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GAS CHROMATOGRAPHIC SEPARATION OF DITERPENE ACIDS MODIFIED WITH MALEIC ANHYDRIDE AND FUMARIC ACID*,**

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SUMMARY

Mixtures of diterpene acids and their Diels–Alder adducts with maleic acid anhydride and fumaric acid were separated on high resolution glass capillary columns, coated with the non-polar stationary phase SE-30. The modified resin acid mixtures were subjected to gas chromatographic–mass spectrometric analysis and a number of adduct compounds could be identified. The presence of some major components in the investigated samples was checked by comparison with pure specimens, independently synthesized or purified by crystallization. The relative retention characteristics of the adducts detected in the differently modified diterpene acid samples are reported.

INTRODUCTION

The analysis of the reaction mixtures of tall oil diterpene acids with dienophiles, such as maleic anhydride (MAA) and fumaric acid (FA), is of great interest. In increasing quantities these products are employed as paper sizes and resin esters[1]. The modified resins have higher softening points, are non-oxidizing and can be used in smaller quantities for paper making purposes than natural pinewood rosin[2]. Scheme I shows the mechanism of Diels–Alder addition of MAA and FA to levopimaric acid leading to the two main products.

Until some few years ago the analysis of the so-called fortified resins was achieved mainly by IR spectroscopy or thin-layer chromatography (TLC)[3–5]. In these methods the quantitative course of the Diels–Alder reaction could not be observed and the distribution of the various adducts formed could not be determined. The use

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** Dedicated to Prof. Dr. J. Schürz on the occasion of his 60th birthday.

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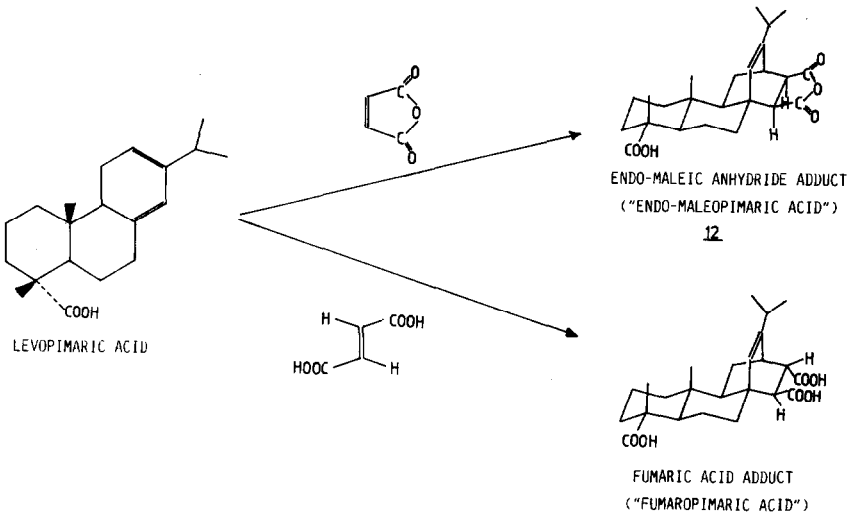


Scheme I. Mechanism of Diels–Alder addition to levopimaric acid. Dienophiles: maleic anhydride and fumaric acid.

of WCOT glass capillary columns would be necessary for the qualitative and quantitative separation of these complex reaction mixtures.

To obtain reasonable retention times of the adducts (the methylated derivatives have molecular weights up to 460 daltons) and best resolution of all components, SE-30 was chosen as a non-polar stationary phase with high thermal stability. The identification of the various substances, mainly compounds with molecular weights of 414 (MAA adducts) and 460 daltons (FA adducts), was possible by a combination of gas chromatography and mass spectrometry (GC–MS).

EXPERIMENTAL

Gas chromatographic measurements

Samples to be analyzed were prepared by Krems-Chemie (Austria) using both natural (colophony) and tall oil resin acid mixtures. The acid samples were methylated with a freshly prepared diazomethane solution and dissolved in diethyl ether or methanol to give 5% (w/w) solutions.

The glass capillary columns were prepared by the methods of Grob *et al.*[6,7] and coated with the non-polar stationary phase SE-30. In all cases the column length was 20 m, with a liquid phase thickness of less than 0.1 μm, to obtain elution temperatures of about 300°C for the adducts. A Carlo Erba Fractovap 4160 gas chromatograph with a flame ionization detector and hydrogen as carrier gas was used. The samples were injected at 300°C with a splitting ratio of 1:30 in an all-glass injector. The best separation was achieved by temperature-programmed analysis, from 150 to 320°C at 5°C/min, and a carrier gas (hydrogen) flow-rate of 1.8 ml/min (corresponding to an average linear velocity of 50 cm/min).

Peak areas and exact retention times, necessary for the evaluation of relative

retention times (RRT) and equivalent chain lengths (ECLS), compared with the retention times of saturated fatty acid methyl esters, were measured with an electronic integrator Model 301 from Laboratory Data Control.

The mass spectra were recorded on a Hewlett-Packard quadrupole mass spectrometer system HP 5992A using the SE-30 column described. Instead of hydrogen, helium was used as carrier gas with a flow-rate of 3.0 ml/min. The injection port temperature was again 300°C; the ion source temperature was 270°C and the ionization energy was 70 eV.

The identification of the various substances, mainly the adducts, was based primarily on mass spectra recorded with the GC–MS system. No further information on the detected modified diterpene acids, *i.e.*, elution patterns or mass spectral data, could be found in the literature.

Chromatographic characterization of the adducts

Following detection the modified resin acids were standardized by relative retention values. The index systems used for characterization of the adducts were relative retention times (RRTS) and equivalent chain lengths (ECLS). The retentions relative to *endo*-maleopimaric acid methyl ester 12 (the adduct numbering corresponds with the GC elution order) were obtained from temperature-programmed analyses. The ECLs were determinated with the following equation

$$\mathrm{ECL}^{\mathrm{SE\text{-}30}} = m \cdot \frac{t_x - t_n}{t_{n+m} - t_n} + n$$

a modification of that first described by Miwa *et al.*[8] where $\mathrm{ECL}^{\mathrm{SE\text{-}30}}$ = ECL for substance x on stationary phase SE-30; n = number of C atoms in the saturated fatty acid methyl ester with lower retention time; t_n = retention time of this fatty acid ester, t_{n+m} = retention time of the fatty acid methyl ester with higher retention time, m = difference in number of carbon atoms between the two fatty acids and t_x = retention time of substance x.

The temperature programme parameters and column pressures were adjusted to obtain a linear relationship between the retention time and number of carbon atoms of the consecutive fatty acid methyl esters. The retention standard was a solution of the saturated fatty acid esters 20:0, 22:0, 24:0, 26:0, 28:0 and 30:0, which was co-injected with the modified diterpene acid samples.

The ECL values seem to be suitable for the characterization of the adducts, because this concept was used by Holmbom[9] for the standardization of the diterpene acids and moreover behenic acid (22:0) and lignoceric acid (24:0) always occur in the tall oil resin acid samples. Table I lists the retention characteristics of the adducts evaluated on the SE-30 column.

RESULTS AND DISCUSSION

Apart from diterpene and fatty acids, a number of different Diels–Alder adducts could be separated and identified by GC–MS. As can be seen from Fig. 1 and 2, the separation of the modified resin acid mixtures on the SE-30 column was excellent. Fig. 1 shows the chromatogram of a sample modified with fumaric acid, Fig. 2 that of a sample fortified with maleic anhydride and fumaric acid. The numbered

TABLE I

ADDUCTS IDENTIFIED FROM MODIFIED TALL OIL RESIN ACID SAMPLES

Retention relative (RRT) to *endo*-maleopimaric acid methyl ester and ECL values on SE-30 column. Temperature programme: 150 to 350°C at 5°C/min. Flow-rate of hydrogen: 1.8 ml/min.

No.	*RRT*	*ECL value*	*MW*	*Structure*	*Modification*
1	0.8426	24.38	460		FA*
2	0.8592	24.70	460		FA
3	0.8636	24.79	460		FA
4	0.8784	25.09	460		FA
5	0.8935	25.39	460		FA and MAA**
6	0.8975	25.47	458		FA and MAA
7	0.9075	25.67	460		FA
8	0.9314	26.15	460	FA adduct	FA
9	0.9359	26.25	414		FA and MAA
10	0.9513	26.57	414	*exo*-MAA adduct	FA and MAA
11	0.9787	27.18	460	*endo*-MA adduct	FA and MAA
12	1.000	27.59	414	*endo*-MAA adduct	FA and MAA

* Modification with fumaric acid only.
** Modification with fumaric and maleic anhydride.

peaks of the adducts are summarized in Table I. The elution pattern of the remaining, unmodified diterpene acids on this stationary phase is known from other investigations[9,11].

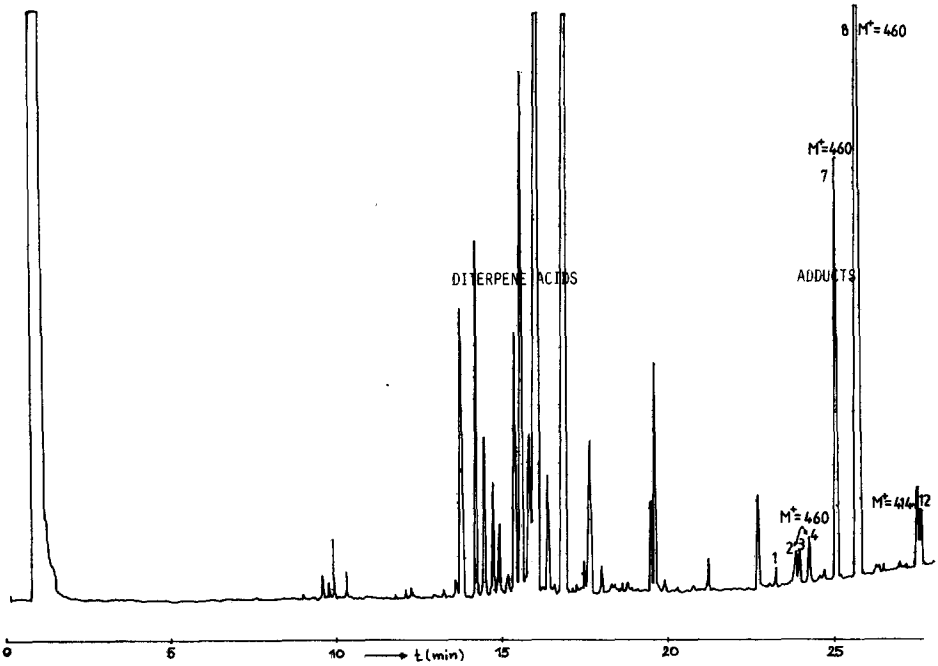


Fig. 1. Chromatogram of a tall oil resin acid fraction modified with fumaric acid (methylated). Column: 20-m SE-30. Temperature programme: 150 to 350°C at 5°C/min. Hydrogen flow-rate: 1.8 ml/min. Peak numbering as in Table I.

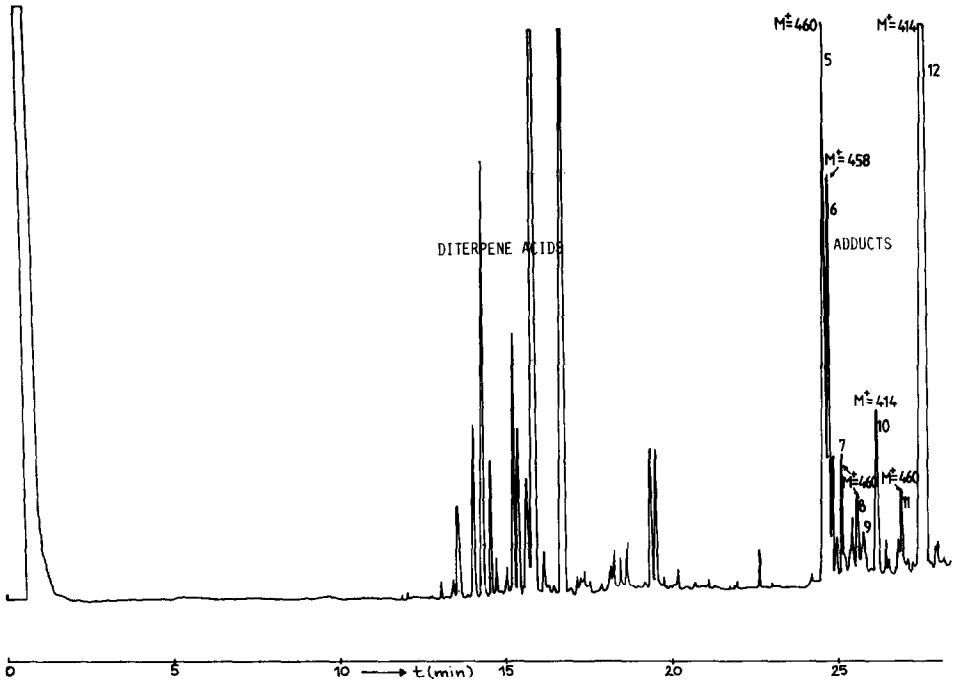


Fig. 2. Chromatogram of a tall oil resin acid fraction modified with maleic anhydride and fumaric acid. Details as in Fig. 1.

Identification of some adducts

In all samples investigated one compound with a molecular weight of 414 could be detected. This adduct was available in high purity by crystallization from a resin modified with MAA only, as described by Langlois and Gastambide[12]. The interpretation of the recorded ^{1}H and ^{13}C NMR spectra[13] confirmed structure of 12 to be a maleic anhydride adduct. This compound is designated as an *endo*-MAA adduct or *endo*-maleopimaric acid.

In several samples, modified with MAA, another substance with a molecular weight of 414 could be detected. This adduct was found only in small amount and could not be concentrated by crystallization or chromatographic methods. The identification of this compound was achieved as follows[14]. The reaction of the diterpene acids —in this case balsamic rosin— with MAA was carried out in the laboratory and the *endo*-maleopimaric acid was precipitated from the reaction mixture with diethyl ether. Apart from the soluble *endo*-MAA adduct and unmodified diterpene acids, another adduct with a molecular weight of 414 remained in the filtrate in high concentration. After removal of the diterpene acid methyl esters by column chromatography on Florisil, the two adducts were separated by preparative TLC on silica gel. The ^{1}H NMR spectrum of the second isolated adduct clearly showed that this compound was identical with *exo*-maleopimaric acid methyl ester 10. By means of co-injection it could be demonstrated that this MAA adduct exists in the fortified resin acid samples.

As is seen in Fig. 1, the fumaric acid modified diterpene acids revealed two

major peaks in the adduct elution range. The identification of the main adduct component 8 was achieved on the one hand by crystallization from benzene solution, and on the other hand by a synthetical method[12]: alkaline epimerization of the *endo*-MAA adduct leads to a homogeneous substance, which also was identified as fumaropimaric acid 8. Concentration experiments on TLC plates showed that the second compound 7 is very unstable and easily isomerizes to other adducts, mainly to compound 8. Nevertheless, the observed molecular weight of 460 for the methylated derivative and the similarity of the recorded mass spectrum with the fragmentation pattern of the adduct 8 allow assignment of the other possible FA adduct, with an '*endo*' configuration of the methyl carboxylate group at the carbon atom 13 and an '*exo*' orientation of the methyl carboxylate group at C-14. The proposed structure is shown in Fig. 5 together with the mass spectrum. It is of interest that a second non-crystalline fumaropimaric acid was described by Halbrook and Lawrence[15], but not studied further.

The other adducts, found mainly in the resin acid sample modified with FA and MAA (Fig. 2), could not be studied further. Only substance 6 with a molecular weight of 458 seems to be a Diels–Alder reaction product of 7,13,15-abietatrien-18-oate[10,16] with fumaric acid.

Finally, the corresponding maleic acid compound was prepared by acidic saponification of the *endo*-MAA adduct[12]. Subsequently, it could be ascertained by co-injection that the fortified resin acid samples contain this adduct 11 (Fig. 8).

Interpretation of the recorded mass spectra

Figs. 3–9 show the mass spectra of the seven most important adduct compounds. In all cases the base peak was found at $m/e = 146$, presumably arising from a retro-Diels–Alder reaction and from a simultaneous ring opening with cleavages at C_5–C_6 and C_9–C_{10} with double H-atom migration. The probable pathway for the formation of this ion is outlined in Scheme II, for the *endo*-maleopimaric acid methyl ester. A similar fragmentation path has been reported by Takeda *et al.*[17], based on

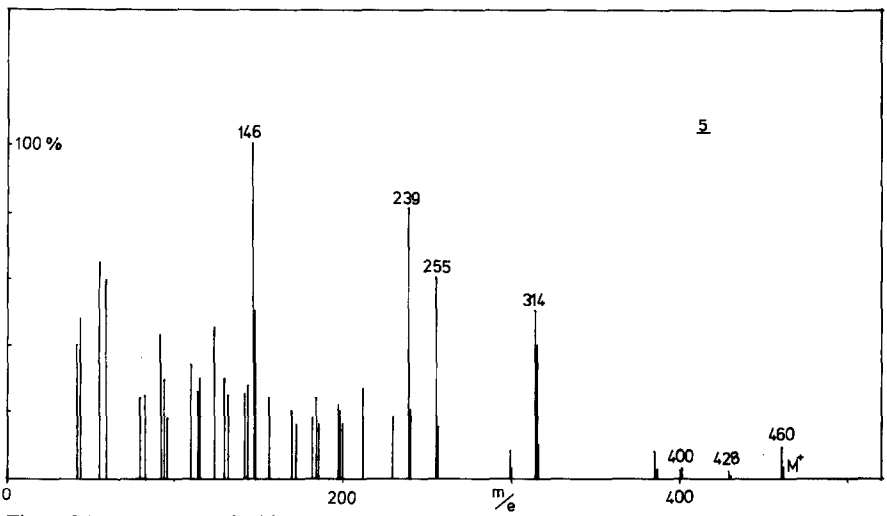


Fig. 3. Mass spectrum of adduct 5.

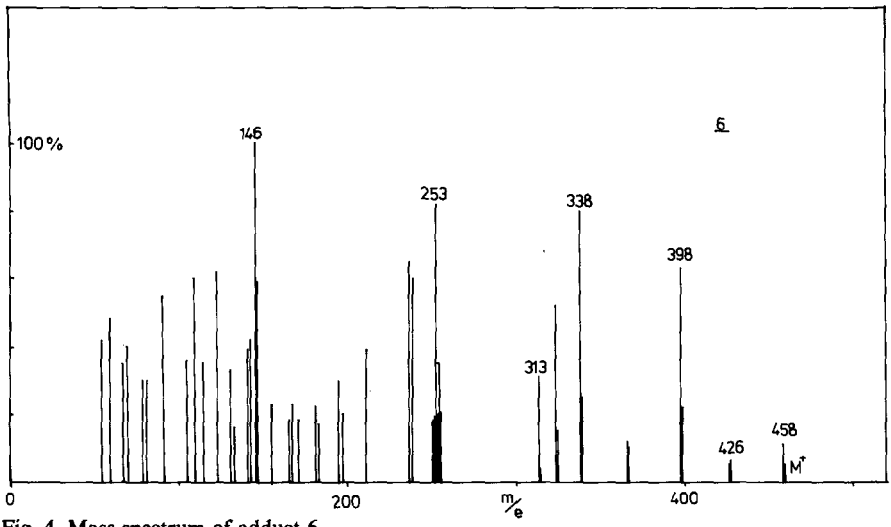


Fig. 4. Mass spectrum of adduct 6.

the mass spectra of the secodehydroabietates, which also give rise to a base peak of $m/e = 146$.

In the mass spectra of the FA adducts (Figs. 5 and 6) significant peaks were observed at $m/e = 428$ and 400, arising from the loss of methanol and methyl formate molecules from the molecular ion.

In the mass spectrum of fumaropimaric acid trimethyl ester 8 another prominent fragment is located at $m/e = 239$. The occurrence of this peak is probably due to the formation of an aromatic ion from the retro-Diels–Alder product levopimarate, $316 - 15(CH_3) - 60(HCOOCH_3) - 2(H_2) \rightarrow 239$, which easily oxidizes to dehydroabietate at high temperature[18].

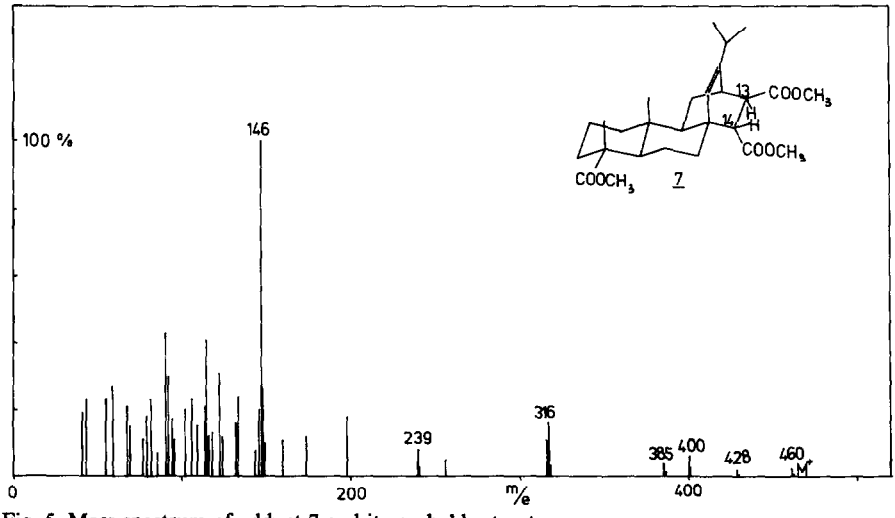
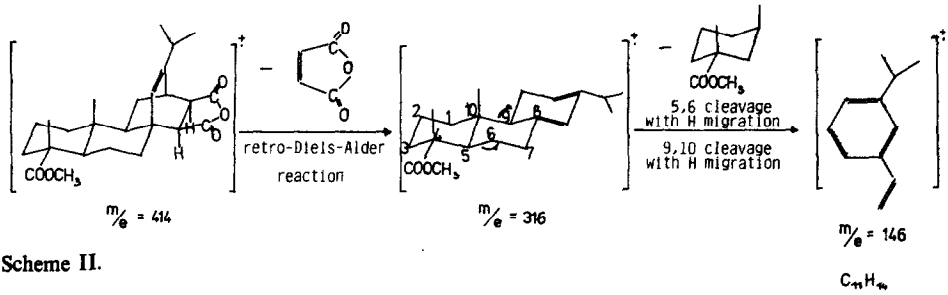


Fig. 5. Mass spectrum of adduct 7 and its probable structure.



Scheme II.

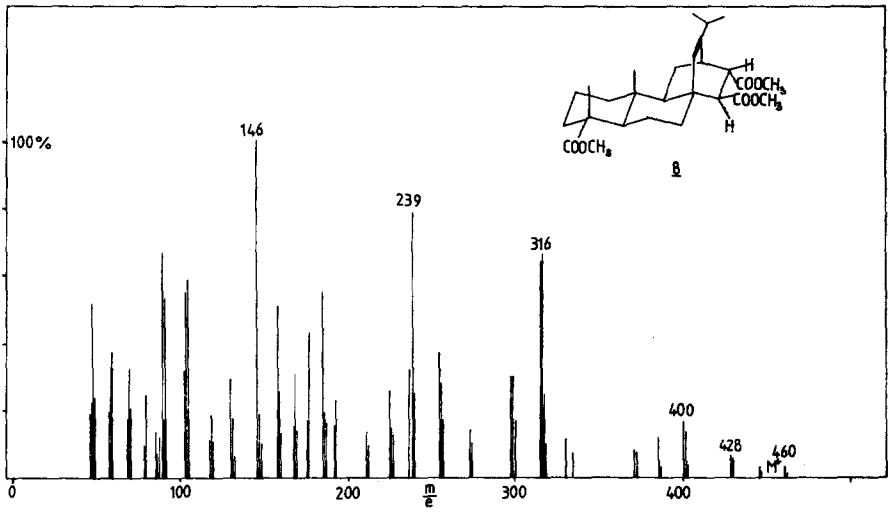


Fig. 6. Mass spectrum of fumaropimaric acid trimethyl ester 8.

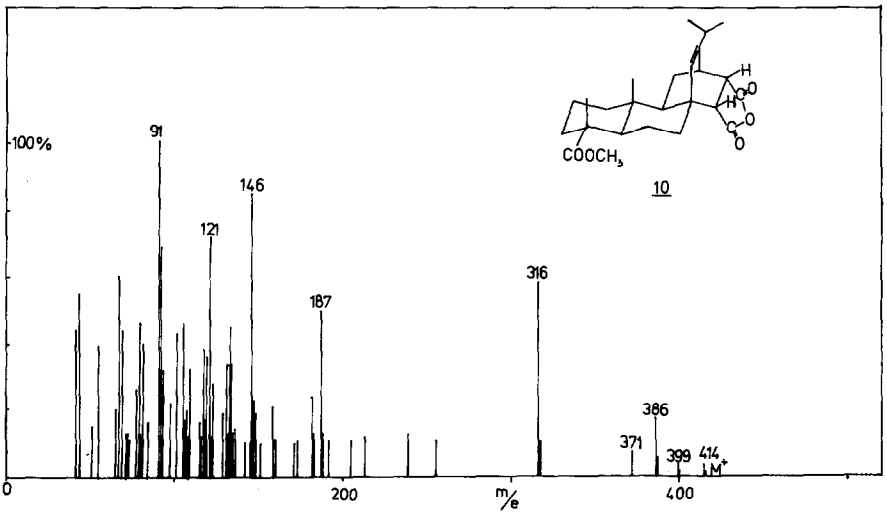


Fig. 7. Mass spectrum of *exo*-maleopimaric acid methyl ester 10.

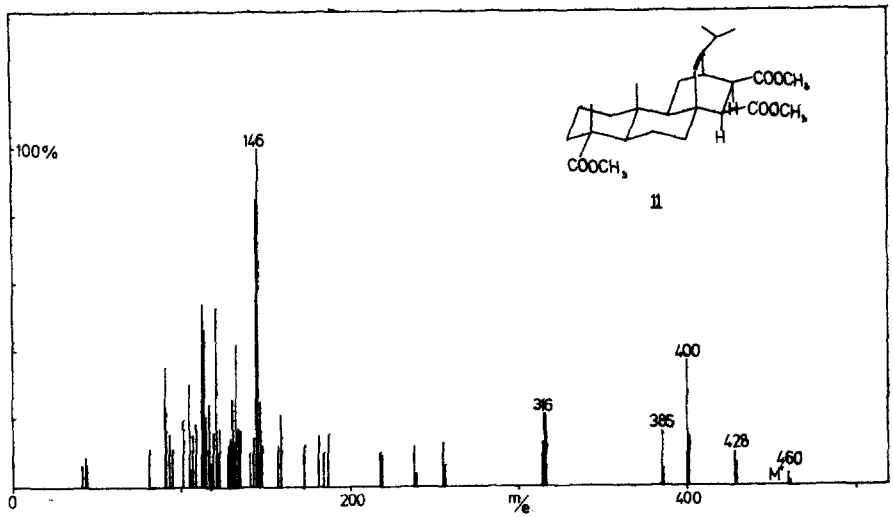


Fig. 8. Mass spectrum of *endo*-maleic acid adduct 11 (trimethyl ester).

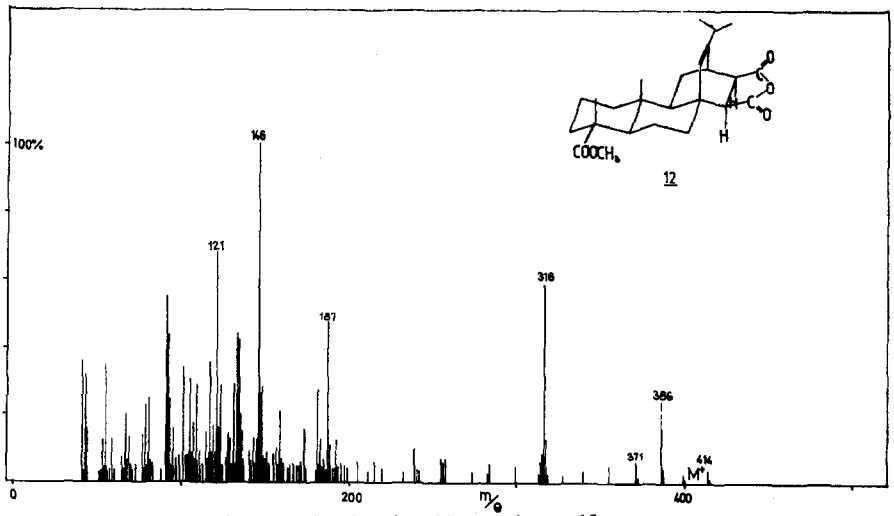


Fig. 9. Mass spectrum of *endo*-maleopimaric acid methyl ester 12.

A very characteristic fragmentation in the mass spectra of the MAA adducts (Figs. 7 and 9) is the elimination of CO, resulting in an ion at m/e = 386 (414 − 28). Apart from the base peak at m/e 146, in the central part of these spectra there are two prominent ions at m/e 187 and 121, which were also found in the fragmentation patterns of different diterpene acid methyl esters[19].

Application

The aim of this study was to achieve as complete a separation as possible of the complex modified resin acid samples using high resolution glass capillary columns. The determination and identification of some adduct components by gas

chromatography–mass spectrometry should be fundamental to other investigations, *e.g.*, of the retro-Diels–Alder mechanism, of the changes in adduct composition upon storage and seasoning of the fortified resins and of the sizing properties of the differently modified tall oil diterpene acids caused by the various distributions of the adducts.

Another objective was to characterize the detected adducts by relative retention times and ECL values. These retention systems also permit automatic analysis and identification without mass spectrometry. This method has proved to be an excellent tool for both detailed component study and routine product control.

ACKNOWLEDGEMENTS

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