Improved Gas Chromatographic Analysis of Fatty and Resin Acid Mixtures with Special Reference to Tall Oil

B. HOLMBOM, Institute of Wood Chemistry and Cellulose Technology, Åbo Akademi, SF-20500 Turku 50, Finland

ABSTRACT AND SUMMARY

A gas liquid chromatography system for the analysis of complex mixtures of fatty and resin acids has been developed. On 30-40 m long, 0.3 mm ID glass capillary columns coated with 1,4-butanediol succinate (BDS) and attaining over 90,000 effective theoretical plates, all main fatty and resin acids in wood extractives and various tall oil products can be separated and quantitatively determined without need of any prefractionation of the acids. Also the *levopimaric acid is well separated. Retention values*, including their temperature dependence, on columns coated with BDS or SE-30 are given for 49 significant fatty and resin acids. Applications on wood extractives, sulfate soaps, and crude and distilled tall oil are presented and discussed.

INTRODUCTION

Fatty acids and diterpene resin acids occur together in coniferous wood species. Mixtures of these acids comprise the major part of the lipophilic extractives from different parts of coniferous trees. In the kraft pulping industry the fatty and resin acids are recovered as the important by-product, crude tall oil. Also the further refined tall oil products contain both types of acids. Mixtures of fatty and resin acids do also occur in pulp and paper mill process streams and effluents and are major factors in effluent fish toxicity (1).

For the analysis of both fatty and resin acids gas liquid chromatography (GLC) is today the prevailing technique. Usually the GLC analysis is carried out separately for the fatty acids and the resin acids after separating these two groups from each other. Separation is achieved by selective esterification of the fatty acids, e.g., by mineral acid-methanol or boron trifluoride-methanol followed by separation of free resin acids and the fatty acid esters. Fatty and resin acids may also be separated by gel permeation chromatography (2). The procedures are cumbersome and are not always completely selective. During selective esterification procedures risks for isomerizations and artifact formations are involved, especially for the resin acids.

Analysis of fatty and resin acid mixtures by GLC have been made with conventional packed columns and such methods are still in use in many laboratories. Recently the use of a support-coated open tubular (SCOT) column, with a somewhat better resolution than packed columns, was demonstrated by Rogers (1).

High-resolution capillary columns have been found necessary in order to achieve good resolution of natural fatty acid mixtures. The advantages of high-resolution capillary columns for analysis of resin acids in tall oil were demonstrated in an earlier report from our laboratory (3). After further improvements in capillary column preparation techniques the analysis of complex mixtures of fatty and resin acids can now be reported. Special emphasis is directed on various tall oil products which are composed of extremely complex acid mixtures including new isomers formed in the technical processes.

EXPERIMENTAL PROCEDURES

Samples to be analyzed were weighed and 5-10 wt% heptadecanoic acid was added to each sample as internal standard. Free acids were separated by extracting diethyl ether solutions with 2-5% aqueous KOH solution. The acidified (pH 3-4) water solutions were extracted with diethyl ether and the acids were converted to methyl esters by freshly prepared diazomethane. Some samples were analyzed by GLC after separation of the methylated acids by preparative thin layer chromatography (TLC). Identical gas chromatograms were obtained with both separation methods. Selective methylations of only fatty acids were for identification purposes made with a 10% boron trifluoride-methanol reagent.

The glass capillary columns used for GLC were prepared by the methods described earlier (4). The columns were of 30-50 m length with internal diameters of 0.30 mm. Liquid phase films of 0.25-0.30 μm thickness were applied. The following phases were tested with fatty and resin acid mixtures: Apiezon L, OV-101, SE-30, SP-1000, 1,4-butanediol succinate (BDS), and Silar 10 C. For detailed investigations SE-30 was chosen as a nonpolar phase and BDS as a polar phase. The separation power of the columns expressed as coating efficiency (5) was 90-100% for the SE-30 columns and 80-90% for the BDS columns. Corresponding values in effective theoretical plates (N) measured at capacity ratios k greater than 10 were 3100-3400/m (SE-30) and 2800-3100/m (BDS).

A Varian 2100 gas chromatograph with flame ionization detector (FID) was used. The columns were connected to an all-glass inlet splitter constructed around a 10 cm long 4 mm ID glass tube. The connection to the detector was arranged so that the column ended inside the flame tip when addition of extra purge gas was not necessary. Peak areas were measured with a Varian 477 electronic integrator. Hydrogen was used as carrier gas. Other chromatographic conditions are given in Figures 1-3.

Mass spectra were recorded with a LKB 9000 GLC-mass spectrometry (MS) instrument as earlier described (3). For operation with glass capillary columns the instrument was equipped with a glass inlet splitter and a device for adding extra carrier gas (He) at the outlet of the column.

The quantitative behavior of the chromatography system was evaluated with the aid of two quantitative fatty acid methyl ester mixtures, (Applied Science Laboratories, Inc., State College, PA, Table II) and with mixtures of methyl heptadecanoate (puriss. Fluka AG, Buchs, Switzerland) and some purified resin acid methyl esters (Prof. H. Bruun of this Institute).

The injection technique was found to influence the quantitative results appreciably. Sample injections were made with a Hamilton, 701 N 10μl syringe. The following injection technique gave reproducible and reliable quantitative results. The syringe was filled in the following order; 1 μl pure solvent (hexane). 1 μl air, 0.4-0.8 μl 1-2 wt % sample solution (hexane or ethyl ether), and 1μl air. The needle of this type of syringe has a volume of 0.9 μl. With injection techniques where the sample solution was not completely pushed out of the needle, remarkably low yields of higher boiling components were obtained.

Determinations of equivalent chain length (ECL) values (6) were made from isothermal analyses using high recorder chart rates. The ECLs were calculated arithmetically with the assumption of linearity of the logarithm of the adjusted

TABLE I

Characteristics of Tall Oil Fatty and Resin Acids Separated on Butanediol Succinate (BDS) and SE-30 Capillary Columns

	Acid as methyl ester	Abbreviation[a]	BDS[b] ECL (195 C)	BDS δECL/10 C	BDS RRT (195 C)	SE-30 ECL (200 C)	SE-30 δECL/10 C	SE-30 RRT (200C)	Argentation TLC fraction[a]	References to aid in identification
1.	Myristic	14:0	14.00	0		14.00	0		0	
2.		15:0ai	14.71	0.010		14.74	0.005		0	
3.		15:0	15.00	0		15.00	0		0	
4.		16:0ai	15.71	0.010		15.74	0.005		0	
5.	Palmitic	16:0	16.00	0		16.00	0		0	
6.		16:1	16.34	0.015		15.74	0.010		I	
7.		16:1	16.41	0.015		15.77	0.010		I	
8.	14-methylhexadecanoic	17:0ai	16.72	0.010		16.73	0.005		0	
9.	Stearic	18:0	18.00	0		18.00	0		0	
10.	Elaidic	9t-18:1	18.30	0.015		17.77	0.005		0-I	
11.	Oleic	9c-18:1	18.32	0.015		17.71	0.010		I	
12.		11-18:1	18.39	0.015		17.75	0.010		I	
13.		5,9-18:2	18.58	0.025		17.51	0.020		II-III A	
14.	Linoleic	9,12-18:2	18.90	0.025		17.64	0.020		II	
15.		9-19:1ai	18.97	0.015		18.41	0.010		I (9)	
16.	Pinolenic	5,9,12-18:3	19.16	0.045		17.43	0.025		III A	
17.	Linolenic	9,12,15-18:3	19.64	0.050		17.61	0.020		III B	
18.	(9c, 11 t ?)	conj-18:2	19.85	0.030		18.05	0.010		I	(10-12)
19.	Cyclopinolenic	cycl FA	19.88	0.110						"CP 2" in (13)
20.	(10t, 12 c ?)	conj-18:2	19.97	0.030		18.14	0.010		I	(10-12)
21.	Arachidic	20:0	20.00	0		20.00	0			
22.		conj-18:3	20.10	0.050		17.85	0.020		II	
23.		conj-18:3	20.13	0.050					II	
24.		11-20:1	20.29	0.020		19.69	0.015		I	
25.	(10t, 12t, + 9t, 11t ?)	conj-18:2	20.48	0.025		18.49	0.010		0-I	(10-12)
26.		conj-18:3	20.57	0.045		18.16	0.020		II	
27.		conj-18:3	20.63	0.040		18.20	0.020		II	
28.		11,14-20:2	20.87	0.030		20.61	0.015		II	
29.	d	Seco 1	21.04	0.140	0.805				-	(14-16)
30.		5,11,14-20:3	21.10	0.055		20.34	0.025		III B	
31.		20:3	21.20	0.055		20.40	0.025		III A	
32.	8,15-Isopimaradien-18-oic	8,15-IP	21.30	0.205	0.875					
33.	e	Seco 2	21.37	0.140	0.895					(16)
34.	8,15-Pimaradien-18-oic	8,15-P	21.68	0.200	0.985					
35.	Pimaric	P	21.72	0.210	1.000	18.96	0.120	1.000		
36.	Behenic	22:0	22.00	0		22.00	0		0	
37.	Sandaracopimaric	Sa	22.10	0.235	1.125	19.11	0.115	1.050		
38.	13-Abieten-18-oic	DiAb	22.28	0.215	1.180					
39.	Abietadienic	xx-Ab	22.51	0.025	1.270					
40.	Levopimaric	Levo	22.69	0.225	1.350	20.53	0.100	1.225		
41.	Palustric	Pal	22.79	0.200	1.390					
42.	7,9(11)-Abietadien-18-oic	7,9(11)-Ab	22.85	0.220	1.420					
43	Isopimaric	IP	22.98	0.245	1.475	20.45	0.125	1.185		
44.	13β-Abieta-7,9(11)-dien-18-oic	13β-7,9(11)-Ab	23.34	0.225	1.630					
45.	Lignoceric	24:0	24.00	0		24.00	0		0	
46	Abietic	Ab	24.25	0.255	2.185	20.21	0.115	1.565		
47.	Dehydroabietic	DeAb	24.40	0.220	2.290	19.75	0.095	1.325		
48.	Neoabietic	Neo	24.68	0.255	2.500	20.67	0.120	1.840		
49.	Dehydrodehydroabietic	DeDeAb	24.84	0.240	2.630					

[a]Abbreviated notation of fatty acids as proposed by Holman (17); resin acids as in ref. 3.

[b]ECL = equivalent chain length; RRT = relative retention time.

[c]Via preparative separation of five distinct fractions: 0, mainly saturated; I, mono-unsaturated; II, diunsaturated; III A, triunsaturated; and IIIB, triunsaturated fatty acids (methyl esters). TLC = Thin layer chromatography.

[d]2α-[2'(m-- Isopropylphenyl) ethyl] - 1β, 3α - dimethylcyclohexanecarboxylic.

[e]2β - [2'(m - Isopropylphenyl) ethyl] - 1β, 3α - dimethylcyclohexanecarboxylic.

TABLE II

Quantitative Analyses of Reference Mixtures

Acid methyl ester[b]	Mixture A		Mixture B		Mixture C	
	Wt composition, %	Recorded peak areas, %[c]	Wt composition, %	Recorded peak areas, %	Wt composition, %	Recorded peak areas,%
14:0	16.7	16.1 16.5				
16:0	16.7	17.1 16.5	20.0	20.0 20.2		
17:0					20.0	22.1
18:0	16.7	16.7 17.5	20.0	20.6 20.6		
9-18:1			20.0	20.0 19.8		
9,12-18:2			20.0	19.9 20.2		
9,12,15-18:3			20.0	19.5 19.2		
20:0	16.7	17.0 16.8				
22:0	16.7	16.8 16.7				
Isopimaric					20.0	21.4
Levopimaric					20.0	14.5
24:0	16.7	16.3 16.1				
Dehydroabietic					20.0	21.0
Neoabietic					20.0	21.0

[a]Carrier gas flow through column 1.8 ml hydrogen/min, split ratio 1:30 injector temperature 250 C.

[b]Acid notations as in Table I.

[c]Analyses on two different butanediol succinate (BDS) columns.

retention times between consecutive fatty acid methyl esters.

The chromatographic characteristics of the BDS and SE-30 columns were investigated in detail by isothermal analysis of fatty and resin acid mixtures of different origin. The influence on the ECL values of the column temperature was determined as the difference in ECL values (δ ECL) between analysis at 195 C and 205 C for BDS and at 180 C and 200 C for SE-30. Relative retention times were calculated for the resin acids to obtain comparison to earlier analyses in our laboratory (3) and to other published data [e.g., (7)].

The identification of the peaks was based primarily on mass spectra, recorded by capillary GLC-MS. Additional information was obtained by comparing the ECL values with those reported in the literature for the same types of columns of similar polarity (8). The fatty and resin acids could be distinguished via selective methylation of only fatty acids. The higher temperature dependence of the ECLs of resin acids confirmed their location in the chromatogram. For all main resin acids and for several fatty acids reference acids were available. Preparative argentation TLC (9) in combination with GLC was carried out on various fatty acid methyl ester mixtures to obtain information on the degree of unsaturation and on the geometrical configurations at the double bonds.

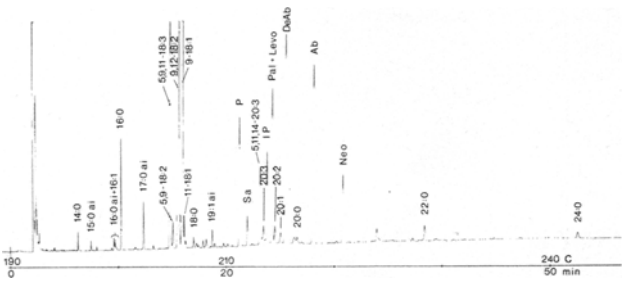

FIG. 1. Analysis of acids from pine wood on SE-30. Samples: total acids from extract of pine *(Pinus sylvestris)* wood chips. Column: SE-30 46 m x 0.30 mm ID. Carrier gas: 1.6 ml hydrogen/min. Acid notations as in Table I.

RESULTS AND DISCUSSION

Chromatographic Characteristics of Columns

Table I lists all the fatty and resin acids present in more than 0.5% in at least one of the samples studied.

On BDS an analysis temperature of 195 C gave an optimal separation in a reasonable analysis time. As experienced earlier by many workers, BDS is of suitable polarity for separation of fatty acid methyl esters. Also positional 16:1 and 18:1 isomers and two 20:3 isomers could be resolved. No overlappings of fatty and resin acids occurred under optimal temperature conditions. All the acids listed in Table I had a difference in ECL of 0.02 or more at 195 C. Acids with ECL differences of 0.03 were clearly resolved on 30-35 m long BDS columns. A small change in polarity may, however, be critical and cause overlappings, e.g., at 190 C the 22:0 acid and sandaracopimaric acid were overlapped and at 185 C the 24:0 acid was overlapped by abietic acid. Notable is the complete separation of the levopimaric and palustric acids which have not been separated earlier as methyl esters. Zinkel has noticed that theses acids can be separated as t-butyl esters on highly efficient Silar 10 C columns (18). These two acids are major resin acids in wood extractives and also in sulfate soaps.

The temperature dependence of retention values was about two times higher on BDS columns than on SE-30 columns. The temperature dependence on BDS expressed as δECL/10 C was 0.015-0.020 for monounsaturated fatty acids, 0.025-0.030 for diunsaturated, 0.045-0.055 for triunsaturated, and 0.110 for a cyclic fatty acid formed during tall oil distillation (13). The values are in good agreement with those reported by Krupčik et al. for BDS (19). Resin acids showed higher values: from 0.140 for secodehydroabietic acids to 0.255 for abietic and neoabietic acids. The temperature dependence can be of value for identification of acid components, especially for distinguishing fatty and resin acids. By appropriate choice of analysis temperature, overlappings of fatty and resin acids can in many cases be avoided. Small differences in polarity between different columns can also be compensated by using different analysis temperatures. A consequence of the temperature dependence of the retention values is also that isothermal analysis and temperature programmed analysis give different elution patterns and may even give a different elution order of components. An isothermal mode can be used for these types of analysis, but programmed operation gives more evenly spaced peaks. It is notable that programmed operation is much more sensitive to changes in operating conditions, e.g., a change in carrier gas flow and also shortening of the column will influence the retention characteristics and may cause overlappings of peaks.

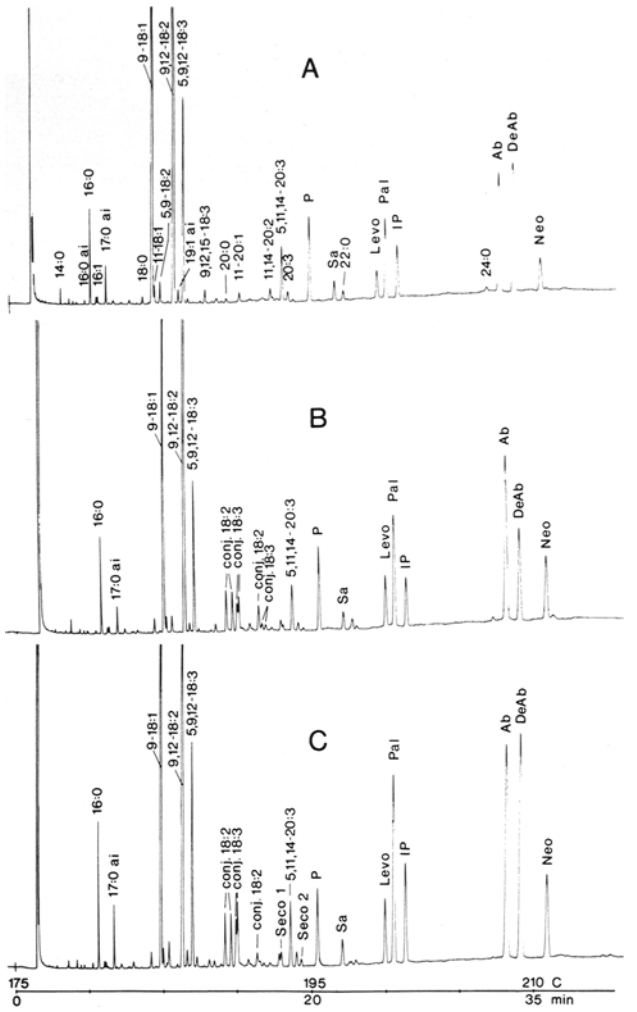

FIG. 2. Analyses of acids from pine wood and sulfate soaps on butanediol succinate (BDS). Samples: A. Sample as in Figure 1. B. Total acids from a Scandinavian pine sulfate soap. C. Total acids from a Canadian sulfate soap. Column: BDS 35 m x 0.34 mm ID. Carrier gas: 2.1 ml hydrogen/min. Acid notations as in Table I.

BDS is one of the most frequently used phases for capillary GLC of fatty acids. Comprehensive compilations of ECL values which have been published (8) can aid in tentative identification of the fatty acids especially by using recently published data for corrections of the differences in polarity of polyester columns (20). BDS columns of steel show a considerable, continuing change in polarity during the lifetime of the columns (21). For the BDS columns of glass used here no notable change in polarity has been experienced even after more than 6 mo daily use up to temperatures of 210 C. Evidently these columns had even and strongly bonded stable stationary phase films. The good stability may be explained by the formation of a "superpolyester" film as reported earlier for packed polyester columns (22).

For SE-30 only acids found in wood extractives, sulfate soaps, and crude tall oils were listed. Several overlappings were found of acids from distilled tall oil products in chromatograms on this column type. Separation of all fatty acids and the resin acid isomers formed in the distillation process could not be achieved. Overlappings of resin acid isomers on the similar liquid phase OV-101 were experienced earlier (3). On SE-30 all major fatty acid components were well separated. All resin acids were separated from the fatty acids at 200 C. At 190 C, however, the 5, 11, 14-20:3 acid and isopimaric acid were overlapped. The SE-30 columns could not resolve levopimaric acid from palustric acid.

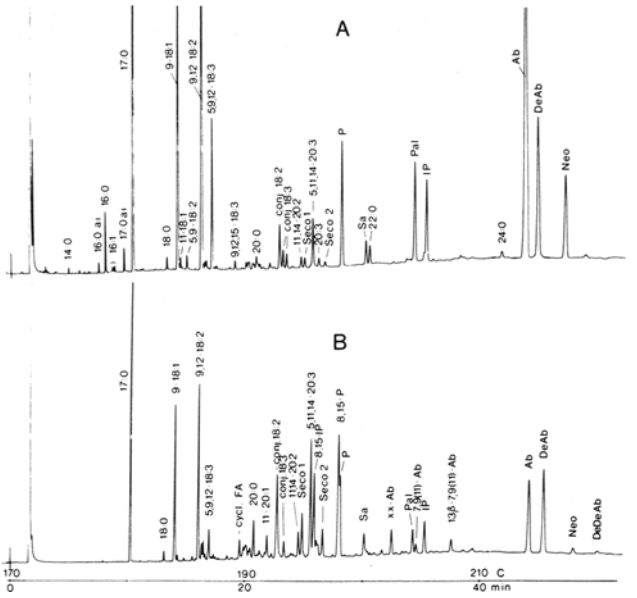

FIG. 3. Analyses of acids from crude tall oil and distilled tall oil on butanediol succinate (BDS). Samples: A. Total acids from a Finnish Crude tall oil. B. Total acids from a distilled tall oil fraction obtained by technical distillation of the crude tall oil in A. Column: BDS 39 m x 0.30 mm ID. Carrier gas: 1.8 ml hydrogen/min. Acid notations as in Table I.

On columns coated with the SP-1000 phase, with a lower polarity than BDS, problems occurred to separate abietic and dehydroabietic acid. On Silar 10 C, with an extremely high polarity, the neoabietic and dehydroabietic acids were overlapped. Levopimaric acid was eluted after palustric acid but was not completely separated even on a column with 120,000 effective theoretical plates.

Aspects on Quantitative Analysis

Heptadecanoic acid is a suitable quantitative internal standard for analysis of fatty and resin acid mixtures. This acid occurs in neglible amounts in samples of wood extractives and tall oil. It is completely separated on both BDS and SE-30 columns.

The quantitative behavior of the chromatographic system is shown in Table II. These tests were made on BDS columns, but tests on SE-30 columns gave similar results. Analyses of a 14:0 to 24:0 fatty acid mixture gave somewhat too low values for both short and long chain acids. Similar responses were found for both saturated and unsaturated fatty acids. The resin acids generally showed the same values as the higher fatty acids eluted in the same region. A significant exception was the levopimaric acid. Only one, normal shaped, peak was obtained for this acid. The quantitative recovery was, however, not complete. A correction factor of 1.50 had to be used for levopimaric acid. A similar lower recovery of levopimaric was earlier observed (23). Uncorrected peak areas can, however, for all other both fatty and resin acids give acceptable quantitative results.

Examples of Application

The samples investigated with the aid of the methods reported here include wood extractives (*Pinus sylvestris, Picea abies,* and *Pinus nigra*), sulfate soaps, and crude tall oils from kraft pulp mills in Finland, Sweden, Turkey, Canada, and the U.S.A., and various tall oil distillation fractions from European distillation plants. More results of these investigations will be published in the near future.

Wood extractives: Most fatty and resin acid components in pine wood extractives were well separated on a high-

efficient SE-30 column (Fig. 1). The palustric and the levopimaric acids are, however, not at all resolved, but are baseline separated on BDS (Fig. 2A).

Sulfate soaps: Several new fatty acid components are formed in the sulfate pulping process. These are alkaline isomerization products evidently formed from the linoleic and pinolenic acids. On BDS seven conjugated fatty acids are well separated and detected. The analysis of a typical Scandinavian sulfate soap (Scots pine, *P. sylvestris*) is shown in Figure 2A and is seen to be notably similar to a typical Canadian sulfate soap (jack pine, *P. banksiana*) (Fig. 2C). It has been reported that levopimaric acid disappears because of isomerization reactions, during the sulfate pulping process (24), but in this work levopimaric acid was found in appreciable amounts in all studied sulfate soaps of different origin.

Crude tall oil, distilled tall oil: In the sulfate soap acidulation process the main component changes are isomerizations of resin acids and of conjugated fatty acids (compare Fig. 2B with Fig. 3A). Levopimaric acid disappears. A redistribution occurs among the conjugated fatty acids. In tall oil distillation several resin acid isomers and cyclic fatty acids are formed. In the intermediate distillate illustrated in Figure 3B the $\Delta 8,15$ pimaric and isopimaric acid both formed during distillation, are dominating components. Notable here is also the disappearance of one of the conjugated 18:3 isomers. This isomer may have been cyclisized to the cyclic fatty acid observed.

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