Identification of Minor Cyclic Fatty Acids in Fractionated Tall Oil

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ABSTRACT

The structure of several minor cyclic fatty acids present in Finnish tall oil fatty acids are elucidated by gas chromatography-mass spectrometry. The origin and mechanism of formation of these cyclic fatty acids are discussed. The cyclic fatty acids identified in tall oil fatty acids are: 4-(5-pentyl-3a,4,7,7a-tetrahydro-4-indanyl)butanoic acid, ω-(o-alkylphenyl)alkanoic acid, 2,6-dimethyl-9-(3-isopropylphenyl)-6-nonenoic acid, 4-(5-pentyl-4-indanyl)butanoic acid, and 4-(2-hexyl-1,2,4a,5,6,7,8,8a-octahydro-1-naphthyl)butanoic acid. In addition, three different branched or cyclic unsaturated C_{19} fatty acids are reported to be present in tall oil.

INTRODUCTION

The structure and formation of bicyclic cyclopinolenic acids [stereoisomers of 4-(5-pentyl-3a,4,5,7a-tetrahydro-4-indanyl)butanoic acids (Ia and IIa in Fig. 1)] present in tall oil were reported earlier (1,2). The combined concentration of these cyclic fatty acids in Finnish tall oil fatty acids (2) is 2.8-3.7% and in Floridan (A. Hase, unpublished work) tall oil fatty acids about 0.4%. These secondary bicyclic fatty acids that are formed during the pulping and distillation processes from pinolenic acid (all cis-5,9,12 octadecatrienoic acid) make up over 90% of all cyclic fatty acids present in tall oil. The rest of the cyclic acids consists of several minor fatty acids. These minor fatty acids are of special interest in studies of tall oil as a nutrient.

By using computerized gas chromatography-mass spectrometry (GC-MS) we have now identified some of those minor cyclic tall oil fatty acids. Especially helpful in the identification work has been the use of the reconstructed mass spectra (3) and mass resolved gas chromatogram (3) as well as the use of mass chromatograms (4).

PROCEDURES

Materials and Methods

The sample of the cyclic fatty acids was prepared from tall oil fatty acids (Enso-Gutzeit OY, Finland) containing ca. 2% of rosin acids and ca. 2% of neutral material. The procedure used was similar to that described earlier (1). Thus urea fractionation was used to remove the straight chain fatty acids as urea adducts. The fatty acids were poured into a boiling solution of urea in 90% aqueous methanol (2.7 ml of 90% methanol and 2.7 g of urea per gram of fatty acids). A high efficiency homogenizing mixer was used to mix the formed adduct. After standing overnight at 5 C the filtrate was separated from the urea adduct by using a cooled centrifuge. The procedure was then repeated for the filtrate fatty acids after the possible adduct present in the filtrate had been split by cold 10% hydrochloric acid solution, the fatty acids extracted, dried with anhydrous sodium sulfate, and the ether evaporated. After a two step urea treatment, the second filtrate was practically free of oleic acid and contained only ca. 4% of linoleic acid. Neutral material was removed by washing the aqueous alkaline solution of fatty acid with ether (5), and the rosin acids were removed by selectively preparing methyl esters from fatty acids and taking the unesterified rosin acids up in aqueous alkaline solution (6).

Ten grams of the resulting fatty acid methyl esters was then fractionated by argentation countercurrent distribution (7,8) using 40 ml of 0.2 N silver nitrate in 90% aqueous methanol and 10 ml of hexane per funnel as solvents in six funnels.

Thus, 200 g of the original tall oil fatty acids gave 25 g of second urea filtrate fatty acids and 16 g of methyl esters from which neutral material and rosin acids had been removed. Ten grams of this concentrate of cyclic fatty acids (containing ca. 45% of cyclic fatty acids by GC-MS) gave ca. 100 mg of fatty acid methyl esters in the methanol phase of the sixth separation funnel after the countercurrent argentation distribution. This 100-mg sample consisted mainly of cyclic fatty acids methyl esters (about 99%), the main component being methyl esters of the two earlier identified cyclopinolenic acids (1) (Ia and IIa in Fig. 1). However, the sample also contained ca. 15% of other cyclic fatty acids methyl esters that were analyzed by computerized GC-MS.

The best source for the two cyclopinolenic acid methyl esters (Ib and IIb) are the hexane phases of the fourth and fifth funnel were they appear in good yield and purity, whereas the methanol phase of funnel one is an excellent source of pinolenic acid methyl ester where it is present in purity of 85 to 90%.

Instrumental Analysis

The GC-MS analysis was performed using instrumentation described earlier (9,11) that consists of a Perkin-Elmer 990 gas chromatograph coupled by a fritted glass Watson-Biemann separator to a modified Hitachi-Perkin Elmer RMU-6L mass spectrometer. It in turn is interfaced with an IBM 1800 computer system which also controls a 16 mm Bolex camera focused at a model 611 Tectronics oscilloscope.

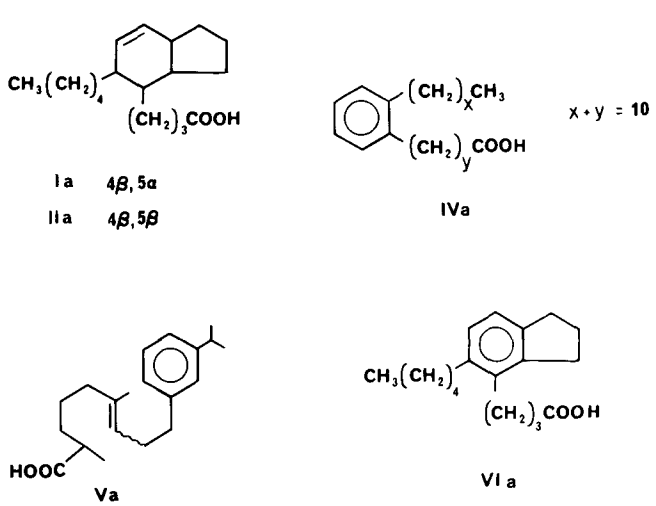

FIG. 1. Structures of two stereoisomers of 4-(5-pentyl-3a, 4,5,7a tetrahydro-4-indanyl)butanoic acids (Ia) and (IIa), structure of ω-(o-alkylphenyl)alkanoic acid (IVa), structure of 9E and 9Z isomers of 2,6-dimethyl-9-(3-isopropylphenyl)-6-nonenoic acids (Va), and structure of 4-(5-pentyl-4-indanyl)butanoic acid (VIa).

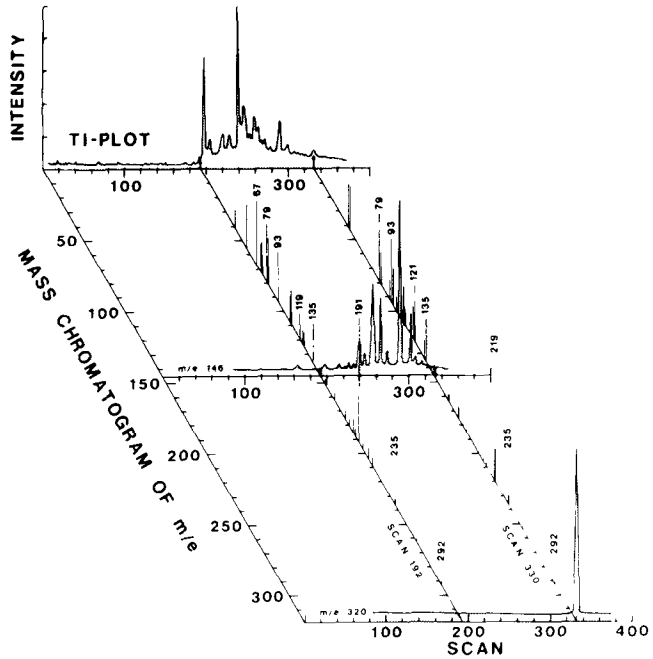

FIG. 2. Three-dimensional representation of a computer analyzed gas chromatography run consisting of a complete set of mass chromatograms and mass spectra (only two of each shown).

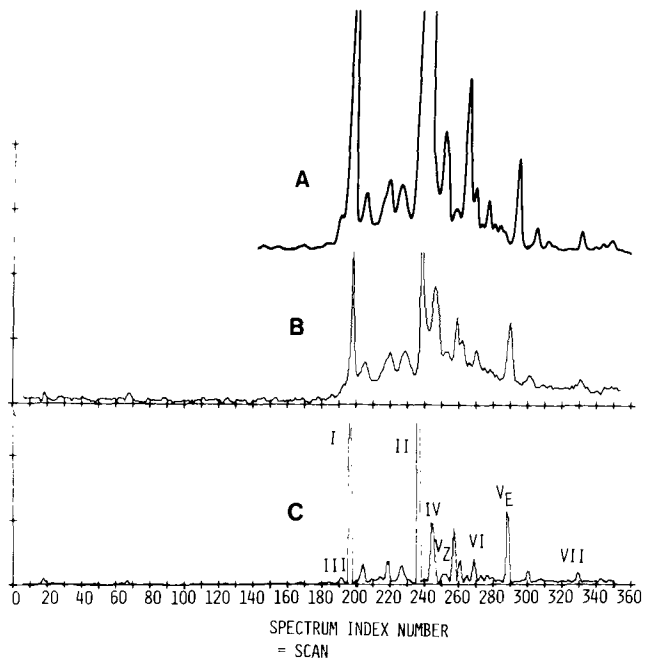

FIG. 3. Chromatograms of cyclic fatty acid ester sample from tall oil. Simultaneous recordings using flame ionization detector (A), total ionization current from the mass spectrometer (B), and mass resolved gas chromatograms (C). The structures of compounds I to VII are presented in Figures 1,4, and 5.

The column was a glass capillary support coated open tubular (SCOT) column prepared using the method of German and Horning (10). The column (100 m x 0.75 mm ID) was drawn from 8 mm Pyrex tubing. The wall was coated with Silanox 101 (Capot Corp., Boston, MA) and SE-30 (Applied Science Laboratories, State College, PA, "GC Grade"). It was used in a Perkin-Elmer 990 GC with a flow of 5 ml/min of helium and a scavenger gas flow of 25 ml/min.

Column oven was programmed from 200 C to 270 C

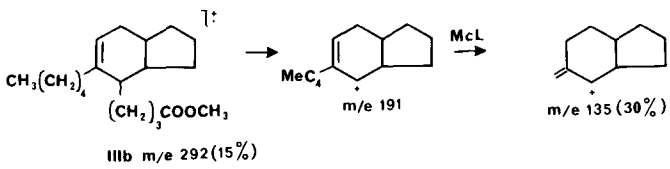

FIG. 4. Formula and fragmentation of 4-(5-pentyl-3a,4,7,7a-tetrahydro-4-indanyl)butanoic acid methyl ester (IIIb).

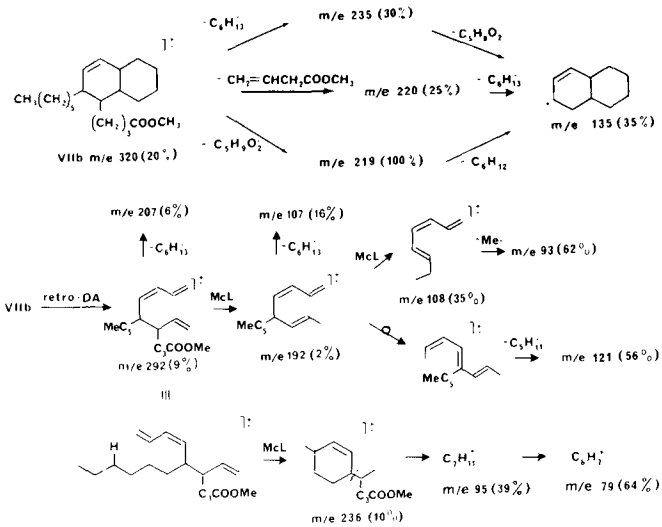

FIG. 5. Formula and fragmentation of 4-(2-hexyl-1,2,4a,5,6,7,8,-8a-octahydro-1-naphthyl)butanoic acid methyl ester (VIIb).

with a rate of 1.5 C/min, and mass spectra (70 eV) were recorded every 4 sec.

The generation of reconstructed mass spectra, mass resolved gas chromatogram, and mass chromatograms are made available by continuous scanning of the mass spectra of the material eluting from the gas chromatograph. After the raw mass spectrometric data accumulated during the gas chromatogram have been processed to generate all mass spectra, the mass chromatograms are generated by representing the abundance of every m/e recorded as a function of spectrum index number, thus resulting in a three-dimensional set of chromatograms each being typical to a certain compound or compound type only (4,11). Figure 2 illustrates the information available in this was from a gas chromatography run.

A peak profile analysis is then performed on each mass chromatogram to identify all scan index numbers where it maximizes. The absolute intensity is then retained for the corresponding scan index number only. After all mass chromatograms are computer analyzed in this manner, the resulting array represents for each scan index number those m/e values which maximize for it along with the absolute abundance of the corresponding ion. A normalized plot of those data corresponds to the mass spectrum of the pure compound eluting at the retention time corresponding to the respective scan index number. This spectrum is called reconstructed mass spectrum (3). Finally the intensities of the ions maximizing at a given scan are summed and plotted v.s. scan index number to produce the mass resolved gas chromatogram (3).

Figure 3 shows the gas chromatogram outputs of the cyclic fatty acid methyl ester sample. The chromatograms are recorded by using the flame ionization detector (A), a simultaneous total ionization plot (B) from the mass spectrometer and mass resolved gas chromatogram (3) (C). The structures of compounds signed with I to VII are presented in Figure 1, Figure 4, and Figure 5. Free carboxylic acids

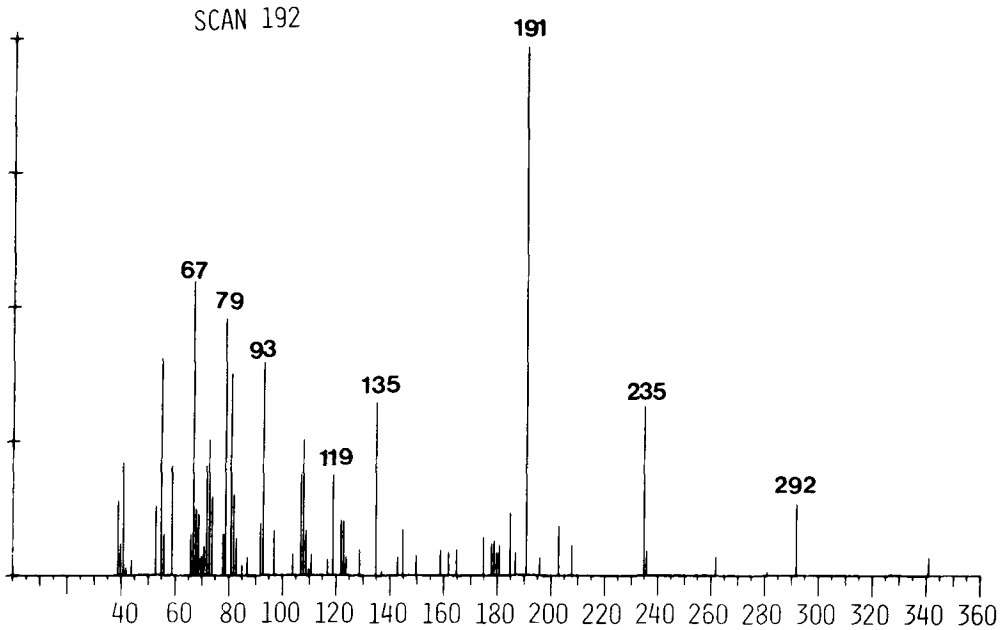

FIG. 6. Scan 192. Reconstructed mass spectrum of methyl 4-(5-pentyl-3a,4,7,7a,-tetrahydro-4-indanyl)butanoate (IIIb).

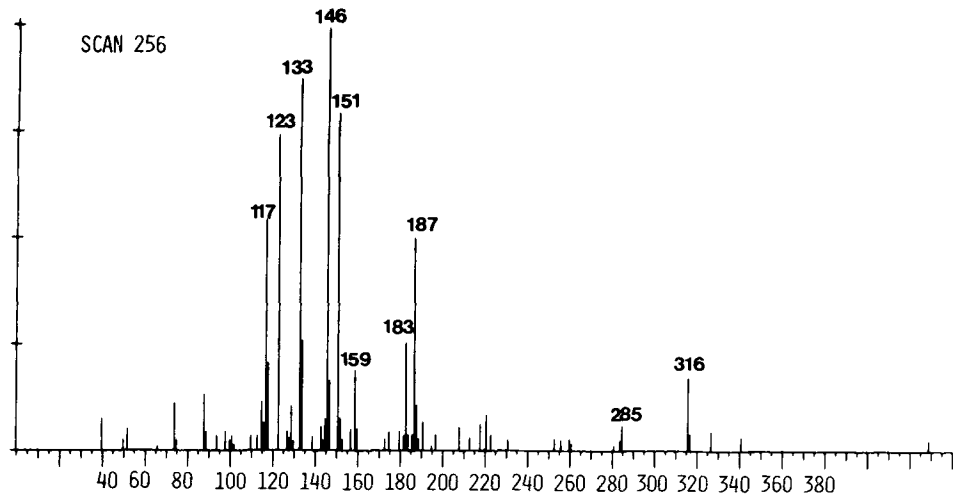

FIG. 7. Scan 256. Reconstructed mass spectrum of the 9Z isomer of 2,6-dimethyl-9(3-isopropylphenyl)-6-nonenoic acid methyl ester (Vb).

are denoted by the letter a and the corresponding methyl esters by the letter b.

ASSIGNMENT AND STRUCTURES

The interpretation of the mass spectra is based on the reconstructed mass spectra (3), some of which are illustrated. The original mixture was complex, and some of the compounds identified were present in such small amounts that the reconstruction was necessary to sort out the ions which belonged to the unknown from the interfering contributions of ions from column bleed and co-eluting compounds.

Occasionally the intensities of some of the minor ions in the reconstructed mass spectra will appear to be distorted, or a peak may be missing altogether. This will happen if the m/e in question is weak relative to the same m/e in neighboring scans. Therefore, the isotope contribution m+1 and m+2 for any given peak m cannot be used with confidence as a support for the fragment ion structures presented.

Scans 195 and *236* belong to the methyl esters of the previously reported (1) two cyclopinolenic acids [stereoisomers of 4-(5-pentyl-3a,4,5,7a-tetrahydro-4-indanyl)-butanoic acid] (Ib and IIb). The mass spectra of these compounds have been published earlier (1,2).

Scan 192: Methyl 4-(5-pentyl-3a,4,7,7a-tetrahydro-4-indanyl)butanoate (IIIb) (Fig. 6). Major fragmentations (Fig. 4) include allylic cleavages directly from M+, giving m/e 235 (32%) and 191 (100%) by the loss of a butyl radical and of $MeO_2C\text{-}(CH_2)_3$, respectively.

Scan 256 and Scan 289: 2,6-dimethyl-9-(3-isopropylphenyl)-6 (Z and E) – nonenoic acid methyl esters (Vb) (Fig. 7). The mass spectra of these scans (with $M^+ = 316$) are identical to the mass spectra of the methyl esters of the two aromatic monocyclic ring opened products Va of levopimaric acid published by Takeda et al. (12). The mass spectrum of scan 256 (Fig. 7) represents the 9Z isomer of V b and the mass spectrum of scan 281 the 9E isomer. The presence of these compounds in tall oil fatty acids has not been reported earlier but two other ring opened products of levopimaric acid, the bicyclic secolevopimaric acids (also with $M^+ = 316$) have been reported to be present in tall oil (13,14).

Scan 265 represents yet another spectrum with a $M^+ = 316$ and peaks at m/e 146 (100%) and m/e 187 (15%). No m/e 101 is present in this spectrum thus indicating that it is

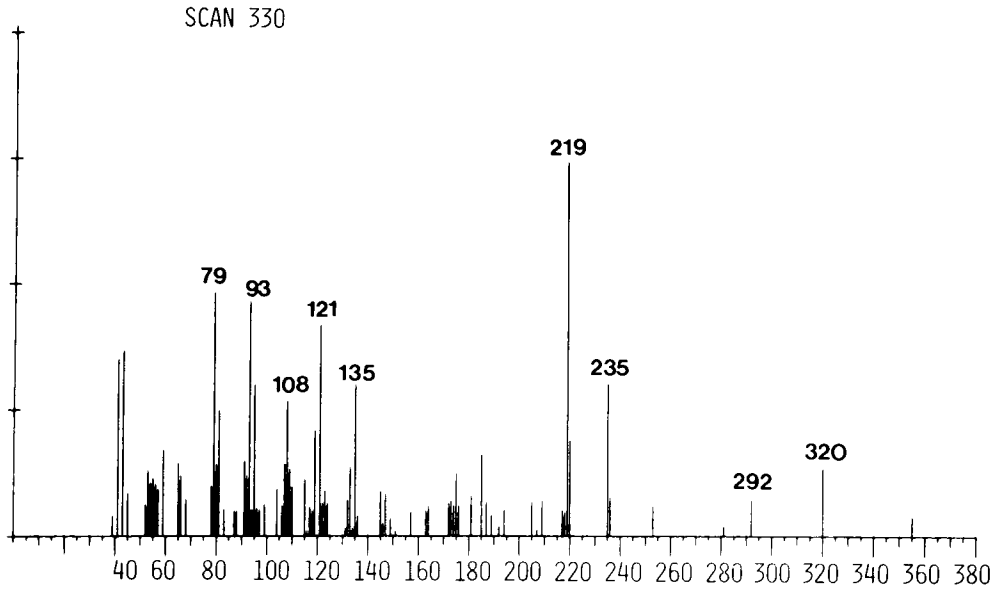

FIG. 8. Scan 330. Reconstructed mass spectrum of methyl 4-(2-hexyl-1,2,4a,5,6,7,8,8a-octahydro-1-naphthyl)butanoate (VIIb).

not identical with the secolevopimaric acid methyl esters reported earlier (13-15). This type of artifact rosin acids have been removed from our sample by the selective esterification method. Scan 265 represents probably an isomer of Vb, but the structure cannot be assigned on the basis of the data available.

Scan 261: Methyl ester of a branched chain C_{19} acid. The general appearance of the mass spectrum is that of an acyclic fatty aicd methyl ester. The molecular ion peak at m/e 308 (27%) belongs to the C_{19} dienoic ester series. The lower mass range of the spectrum features prominent C_nH_{2n-1} and C_nH_{2n-3} series at m/e 41 (100%), 55 (41%), 69 (54%), 83 (27%), and 53 (13%), 67 (73%), 81 (40%), 95 (70%), 109 (53%), respectively. The presence of an ethyl branch or an allylic ethyl group is indicated by a M-29 peak (m/e 279, 23%). The location and nature of branching and of the two double bonds remains open. However, the usual ester McLafferty peak at m/e 74 (8%), and the absence of an analogous peal m/e 102 indicate that an ethyl group is not located at C-2. Although an allylically situated ethyl group would give a M-29 peak, the origin of the sample excludes straight chain C_{19} dienoic acids, since they have been removed by the isolation technique used.

Scan 247: Methyl ω-(o-alkylphenyl)alkanoate (IVb). A fairly stable M^+ at m/e 290 (22%) and the overall scarcity of intense fragment ions are indicative of an aromatic character. The base peak at m/e 105 and its companion McLafferty peak at m/e 106 (15%) show that this C_{18} acid methyl ester is a ω(o-alkylphenyl)alkanoate, its mode of formation requiring that the substituents at the benzene ring are ortho to each other. The mass spectra of several such esters have been reported (16-20). It is not possible here to assign chain lengths to the benzene substituents, but the presence of a peak at m/e 197 (7%) which is lacking (16,17) in isomers with the alkyl side chain $> C_4$ indicates that the alkyl chain is C_1-C_4. The reported (18,19,20) mass spectra of the o-methyl- and o-propyl isomers agree reasonably well with scan 247. To our knowledge, the mass spectrum of methyl 10-(o-ethylphenyl)decanoate has not been reported.

Scan 269: Methyl 4-(5-pentyl-4-indanyl)butanoate (VIb). This scan apparently includes four compounds with molecular ions at m/e 288, 310, 292, and 308. The mass spectrum of the disubstituted C_{18} indanebutanoate (M^+ m/e 288) has been reported earlier (1) and its contribution to the composite spectrum is readily discernible here.

Scan 330: Methyl 4-(2-hexyl-1,2,4a,5,6,7,8,8a-octahydro-1-naphthyl)butanoate (VIIb). The parent ion at m/e 320 (Fig. 8) is indicative of a C_{20} acid methyl ester with three double bonds plus rings. The general appearance is that of a cyclic compound, substantiated by a M-28 peak (m/e 292, 9%), presumably due to a retro-Diels-Alder cleavage of a substituted cyclohexane system. Major fragmentation pathways are assigned as shown in Figure 5.

DISCUSSION

The mechanism of formation of VIIa in tall oil is obviously similar to the formation (2) of cyclopinolenic acid (Ia and IIa) except that the precursor in formation of structure VII is the all-cis-5,11,14 eicosatrienoic acid known (21) to be present in tall oil. This acid isomerizes to 5,11,13-eicosatrienoic acid under the alkaline pulping conditions and then cyclisizes during tall oil distillation process via a Diels-Alder mechanism to form the bicyclic VIIa.

The acid VIa has presumably been formed from Ia and IIa via hydrogen transfer and disproportionation reactions during the distillation processes. VIa could also have been formed via conjugation and cyclization from a pentaenoic C_{18} fatty acid. The presence of such pentaenoic acids in tall oil has not been reported but this does not exclude the possibility that the acid of this type would only occur in cyclisized form in tall oil fatty acids.

The ω-(o-alkylphenyl)alkanoic acid (IVa) is presumably formed from the corresponding acid of a cyclohexadiene structure via hydrogen transfer and disproportionation reactions. The precursor of this intermediate cyclohexadienyl acid is obviously linolenic acid which isomerizes and cyclisizes during the pulping and distillation processes via a mechanism presented earlier (16). It should be pointed out that the chromatogram peak appearing at scan 247 is a mixture of IVb and other methyl esters, and thus the size of that peak does not represent the true amount of IVb in the sample.

IIIa has obviously been formed from Ia or IIa by isomerization. The presence of two other bicyclic fatty acid esters with molecular weight 292 (at scan 245 and scan 226) and a mass spectrum very similar to that of Ib and IIb may indicate the presence of two further cyclohexene ring double bond position isomers. The mass spectra of those

methyl esters (scan 245 and 226) do not, however, permit the determination of the double bond position.

The presence of a branched chain C_{19} enoic acid in tall oil has been proposed earlier (22), and the weak m/e 310 at scan 269 belongs to the M^+ ion of the methyl ester of this acid. The mass spectrum of scan 261 now confirms that there is also another branched C_{19} dienoic acid present in tall oil. It is not possible to establish the site of branching and unsaturation at the hydrocarbon chain. A weak m/e 306 at scan 267 indicates the presence of either a C_{19} trienoic acid of a branched structure or more probably an unsaturated cyclic C_{19} fatty acid formed from a triunsaturated C_{19} fatty acid reported earlier (22) to occur in tall oil.

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