Gas Chromatographic Separation of Diterpene Acids on Glass Capillary Columns of Different Polarity[1]

M. MAYR, E. LORBEER and **K. KRATZL**, Institut für Organische Chemie der Universitat Wien, Wahringer Strasse 38, A-1090 Vienna, Austria

ABSTRACT

Mixtures of resin acids from tall oil distillation products and from naturally occurring rosins (colophony) were separated on WCOT glass capillary columns coated with SE 30, FFAP, DEGS and BDS, and the resin acids were identified by gas chromatography-mass spectrometry (GC-MS). In contrast to previous investigations, the identified diterpene acids are characterized by Kovats index values. These retention values are correlated with the different polarity of the stationary phase. The advantages of columns coated with FFAP for the separation of crude and distilled tall oil and fatty acid samples are discussed.

INTRODUCTION

Tall oil and rosin products consist of a large number of resin acids. Such mixtures have been analyzed previously, but in most cases, the separation was achieved on packed columns (1-3). The use of capillary columns has led to much better resolution of the components of the complex tall oil samples and an improved analysis of resin acids and of other compounds (e.g., fatty acids). The additional determination of equivalent chain length (ECL) values on glass capillary columns has been reported recently (4).

The aim of this study was to achieve as complete a separation as possible of the complex natural and tall oil resin acid mixtures using high-resolution glass capillary columns with different stationary phases. Further improvements in the analysis of isomeric diterpene acids formed during the tall oil distillation process were attempted. In this case, a special resin acid fraction from Krems-Chemie Co., Austria, was used for all experiments.

To obtain detailed information on the overall composition of the fractionated diterpene acids, we decided to analyze the samples on a stationary phase of medium polarity, i.e., free fatty acid phase (FFAP). Additionally, levopimaric and palustric acid methyl esters, which previously could only be separated on very polar coated columns, are completely resolved on the FFAP column.

Combination of gas chromatography with mass spectrometry (GC-MS) also follows identification of compounds other than resin acids—mainly fatty acids and resin acids oxidized during the distillation process. Furthermore, one objective was to characterize the identified diterpene acids using Kovats index values, determined for the 4 capillary columns of different polarity. Minor changes in polarity during the lifetime of a column leading to different retention times are compensated by this index system, which also permits automatic analysis and identification without mass spectrometric detection. This method has proved to be excellent both in detailed component study and in routine product control.

EXPERIMENTAL

Samples to be examined were balsamic rosin (colophony) of Polish origin and a special resin acid fraction, distilled from crude tall oil by Krems-Chemie, Austria. Pure diterpene acids for comparison, such as levopimaric and palustric acid, were made available by Krems-Chemie.

The acids were converted to their methyl esters by freshly prepared diazomethane in ether. As resin acids easily isomerize in chlorinated solvents (CH_2Cl_2, $CHCl_3$), aromatic solvents were employed, and the samples were dissolved in toluene or xylene to give 5% (w/w) solutions.

To improve the chromatographic separation and to obtain reproducible results, glass capillary columns were prepared using the methods of Grob et al. (5-7) with SE 30, FFAP, DEGS and BDS as stationary phases. After deactivation of the glass surface with hexamethyldisilazane, one column was statically coated with the silicone rubber SE 30, the other columns were prepared by the barium carbonate treatment, applying the dynamic coating method. Column length was 25 m in all cases, with 0.28 mm id and thickness of the liquid phase, 0.15-0.20 μm.

A Carlo Erba Fractovap 2300 gas chromatograph with flame ionization detector (FID) and hydrogen as carrier gas was used. The samples were injected at 250 C with a split of 1:50 in an all glass Grob injector. Table I shows the selected GC operating conditions to achieve spaced peaks and best separation of the components on the 4 different columns. Flow rate and average linear velocity of the carrier gas, $\bar{u}$m, were measured at the initial temperature of the temperature programming. In order to remain in

TABLE I

Gas Chromatographic Conditions for the Separation of Resin Acids

Stationary phase	Initial temperature (C)	Program rate[a] (C/min)	Final temperature (C)	Carrier flow (mL H_2/min)	Average linear velocity[b] (cm/sec)
SE 30	140	4	280	1.8	50
FFAP	100	4	240	2.3	62.5
DEGS	80	5	220	3.0	83.3
BDS	100	3	220	3.0	83.3

[a]Temperature programs were started when the solvent peaks were detected.
[b]Evaluated at the initial temperature.

[1] Dedicated to Prof. Dr. Erich Ziegler at his 70th birthday.

the flat part of the Van-Deemter curve and not to drop below $\bar{u}_{opt}$ during the course of the runs, high values for the carrier gas velocities were chosen.

Peak areas and exact retention times, necessary for the calculation of Kovats indices, were determined with an attached integrator (Minigrator, Spectra Physics).

GC-MS was done on a Hewlett-Packard quadrupole mass spectrometer System 5922 A, equipped with an all-glass injector, on the 4 columns previously described. The injection port temperature was again 250 C; helium was used instead of hydrogen as carrier gas. In all cases, the column pressures were adjusted to have comparable hold-up times evaluated during the FID-measurements, i.e., the average linear velocities of the carrier gas at the beginning of the temperature programming were equal. The end of the glass capillary column was connected by a restriction (split 1:3) to the ion source. The ion source temperature was 270 C, ionization energy was 70 eV.

Identification of the various substances was possible by the analysis of their mass spectra. The identification of the 7 diterpene acids available in pure form (abietate, palustrate, levopimarate, neoabietate, dehydroabietate, pimarate and isopimarate) was trivial and was ascertained by coinjection. The mass spectra of the other resin acids were compared with unambiguous spectra reported in the literature (1,2,8). Further information on the structure of the unknown acids was obtained by comparing the retention data with results reported (4,9) for identical columns. MS data of the 22 identified compounds—the 5 most prominent peaks and the relative abundance of the parent ion M^+ are given in Table II.

Following identification, the compounds were standardized by relative retention values using the retention index concept of Kovats (10). These values are independent of column length and film thickness of the stationary phase. Originally, retention indices of Kovats were determined at constant temperature, but in this work, a temperature program was used. By suitable choice of this program, a linear relationship between the retention time and number of carbon atoms of the hydrocarbons used as retention standards was observed.

Retention standard was a 5% (w/w) solution of the *n*-hydrocarbons C_{18}-C_{20}, C_{22}, C_{24}, C_{26} and C_{28} in cyclohexane, which was coinjected with the diterpene acid samples to determine Kovats indices as exactly as possible. The retention of the resin acids to be characterized could be linearly interpolated between the 2 *n*-paraffins with lower and higher retention time. In this way, an index value could be assigned to each resin acid component using the equation:

$$I^y = 100 \cdot z + n \cdot 100 \cdot \frac{t_x - t_z}{t_{z+n} - t_z},$$

where I^y = Kovats index for substance x on stationary phase y; z = number of C atoms in the standard paraffin with lower retention time; t_z = retention time of this paraffin; t_{z+n} = retention time of the paraffin with higher retention time; n = difference in number of carbon atoms between the 2 paraffins; and t_x = retention time of substance x.

Table III lists the retention characteristics of the tall oil resin acid methyl esters separated on SE 30, FFAP, DEGS and BDS columns. Because of different polarities and temperature limits of the coatings, GC conditions vary in each case. Table I lists the different GC conditions.

RESULTS AND DISCUSSION

Composition of the Resin Acid Mixture

To investigate a broad spectrum of diterpene acids, a distilled tall oil resin acid fraction (Sacotan 90 from Krems-Chemie Co., Austria) was analyzed (Fig. 1 and 2). This sample contains fatty acids in less than 3% of the total amount. Also detected were 2 secodehydroabietates ("Seco 1," "Seco 2"), formed by ring cleavage of dehydroabietic acid during the tall oil fractionation process. These 2 substances were identified by comparing the mass spectra with those published by Takeda et al. (11,12): Seco 1 = methyl-2α-[2′(*m*-isopropylphenyl)ethyl]-1β,3α-dimethylcyclohexanecarboxylate; Seco 2 = methyl-2β [2′(*m* isopropylphenyl)-ethyl] 1β,3α-dimethylcyclohexanecarboxylate.

Levopimaric acid is known to be very unstable toward thermal treatment (13) and rapidly isomerizes to abietic acid, only a small amount (less than 0.2%) was found in the tall oil rosin. To characterize this diterpene acid by Kovats index value, a Polish colophony was investigated,

TABLE II

Relative Abundances of the Recorded Mass Spectra of the Identified Resin Acid Methyl Esters

GC peak number	Relative abundances of the 5 most prominent peaks (% of base peak)	Parent ion M^+ (m/e/%)
1	146/100, 187/53, 133/45, 101/40, 92/31	316/ 5
2	146/100, 133/41, 109/26, 131/26, 123/23	316/ 5
3	241/100, 257/47, 316/42, 301/39, 91/37	316/42
4	241/100, 301/37, 105/34, 91/33, 316/31	316/31
5	121/100, 180/25, 91/18, 119/14, 105/14	316/ 6
6	121/100, 91/16, 119/15, 133/15, 93/14	316/ 7
7	243/100, 121/46, 91/33, 105/40, 186/36	318/20
8	121/100, 91/46, 81/41, 146/32, 237/31	316/ 5
9	241/100, 247/52, 256/51, 187/41, 105/37	316/25
10	241/100, 148/84, 301/82, 105/79, 149/68	316/61
11	131/100, 241/65, 105/53, 201/51, 256/45	316/39
12	121/100, 136/90, 105/67, 91/49, 92/43	316/31
14	256/100, 121/82, 241/72, 213/71, 105/68	316/48
15	239/100, 240/21, 299/12, 141/11, 129/10	314/10
16	237/100, 312/23, 238/21, 297/12, 181/ 8	312/23
17	135/100, 121/32, 148/25, 134/19, 91/18	316/ 7
19	237/100, 197/53, 195/31, 312/31, 141/22	312/31
20	254/100, 121/83, 314/68, 132/62, 134/60	314/68
21	253/100, 328/48, 187/26, 254/21, 269/17	328/43
Levopimarate	121/100, 146, 95, 91/90, 92/71, 133/48	316/32

TABLE III

Retention Characteristics (Kovats Index Values) of Diterpene Resin Acid Methyl Esters Determined on Different Glass Capillary Columns

Peak number[a]	Systematic name (methyl)	Common name	Kovats index			
			$I^{SE\ 30}$	I^{FFAP}	I^{DEGS}	I^{BDS}
1	Seco 1		2130	2312	2269	2262
2	Seco 2		2150	2344	2302	2290
3	8,15-Isopimaradien-18-oate		2158	2473	2391	2376
4	8,15-Pimaradien-18-oate		2184	2511	2433	2420
5	8(14),15-Pimaradien-18-oate	Pimarate	2198	2511	2429	2411
6	8(14),15-Isopimaradien-18-oate	Sandaracopimarate	2214	2546	2470	2450
7	13-Abieten-18-oate		2244	2571	2477	2466
8	xx-Abietadienoate		2224	2582	2511	2489
	8(14),12-Abietadien-18-oate	Levopimarate	2264	2622	2538[b]	2528[b]
9	7,15-Isopimaradien-18-oate	Isopimarate	2252	2628	2561	2540
10	8,13-Abietadien-18-oate	Palustrate	2264	2635	2540	2530
11	13β-Abieta-7,9(11)dien-18-oate		2278	2684	2604	2585
12	xx-Abietadienoate[c]		2312	2696	2655	2643
13	Docosanoate	Behenate (22:0)	2517	2727	2629	2602
14	7,13-Abietadien-18-oate	Abeitate	2339	2766	2709	2679
15	8,11,13-Abietatrien-18-oate	Dehydroabietate	2288	2779	2746	2704
16	6,8,11,13-Abietatetraen-18-oate		2378	2807	2806	2745
17	8(14),13(15)-Abietadien-18-oate	Neoabietate	2387	2820	2755	2716
18	Tetracosanote	Lignocerate (24:0)	2715	2947		
19	xxxx-Abietatetraenoate		2433	2983	2962	2890
20	7,13,15-Abietatrien-18-oate		2466	3017	2996	2902
21	7-Oxo-8,11,13-Abietatrien-18-oate	7-Oxodehydroabietate	2507			

[a]Used in all FID-chromatograms.

[b]Approximate value as a result of poor resolution from palustrate.

[c]Probably 8, 12 abietadien 18 oate.

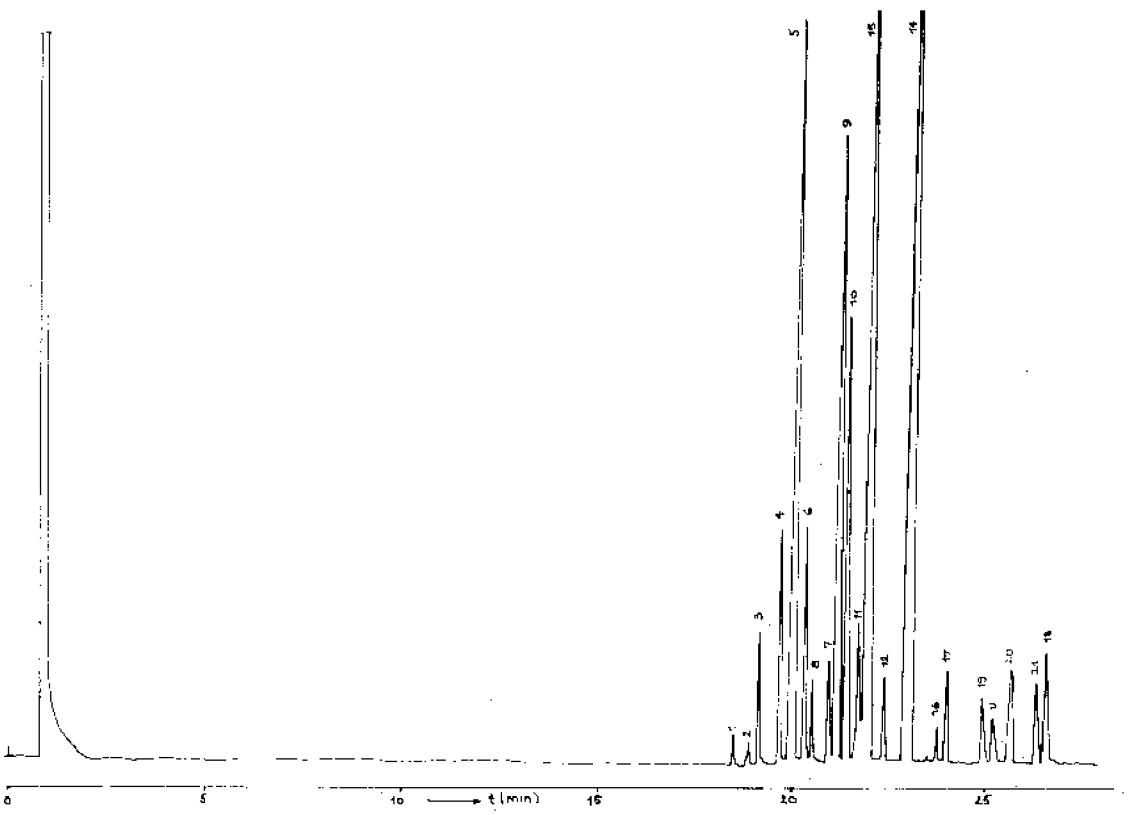

FIG. 1. Gas chromatographic analysis of diterpene acid methyl esters from a crude tall oil resin acid fraction (Sacotan 90, Krems-Chemie Co.): FID-chromatogram on SE 30. Peak numbering as in Table III; U = unidentified substance. GC conditions see Table I.

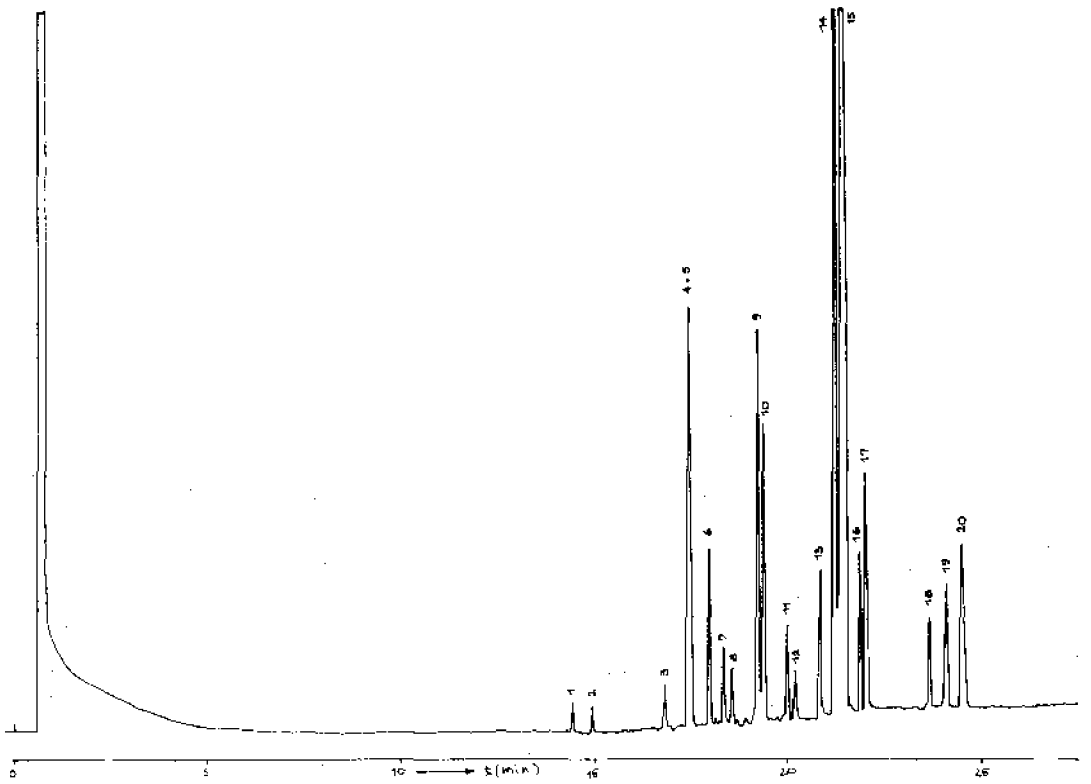

FIG. 2. Gas chromatographic analysis of diterpene acid methyl esters from a crude tall oil resin acid fraction (Sacotan 90, Krems-Chemie Co.): FID-chromatogram on FFAP. Peak numbering as in Table III; U = unidentified substance. GC conditions—see Table I.

The levopimaric acid was found in an amount of 2.4%, based on the total volatile mixture in this naturally occurring rosin. In addition, the extent of separation of levopimaric and palustric acids could be examined with this sample. The gas chromatograms of the methylated colophony showed no separation of the 2 acids on the SE 30 column and poor resolution—in contrast to reports by Holmbom (4)—on DEGS and BDS columns. On the other hand, on the column coated with the FFAP phase (with lower polarity than DEGS and BDS), levopimaric and palustric acid were nicely separated. In addition, the elution of the isopimaric acid between the other 2 acids was observed (Fig. 3). Oxidized resin acids formed during the distillation process were found in small amounts in the Sacotan 90 sample. With the exception of 7-oxodehydroabietic acid (14) on the SE 30 column, these substances did not have reasonable retention times, but were eluted considerably only after the GC temperature programs had ended.

Identification of the Resin Acids

The objective of this investigation was to achieve as complete a separation as possible of all resin acid components. As can be seen in Table III, more than 20 diterpene acids could be identified in the tall oil resin acid mixtures.

While resolution of all components was sufficient when an FID was used as a detector, the maximal scan speed of the MS equipment used was only 200 amu/sec for the range of 33-400 mass units, and some smaller peaks were not separated in the sum plot chromatograms (i.e., sum of the intensities of all ions detected). Identification of all compounds in such a case was achieved by comparing the elution patterns of 2 or more columns of different polarity. Because corresponding peaks on the FID-chromatograms could be identified by their relative intensities, and because resolution of components was satisfactory for GC-MS analysis on at least one column, the mass spectroscopic identification of all components was possible.

Characterization of Resin Acids: Kovats Indices—Conclusions on Qualitative Separation

After identification of the single components using polar and nonpolar phases, the diterpene acids could be characterized on each column by retention values. These retention values were calculated by programmed analysis applying the Kovats index concept (10).

The Kovats indices obtained with the 4 columns show that the resin acids were eluted in a more or less broad range. This range increases with the polarity of the stationary phase. As seen in the FID-chromatograms the high polarity of DEGS and BDS columns did not improve the resolution of the diterpene components and the resulting peaks were broadened. Both these columns also showed considerable bleeding of phase upon repeated operation up to 200 C.

Improved separation could be obtained on the FFAP column. Similar to the column coated with SE 30, this phase gave the properties required for high-resolution GC: (a) close to perfect resolution of all diterpene acids with peaks of satisfactory ratio of height to half-width, (b) suitability for quantitative analysis, and (c) high thermostability and long column life.

In order to test the superior properties of the FFAP-coated column, various samples containing fatty acids, terpenes and related substances were injected. Among them were crude tall oils, wood extractives, various tall oil distillation produces, and finally, a sample of oleoresin freshly obtained by exudation from spruce immediately prior to injection (Fig. 4). In all cases, separation of components, especially of the fatty acid mixtures of mainly

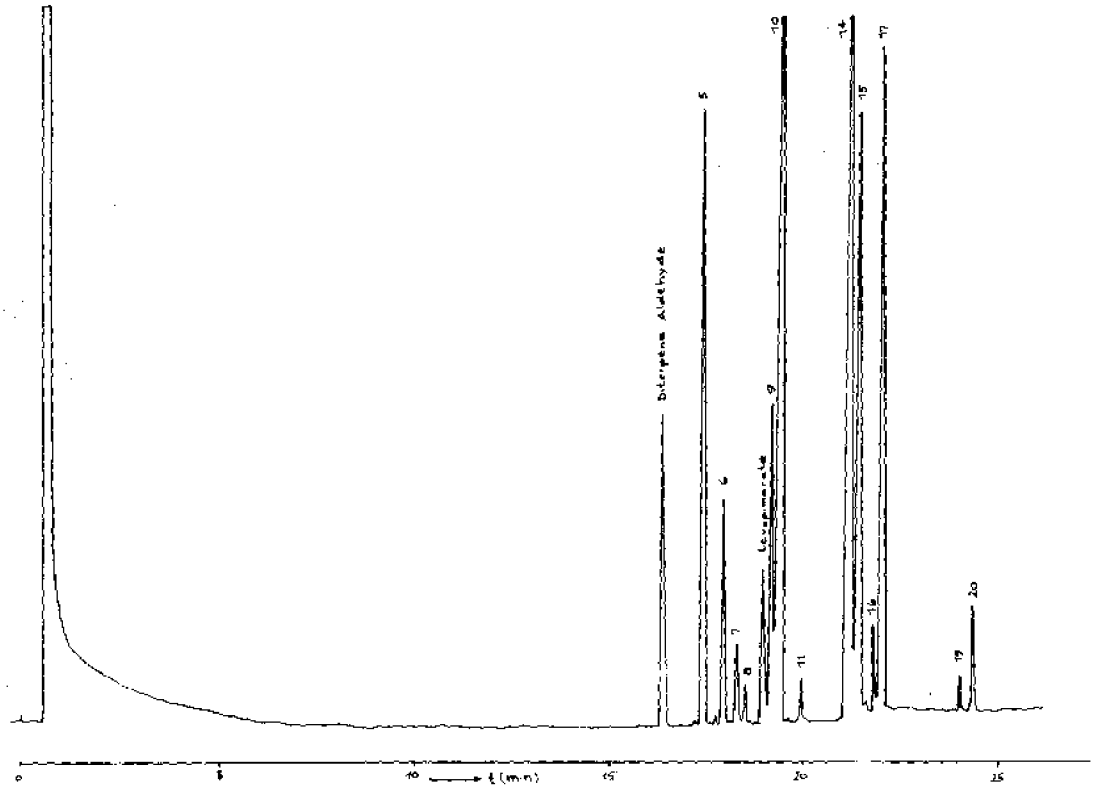

FIG. 3. FID-chromatogram of methylated Polish colophony on FFAP. GC conditions—see Table I.

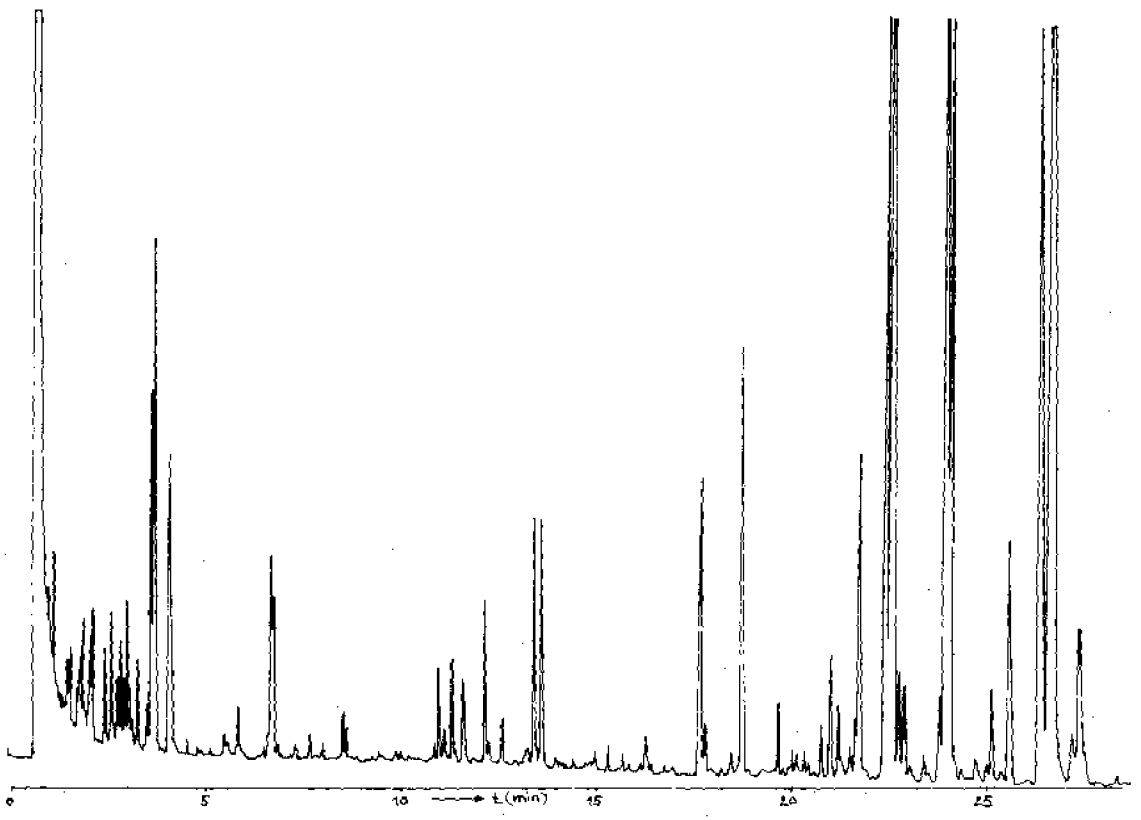

FIG. 4. FID-chromatogram of methylated oleoresin from spruce on FFAP. Column, 25 m FFAP, 0.28 mm id; temperature, 80-240 C, 4 C/min; carrier gas, H_2 ($\bar{u}$ = 62.5 cm/sec). Identified fatty acid and resin acid methyl esters are indicated.

octadecadienic and octadecatrienic acids formed during the Kraft process, was very satisfactory. This stationary phase therefore seems very well suited to analyze the complex changes in fatty and resin acid composition during tall oil isolation and distillation processes. More detailed information on these isomerization and disproportionation reactions will be reported in a forthcoming publication.

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Fatty Acid Composition and Cyclopropene Fatty Acid Content of China-Chestnuts (*Sterculia monosperma*, Ventenat)

S.K. BERRY, Department of Food Science and Technology, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia

ABSTRACT

The China-chestnuts (*Sterculia monosperma*, Ventenat) were examined for their fatty acid composition by gas liquid chromatography, infrared and nuclear magnetic resonance spectroscopy. The oil in nuts contained cyclopropene fatty acids (CPFA) determined as silver nitrate derivatives of their esters. The values (area %) for the major fatty acids as methyl esters were 23.47% C16:0, 1.25% C16:1, 2.56% C18:0, 24.89% C18:1, 18.24% C18:2, 5.40% dihydrosterculic, 3.21% C18:3 + C20:0 and 19.15% sterculic. The proportion of CPFA in the oil did not decrease upon cooking the nuts.

INTRODUCTION

Sterculia monosperma, Ventenat (China-chestnut) is a small evergreen tree found in the home gardens of Chinese people in Malaysia. The ripe fruits are scarlet-colored pods containing 1 to 3 glossy black nuts. The nuts are oblong shaped measuring to 2.5-5 cm long and 1.5 2.5 cm wide. Each nut contains a mealy kernel surrounded by 3 layers of skin with a black sticky resinous substance on the outermost shell. The nuts are consumed after boiling or roasting and removing the 3 outer skins, and are reported to taste pleasant, resembling the European chestnut (1).

The chemical composition of these nuts has not been investigated. As they belong to the family *Sterculiaceae*, they may contain cyclopropene fatty acids (CPFA) in their oil. The adverse physiological effects of CPFA in experimental animals are well documented (2,3). Sinnhuber and coworkers (4,5) have found these fatty acids to be carcinogenic in rainbow trout, and atherosclerotic to rabbits. Of the 2 CPFA, sterculic and malvalic acids, the sterculic has been reported to exhibit higher biological activity in animals (2,6,7). In view of these reports, this study was prompted to examine China-chestnuts for their fatty acid composition and CPFA content.

EXPERIMENTAL PROCEDURES

Materials

China-chestnuts and *Sterculia foetida* L. seeds were procured locally. Methyl fatty acid ester standards were obtained through Sigma Chemical Co., St. Louis, MO. Sodium methoxide reagent (0.5 N) was purchased from Supelco, Inc., Bellefonte, PA. All other reagents required for analyses were of analytical grade.

Extraction of Oil and Analyses

Fresh, whole nuts were weighed and average nut weight calculated. The nuts were then divided into 2 equal portions of which one portion was boiled in distilled water for 40 min. All the nuts were dried in the oven at 45 C. The dried nuts were shelled manually, and kernel-to-shell ratio was calculated. The kernels were pulverized in a mortar and extracted for oil as described previously (8). The moisture and protein content of kernels were determined according to AOAC (9) procedures 7.008 and 2.049, respectively.

The Halphen color test, preparation of methyl esters plus argentation, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies and gas chromatographic (GC) analyses of the mixture of normal fatty acid methyl esters and CPFA ester derivatives were done as described elsewhere (8).