the silylated neutral fraction obtained after saponification of 50-year-old yellow ochre/egg yolk – stand oil paint film. The three lower plots show mass-scans for 369,386, and 458; masses characteristic for cholesterol trimethyl silyl ether (cholesterol TMSE) under ammonia chemical ionization conditions as used here.

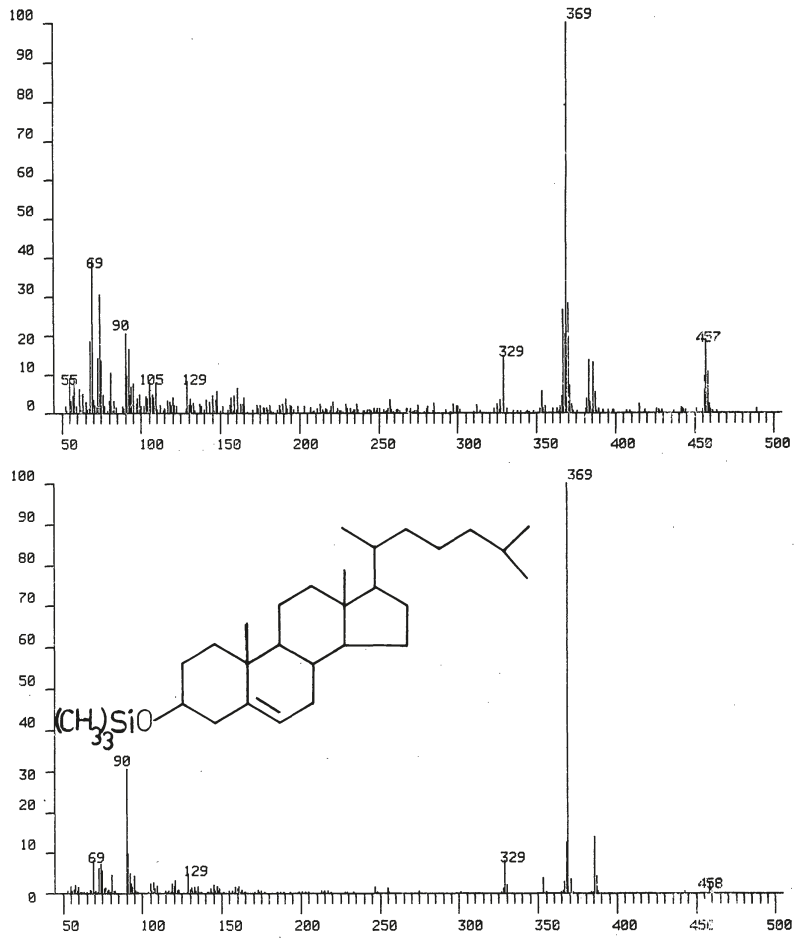

Figure 7 Top: the spectrum given by scan 158 of Fig. 6 after background-subtraction (using scan 156). Bottom: spectrum of cholesterol TMSE under similar ammonia chemical ionization conditions.

the more sensitive and specific approach of mass-spectrometry. This had already been demonstrated some years ago [10] but without any indication of the size of sample needed.

There are two possible approaches to the detection of cholesterol. The simpler is to introduce the sample, without any pre-treatment, directly into the source using the direct insertion probe and heat it up through a suitable temperature range to drive off volatile components. This would involve the possible disadvantage of having to detect the cholesterol amongst much volatile matter from the oily component of the medium, but it might also serve to show up other characteristic differences in mass-spectra of different media besides presence or absence of cholesterol. The other approach is to isolate the non-saponifiable fraction of the sample and examine this, again either using the direct probe or by employing combined gas-chromatography – mass-spectrometry. We tried both of these approaches with mixed success. As test samples we used the 1978 paint films mentioned above and also the Fogg Museum paint samples prepared in the 1930s which we had used in our previous sterol study [9].

Direct insertion

Very small samples of paint (rather smaller than those usually taken in practice for medium analysis) were placed in capillary tubes, mounted in the probe and inserted into the source. The probe heating system is ballistic in character and we found that a dial setting of 7 gave a suitable heating rate with an upper probe temperature limit of about 320°C. The spectrometer was set up for chemical ionization rather than electron impact ionization since we wanted to minimize fragmentation and maximize formation of characteristic ion masses of cholesterol. Ammonia was used as reagent gas. Scanning was effected at a rate of 1 second/decade and carried out continuously during the heating schedule. Rather than use the total ionization plot for following the course of the experiment we found it more convenient to use the 'miniplot' mode which shows in real-time a small mass-spectrum bar-chart of each scan on the VDU immediately after it is made, four to a page. Appearance of relevant masses can therefore be seen immediately. Characteristic masses for cholesterol, when observed, usually appeared at a probe temperature of about 280°C and upward.

Cholesterol was readily detected in the paint sample most favourable to its survival namely the egg yolk – verdigris film (three years old). The most characteristic masses, observed over several scans, are 369 (the molecular weight of 386 minus 18 from loss of the elements of water, plus 1 from the addition of H^+) and 386 (386 minus 18 from water plus 18 from (NH_4^+). Less abundant and not always present are 387 and 404 (386 plus H^+ and NH_4^+ respectively). In Fig.5 one of the scans is compared with that for cholesterol

itself. Of course many other masses are also present in the sample scan since other materials are evaporated from the sample simultaneously with cholesterol, and this is undoubtedly the limiting factor for this approach. As the proportion of cholesterol in the sample diminishes its characteristic masses will be swamped by those of other compounds or even if present could not be confidently attributed to cholesterol itself. Use of greater sensitivity will not solve the problem since it cannot be made selective.

Cholesterol was also detectable in this way in a film of verdigris in a mixed medium of egg yolk and linseed oil; also in a lead white – egg yolk film. With a lead white and mixed medium of egg yolk and linseed oil however the characteristic masses were beginning to be submerged and in the Fogg yellow ochre – egg yolk film (1933) they could not be detected.

An extensive analysis of the results was made to try to detect particular masses which, in an empirical way and without attribution to particular compounds, were characteristic for egg and for oil media. It was thought that proteinaceous material ought to yield some characteristic features. After several hopeful but false leads however it was concluded that probably the lipid (fatty) components of egg yolk are the dominant provider of volatilizable material and that this results in an overall similarity of mass-spectra, over the heating range employed, for egg yolk and oil. Another possibility is that the protein needs higher temperatures — pyrolysis in fact — to yield characteristic masses.

Non-saponifiable fraction

The second approach was to examine the non-saponifiable fraction of the paint samples, thus avoiding interference from the triglyceride or fatty acid material. The small samples were saponified with methanolic potassium hydroxide in the way normally adopted for fatty acid analysis. Then after dilution with water but before acidification the neutral material was extracted with ether, and the ether extract backwashed with water. It was found difficult to introduce the extracted material into capillary tubes for examination using the direct probe and we concentrated on the chromatographic method. Separation was effected on a short (6 meter) quartz capillary column using a very rapid temperature programme so that the cholesterol should be eluted during only a few scans rather than be dissipated over several. We soon found though that cholesterol was being lost somewhere in the system by irreversible absorption or decomposition and it was necessary to derivatize the sample to protect it. Trimethylsilylation was therefore carried out before chromatography [11].

The results of this approach were a great advance on those obtained with the direct probe. We were able to demonstrate the presence of cholesterol, not only in the recent paint samples, but also in two of the Fogg paint films including what may even be the 'worst case' sample from a yellow ochre with mixed medium of egg yolk and stand oil paint film. Results for this last are illustrated in Fig.6 which shows the total ion current trace and also mass scans for 369, 386, and 458 which permit localization of the cholesterol trimethylsilyl ether peak on the chromatogram (the ether gives a rather similar mass spectrum to the parent compound since it readily loses the functional group in the same way that cholesterol loses water). Fig.7 shows the mass-spectrum afforded by this very small peak, after background subtraction, compared with the spectrum of a reference sample of cholesterol TMSE. It is certainly good enough for a positive identification.

The main reason for this success when the direct probe approach failed is undoubtedly that the cleaning-up of the sample by isolating the non-saponifiable fraction and separating out the cholesterol by GLC meant that much higher sensitivity settings could be used on the spectrometer. In fact we used a setting of 280 instead of 230, a seven-fold increase. As can be seen from Fig.6 a number of other peaks appear on the chromatogram before the appearance of cholesterol TMSE. These have not been looked at carefully but some are simply fatty acid methyl esters. We have recently confirmed, rather to our surprise, that such are formed directly to a certain extent when *methanolic* potassium hydroxide is used for the saponification and we now tend to add a proportion of water to reduce or eliminate this.

This approach does then seem a viable way of detecting egg yolk in the presence of oil. However 50 years are not 500 years and we have yet to demonstrate its success with really old samples. Also, to blur the picture somewhat, we did find that some mass 369 was appearing in the chromatogram yielded by some recent oil-pigment films even though the relevant spectrum as a whole did not resemble that of cholesterol TMSE. It would thus be unwise to rely on the appearance of this mass alone for identification of cholesterol.

Resin in paint media

The identification of small amounts of resin in paint media is another problem which has been rather uncertain when relying on gas-chromatography alone. It must be said that resins such as Baltic amber or the true copals would still be hard to detect and identify since these are mostly polymeric even before use. The diterpenoids of Conifer resins are however potentially detectable especially, as has been explained elsewhere [12], those such as pine resin which contain or give rise to the relatively stable diterpenoid, dehydroabietic acid. Even this compound however is liable to have disappeared, or been greatly reduced in quantity, in oil paint films in which vigorous free-radical autoxidation reactions have taken place. A small peak in the dehydroabietate region of the fatty acid ester chromatogram is not really sufficient proof for its presence especially as other unidentified compounds often appear in the region. Confirmation by mass-spectrometry is needed. We briefly reported before [13] on the detection of pine resin in this way in a sample of green paint from Veronese's *Allegory of Love, II* (No. 1324), and we show in Fig.8 part of the

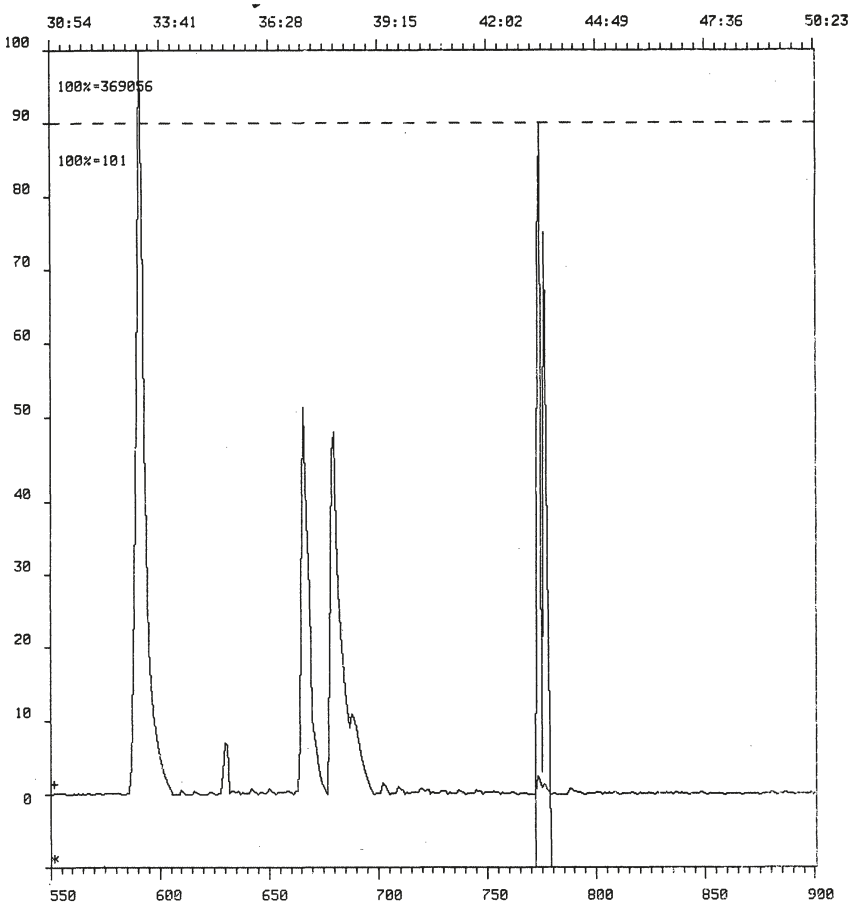

Figure 8 Part of the chromatogram yielded by a sample of green paint from Veronese's *Allegory of Love II* (No.1324) after saponification and methylation. The mass-scan for mass 314 locates the position of methyl dehydroabietate on the chromatogram. The three main peaks are palmitate, oleate and stearate and the small size of the dehydroabietate can be judged from them.

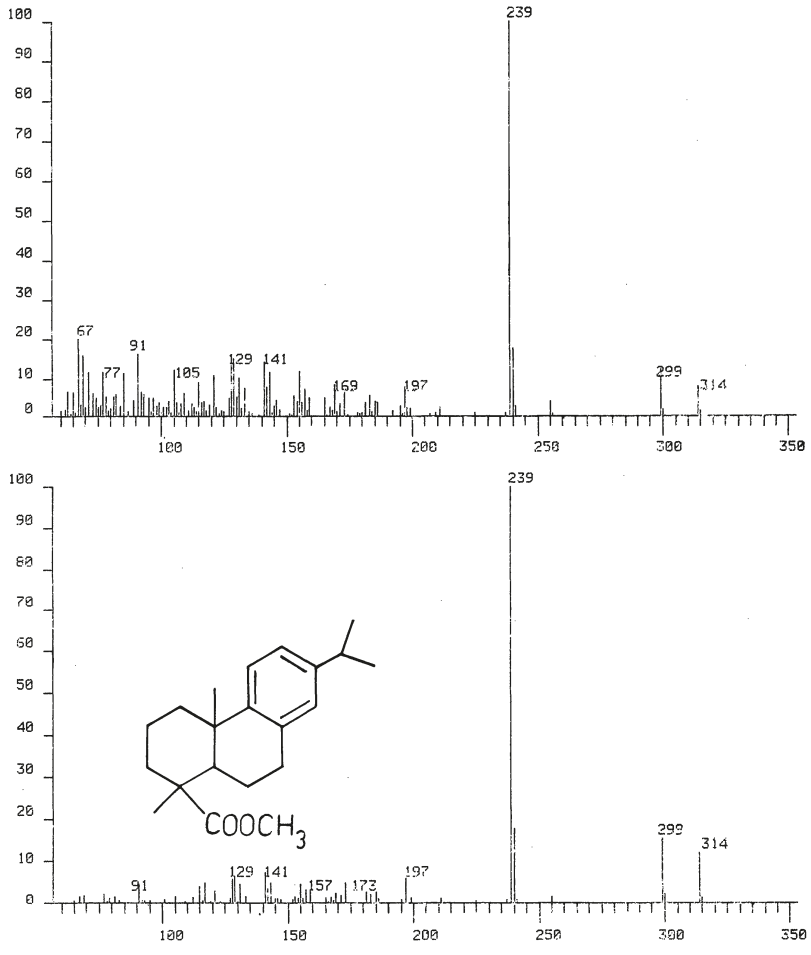

Figure 9 Top: the mass-spectrum of scan 773 of Fig. 8 compared with, bottom: mass-spectrum of methyl dehydroabietate as observed during the chromatography of methylated wood rosin.

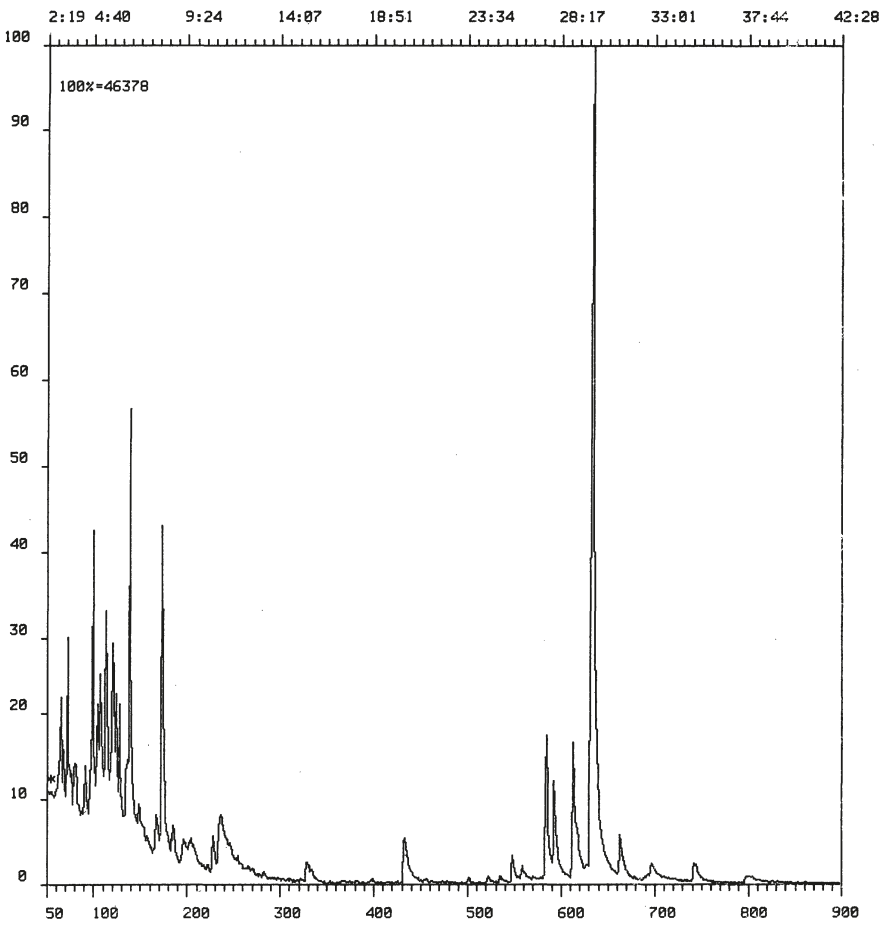

Figure 10
Chromatogram yielded by a green 'European lacquer' of 1770 after saponification and methylation. The early peaks, up to about scan 200, are monoterpenoids while those from around scan 500 are diterpenoid resin components.

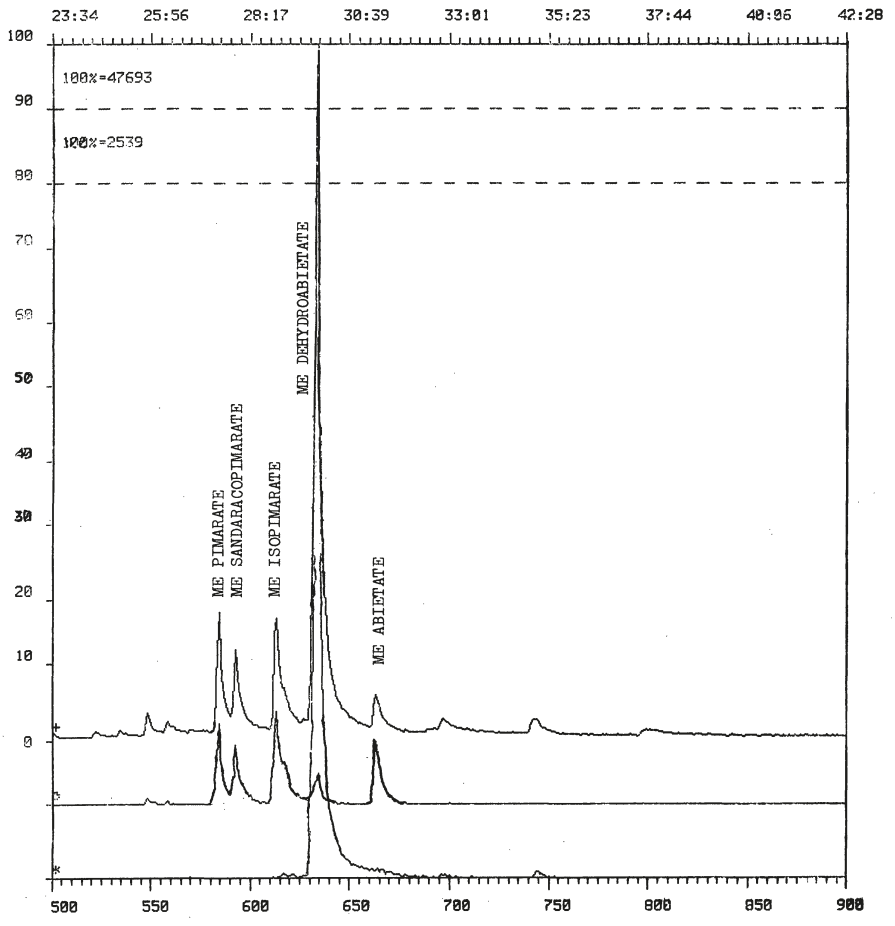

Figure 11 The diterpenoid section of the chromatogram of Fig. 10. Mass-scans for 314 and for 316 indicate the positions of methyl dehydroabietate, and the abietadiene and pimaradiene esters respectively.

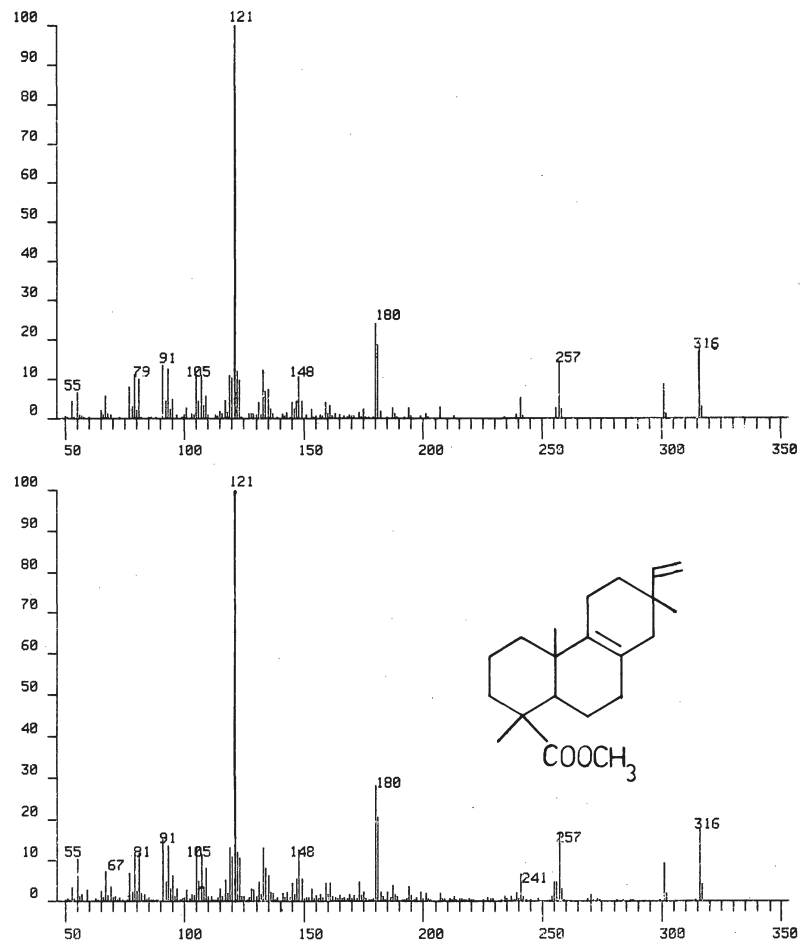

Figure 12 Top: the spectrum of scan 584 of the chromatogram of Figs. 10 and 11. Bottom: the mass-spectrum of methyl pimarate as observed during the chromatography of methylated wood rosin.

total ion current chromatogram of the sample together with the 'mass-chromatogram' for mass 314, the molecular ion for methyl dehydroabietate, which serves to pick it out on the chromatogram. Fig.9 shows the mass-spectrum of scan 773 compared with that of methyl dehydroabietate in wood rosin. Considering the small sample size and the minute amount of the ester which must be present in it, the match is remarkably good. Pine resin has also been identified in this way in other paint samples including that remaining on Constable's palette in the collection of the Tate Gallery.

Analysis of old varnishes

Varnishes can prove to be simple or quite complex. In the latter case mass-spectrometry is almost indispensable in identifying individual components and attributing them to particular resins. 'Simple', also, is a relative term for even single resins can show a pattern of many peaks and this may be considerably changed from that of the fresh material. Generally speaking the resin components of varnishes seem to be better preserved than when they are incorporated into oil paint films and, being the main component, are present in larger amount. In favourable cases, as in the first example to be described, the virtually intact pattern of the original resin composition is preserved. In other cases interpretation of the composition found is not so obvious.

Chippendale lacquer

It is very unusual to find old picture varnish samples of known date since varnish is liable to be often renewed both with and without removal of the old. One has not come our way so far and therefore we give as our first example a material which, while not from a painting nor strictly speaking a varnish, is precisely dated and uncontaminated by later layers. It consisted of a green 'European lacquer' from a privately owned washstand or commode by Thomas Chippendale dating from 1770. The green pigment was verdigris and this had had its now familiar antioxidant effect and preserved the resin components well. Fig.10 shows the chromatogram given by the lacquer after saponification and methylation (that given by simple extraction and methylation was similar). The group of early peaks is due to monoterpenoids — components of oil of turpentine — which, though relatively volatile, still remained in the film. Many were identified but space prevents us from detailing them here. The scans from about 560 onward show a typical pattern of pine resin acid esters. Fig.11 shows this section of the chromatogram together with mass scans for 314 (showing up dehydroabietate) and 316 (which shows up the peaks due to pimaradiene and abietadiene acid esters). These were all readily identified from their individual mass-spectra and as an example we show in Fig.12 that of scan 584 compared with that of methyl pimarate from a chromatogram of wood rosin.

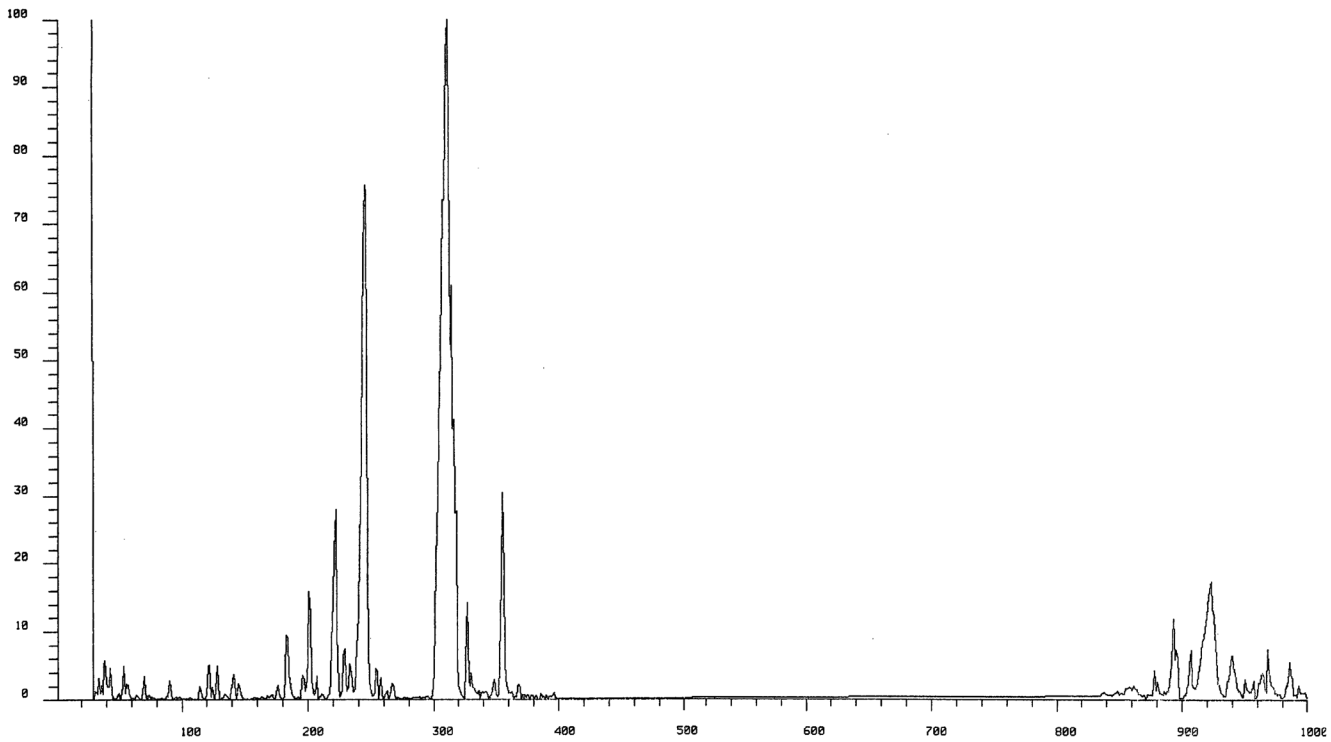

Figure 13 Chromatogram afforded by a varnish removed from Pieter Bruegel's *Adoration of the Kings* (No.3556) after automatic background-subtractions. For conditions see text. Peaks between about scans 150 – 400 are diterpenoid resin components, those from scan 800 upward are triterpenoid resin components.

Between the mono- and diterpenoid peaks are small peaks of the usual dried oil esters. One can say with confidence then that this lacquer was formulated simply of pine resin dissolved in oil of turpentine with a very small addition of drying oil.

Varnish from a painting

Our second example is the analysis of a varnish removed some years ago by the restorer from Pieter Bruegel's *Adoration of the Kings* (No.3556). At that time analysis was not practicable and the sample was kept until it might prove to be so. It consisted of a lower layer of somewhat insoluble varnish of dark colour which had remained after removal of upper, paler and more soluble varnish layers. It presumably comprised an early varnish remaining from an earlier incomplete cleaning but of quite uncertain date.

Preliminary fatty acid analysis on part of the sample had shown that it contained some drying oil, probably linseed, and higher boiling components. For examination by GLC – MS another portion was extracted overnight with cold ether/methanol (9:1) and then diluted with further methanol to precipitate less polar polymeric material. The solution was then treated, in the fume cupboard, with diazomethane to methylate the acids and evaporated carefully to small volume. 0.5 μl was injected under cold-trapping conditions using a Grob splitless injection system at 260°C with solvent tail cutting after 20 seconds. A 6 m flexible quartz capillary column coated with SE30 silicone gum was used for the analysis. The column

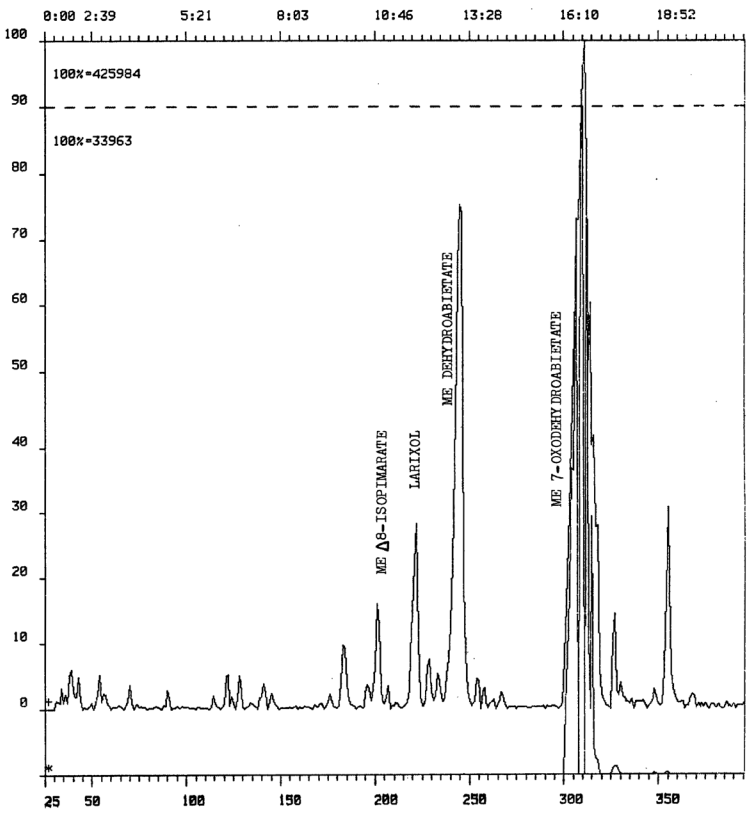

Figure 14 A section of the chromatogram of Fig.13. A mass-scan for mass 328 shows that the largest diterpenoid peak consists predominantly of methyl 7 – oxo – dehydroabietate, a further oxidized derivative of methyl dehydroabietate.

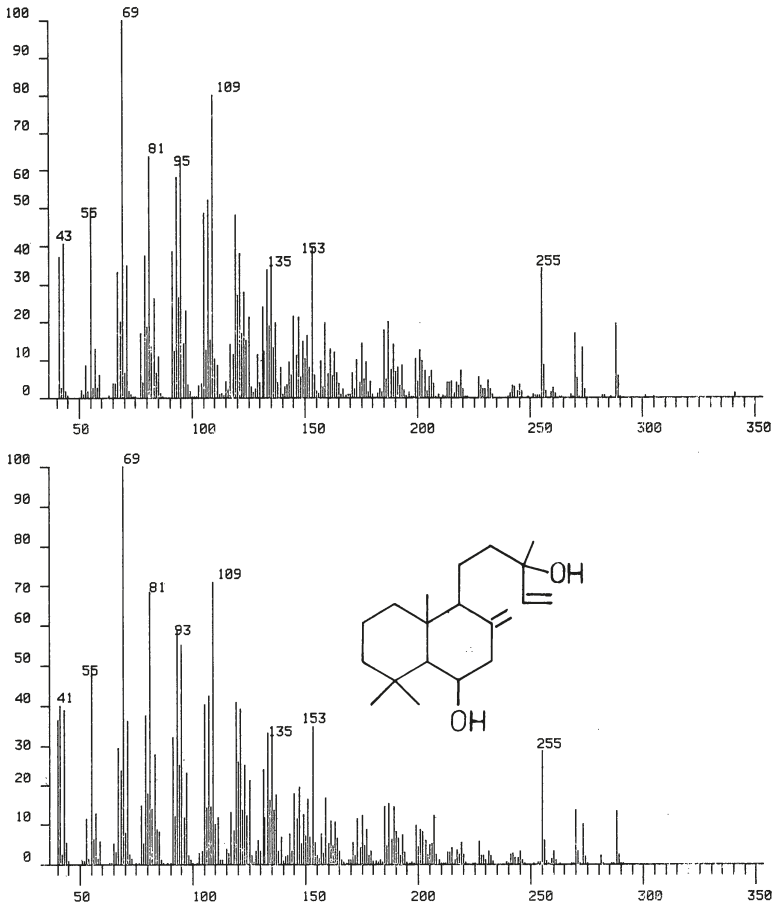

Figure 15 Top: mass-spectrum of scan 222 of the chromatogram of Figs. 13 and 14. Bottom: spectrum of larixol as observed during chromatography of larch resin (Venice turpentine).

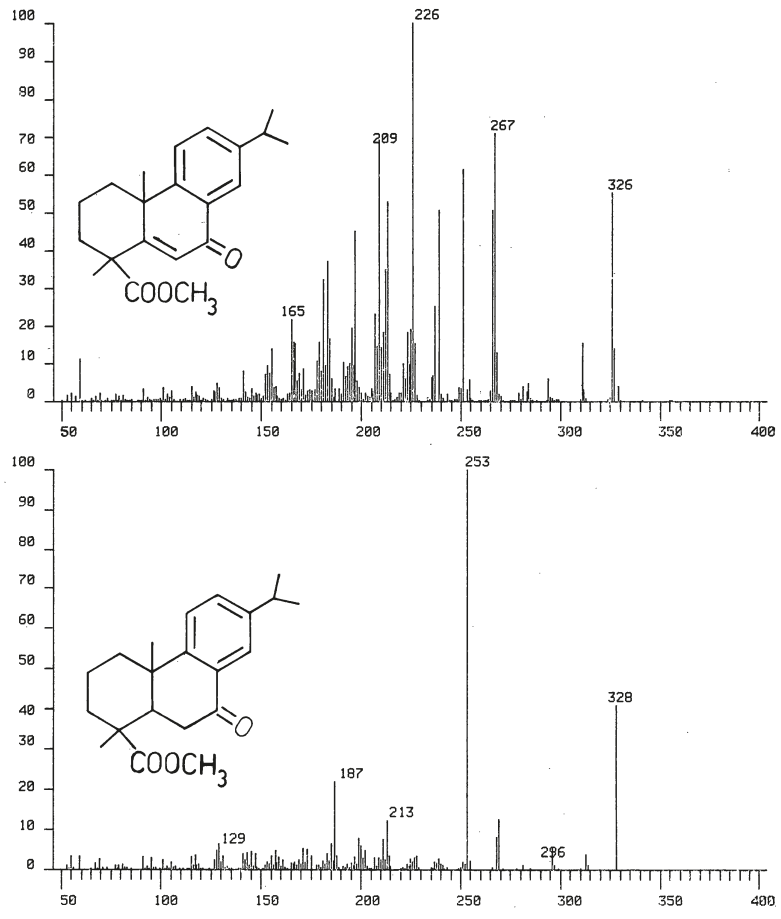

Figure 16 Separation of the spectrum of an individual component from a mixture by using the subtraction routine. Top: the spectrum which results from subtracting that of methyl 7 – oxo – dehydroabietate (scan 310) from that of scan 314 of the chromatogram of Figs. 13 and 14. It is close to that of methyl Δ5 – 7 – oxo – dehydroabietate.

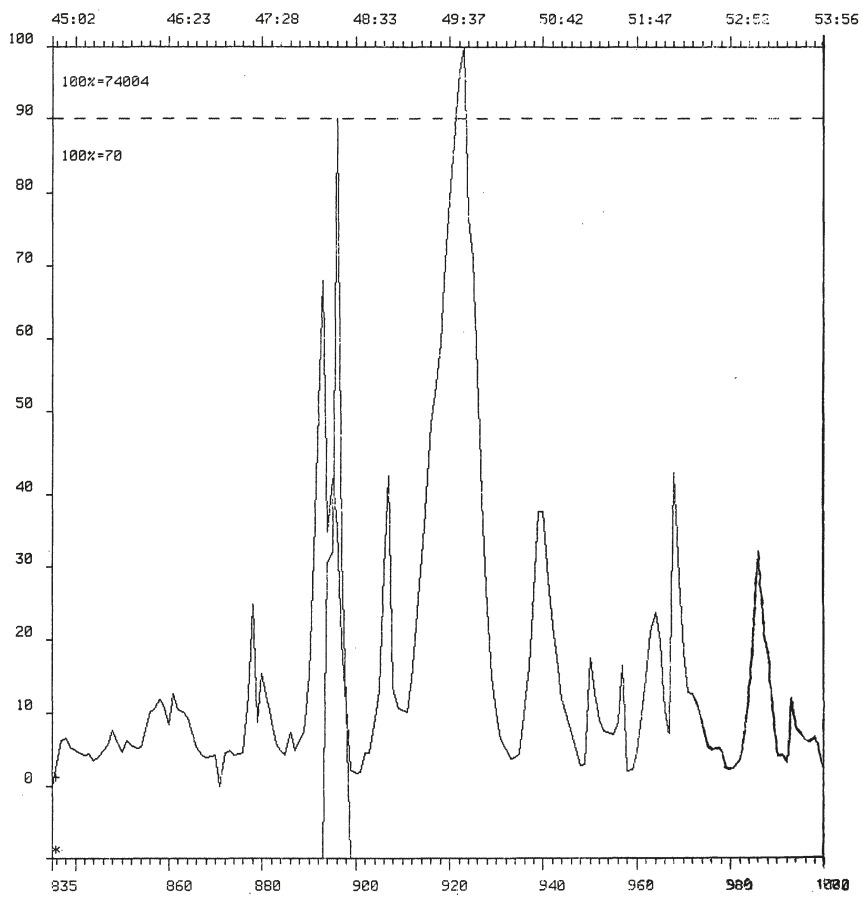

Figure 17 The triterpenoid section of the chromatogram of Fig.13. A scan for mass 426 picks out the position of a possible triterpene alcohol.

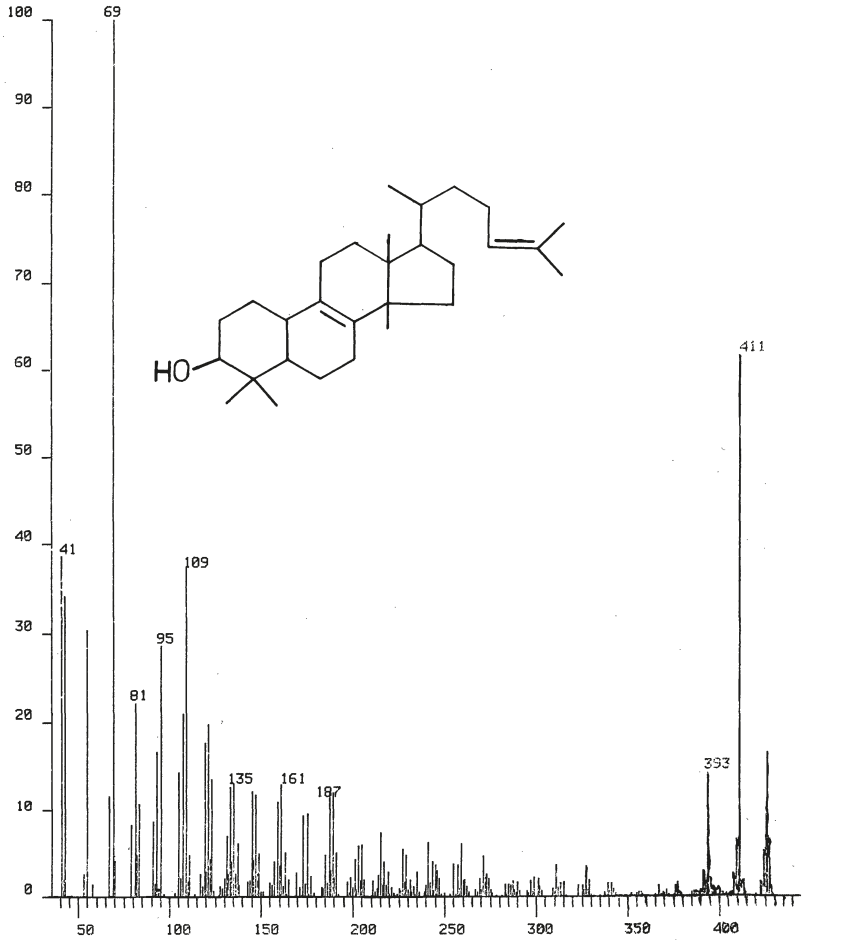

Figure 18 Mass-spectrum of scan 896 of Fig.17. It agrees well with that of tirucallol, a component of mastic resin.

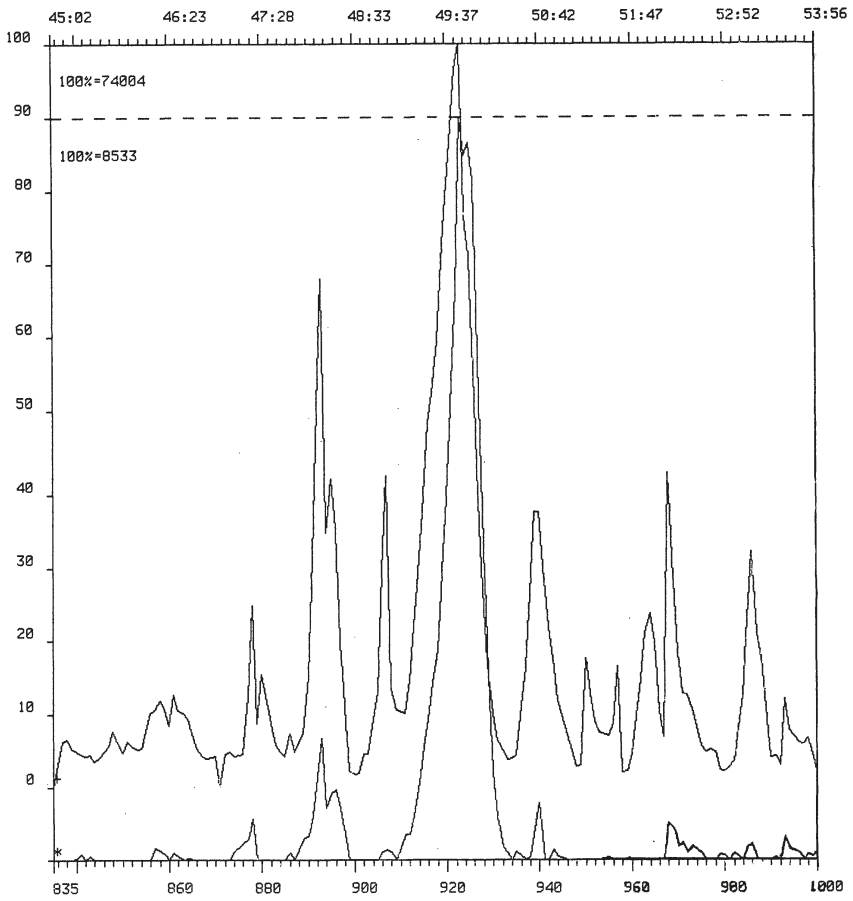

Figure 19 The same region of the chromatogram as in Fig.18. A scan for mass 203, the strongest (base) peak of methyl ursonate, shows how this lies under the composite keto-ester chromatographic peak centred at around scan 923.

carrier gas was helium at 0.6 bar pressure; the oven temperature was programmed from 120°C at 5°/minute to 260°C. The spectrometer was set up for electron impact ionization at 70 eV with a source temperature of 220°C. Scanning was 1 second/decade with a 30 μsec sampling rate. Gain 10^4.

Fig.13 shows the chromatogram obtained after subjecting the scans to automatic background subtraction. Use of the short capillary column means that retention times are relatively short and high column temperatures are not needed to elute the less volatile high molecular weight compounds. Thus distribution by one non-polar column has permitted the analysis of compounds ranging from monoterpenoids through the fatty acid esters and diterpenoids to the triterpenoids. However this is clearly at some cost in chromatographic resolution for there are several components which overlap to some extent. Nevertheless by judicious use of the subtraction routines and the use of mass chromatograms individual spectra of these overlapping compounds can still be separated out.

As with the Chippendale lacquer, mass scans for 314 and 316 served to locate dehydroabietate and the pimaradiene and abietadiene acid esters in the diterpenoid region of the chromatogram. Scanning for mass 328, the molecular weight of methyl 7 – oxo – dehydroabietate, showed that this occurred in the major peak centred at around scan 311. The high proportion of this more highly oxidized compound here, as compared with the preceding example, reflects the vulnerability of exposed varnish films. In Fig.14 some of the other peaks are also identified. The identification of the peak centred at scan 201 as methyl Δ8 – isopimarate was quite clear from its spectrum. This is usually only a minor component of most conifer resins and the significance of its relative abundance in this sample is not entirely clear.

An important neutral component giving rise to the peak centred at scan 222 is interesting and significant. This proved to be the bicyclic diol, larixol, as can be seen from the comparison of spectra in Fig.15. This compound, molecular weight 306, contains a tertiary hydroxyl group easily lost to give a highest observed mass of 288. Larixol is only known to occur in certain larch (*Larix* spp.) resins and consequently its presence here shows that Venice turpentine from the European larch is a component of the varnish. Whether pine resin in the form of rosin was also present cannot definitely be said since all the pine resin components are also to be found in larch resin and we cannot be certain about changes with time of proportions. Examination of the scans between nos. 310 to 320 indicated that the peak due mainly to methyl 7 – oxo – dehydroabietate included another compound with parent ion at 326 rather than 328 which was at a maximum at scan 314. It was found possible to get a reasonably pure spectrum of this second compound by subtracting the contribution of the 7 – oxo – dehydroabietate using scan 310 and normalizing to mass 328. The result was the upper spectrum shown in Fig.16

which agreed well with that of methyl Δ5 – 7 – oxo – dehydroabietate, a still further oxidized derivative.

Moving to the higher molecular weight region of the chromatogram, the scans above 850, we are observing compounds with molecular weights of the order of 400 – 500, representing triterpenoid resin components. Mass scanning at 426 (Fig.17) shows up triterpenoid alcohols. That located as a shoulder on the peak centred on scan 896 gave the spectrum shown in Fig.18 and appears to be tirucallol, a component of mastic.

Keto-esters such as oleanoic, ursonic, and masticadienonic methyl esters have molecular ions of 468. A mass scan for this showed that there were several compounds of this molecular weight within the broad peak centred at scan 923. Mass scanning at 203, the base (strongest) peak of the spectrum of methyl ursonate showed up the occurrence of this compound within the composite chromatographic peak (Fig.19). It must be said that the chromatographic conditions used were not ideal for triterpenoids. Some decomposition of hydroxy compounds was probably occurring during chromatography and these are best first protected by silylation. While not all the triterpenoids were identified they are generally suggestive of the use of mastic. The presence of mastic together with Venice turpentine suggests that the latter was included probably on account of its supposed plasticizing effect.

Conclusion

We hope that the foregoing account of some applications of mass-spectrometry to the study of the organic materials of art has served to indicate how powerful the technique is to reveal details of composition on even minute samples. While the apparatus is too expensive, and needs too specialized and dedicated attention, for it ever to become commonplace in the museum laboratory, its use here and in a few other centres will surely provide results of general interest and usefulness which could not be otherwise obtained.

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