GAS CHROMATOGRAPHIC ANALYSIS OF CYCLOPENTENYL FATTY ACIDS

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Some seed oils of the Flacourtiaceae species contain cyclopentenyl-substituted fatty acids in their glycerides[1]. The fatty acid composition of these oils was first studied by POWER AND GORNALL[2], who also determined the presence of a cyclopentenyl structure in the fatty acid molecules. The major components are chaulmoogric (13-cyclopent-2-enyltridecanoic), hydnocarpic (11-cyclopent-2-enylhendecanoic) and gorlic (13-cyclopent-2-enyltridec-6-enoic) acids.

The composition of Flacourtiaceae seed oils was later investigated by COLE AND CARDOSO[3]. In addition to the above fatty acids, they found traces of lower homologues, *i.e.* alepric (9-cyclopent-2-enylnonanoic), aleprylic (7-cyclopent-2-enylheptanoic), aleprestic (5-cyclopent-2-enylpentanoic) and aleprolic (cyclopent-2-enylcarboxylic) acids and small amounts of normal straight-chain fatty acids, mainly palmitic and oleic acids.

Chaulmoogra oil is the best known product prepared from the seeds of various Flacourtiaceae, formerly obtained mainly from *Taraktogenos Kurzii*, now mostly from *Hydnocarpus Wightiana*. Chaulmoogra oil is used in medicine for the treatment of leprosy. The technical product, however, is rarely sufficiently pure and most frequently contains a mixture of extracts from several related plants. In spite of their importance in medicine, very little attention has been payed to the further investigation of this group of oils.

It is assumed that the fatty acid composition of Flacourtiaceae seed oils may conveniently be determined by means of gas–liquid chromatography, but it was necessary to investigate the chromatographic behaviour of cyclopentenyl fatty acids and to compare them with the normal straight-chain fatty acids.

EXPERIMENTAL

Material

All the chemicals used were analytical reagents, produced by Lachema, nat. corp., Brno.

Chaulmoogra oil was extracted from the seeds of *Hydnocarpus Wightiana* with hexane, and had the following properties: acid value 27.2, ester value 159.9, saponification value 187.1, iodine value (Hanuš) 94.8, unsaponifiable matter 2.4%, melting point 22.8°, water and volatile products 2.2%, total fatty acids 91.4%, iodine value of isolated fatty acids (Hanuš) 97.7, acid value of fatty acids 202.0, peroxide value (iodometric "cold" method) 2.4 mequiv./kg.

Preparation of methyl esters

Approximately 5 g of chaulmoogra oil were refluxed for 2 h with 100 ml of 5 % ethanolic potassium hydroxide solution, 100 ml of 10 % aqueous hydrochloric acid were added and the liberated fatty acids extracted with pentane. The residue after the evaporation of pentane was esterified by refluxing for 2 h with 100 ml of anhydrous methanol containing 2 % of sulphuric acid. About 100 ml of water were then added and the methyl esters were extracted with pentane. The unreacted fatty acids were removed by washing with 5 % aqueous potassium carbonate solution. After the removal of the solvent the methyl esters were used for analysis by gas chromatography.

Analysis by gas chromatography

The analysis was carried out with the Chrom I apparatus manufactured by Laboratorní přístroje, nat. corp., Prague, with a flame ionization detector, described by NOVÁK, RUSEK AND JANÁK[4]. The conditions of the determination using a non-polar stationary phase were: 8.50 g of packing (Celite, particle size of 0.2–0.3 mm, impregnated with 20 % of Apiezon M), temperature of 240°, column length of 80 cm and flow rate of 35 ml N_2/min. The conditions of the determination using a polar stationary phase were: 15.23 g of packing (Celite of the same particle size, impregnated with 20 % of polyethylene glycol succinate), temperature of 190°, column length of 160 cm and flow rate of 35 ml N_2/min. The fatty acid composition was calculated on the basis of the areas of the peaks.

RESULTS

Typical chromatograms on Apiezon and polyester stationary phases are shown in Figs. 1 and 2. Numerical values of relative retention volumes of chaulmoogra fatty acids are summarized in Table I. Normal straight-chain fatty acids were identified by means of pure standards: even-numbered saturated fatty acids of 10–22 carbon atoms, oleic and linoleic acids. Individual fatty acids were identified by comparing graphically logarithms of relative retention volumes on both phases tested, according

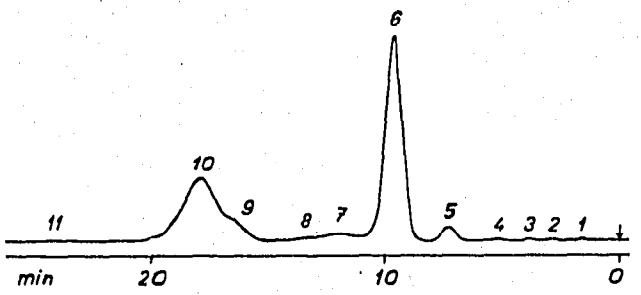

Fig. 1. Separation of methyl esters of fatty acids from *Hydnocarpus Wightiana* seed oil on Apiezon M. For operating conditions see text. Fatty acids: 1 = aleprestic; 2 = aleprylic; 3 = myristic; 4 = alepric; 5 = palmitic; 6 = hydnocarpic; 7 = oleic; 8 = stearic; 9 = gorlic; 10 = chaulmoogric; 11 = eicosanoic.

to JAMES[5]; small amounts of myristic, palmitic, stearic, eicosanoic and oleic acids were detected. No linoleic acid was found by gas chromatography, although 0.2–0.3 % of dienoic acids with isolated double bonds and 0.1 % of dienoic acids with conjugated double bonds were determined by spectral methods. Six other components (three

TABLE I

RELATIVE RETENTION VOLUMES OF NORMAL AND CYCLOPENTENYL FATTY ACID METHYL ESTERS

Methyl ester of fatty acid	*Shorthand designation*	*Relative retention volumes in*	
		Apiezon M at 240°	*Polyethylene glycol succinate at 190°*
Decanoic	10:0	0.15	0.20
Lauric	12:0	0.28	0.34
Myristic	14:0	0.52	0.58
Palmitic	16:0	1.00	1.00
Stearic	18:0	1.88	1.69
Eicosanoic	20:0	3.51	2.92
Behenic	22:0	6.60	5.00
Oleic	18:1	1.72	2.03
Linoleic	18:2	1.70	2.45
Aleprestic		0.19	0.45
Aleprylic		0.37	0.73
Alepric		0.70	1.21
Hydnocarpic		1.31	2.04
Chaulmoogric		2.51	3.38
Gorlic		2.33	3.92

major components and three trace components) were detected, possessing unusual retention volumes on the JAMES' diagram and differing substantially from normal fatty acids (Fig. 3).

As is evident from Fig. 3, the specific component fatty acids of chaulmoogra oil (A–E) lie on one straight line at distances corresponding to an increase of chain

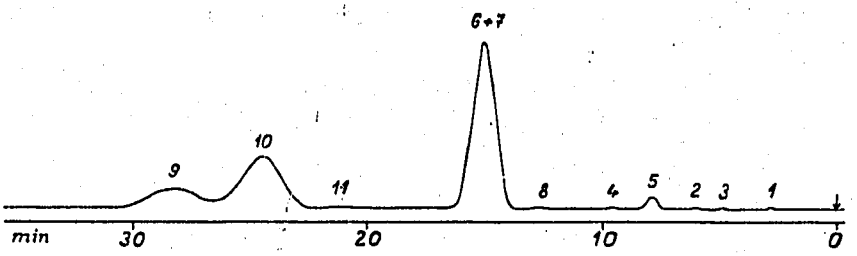

Fig. 2. Separation of methyl esters of fatty acids from *Hydnocarpus Wightiana* seed oil on polyethylene glycol succinate. For operating conditions see text. Fatty acids: the same as in Fig. 1.

length by 2 carbon atoms, similarly to the case of normal aliphatic fatty acids, which are situated on a nearby line. The component F alone lies outside and its position with regard to the nearest component (E) is analogous to that of oleic acid to stearic acid. The series A–E evidently corresponds to the homologous cyclopentenyl fatty acids with saturated straight chains (aleprestic, aleprylic, alepric, hydnocarpic, and chaulmoogric acid, the lowest homologue, *i.e.* aleprolic acid, was not detected), whilst the component F corresponds to an unsaturated derivative of chaulmoogric acid (gorlic acid).

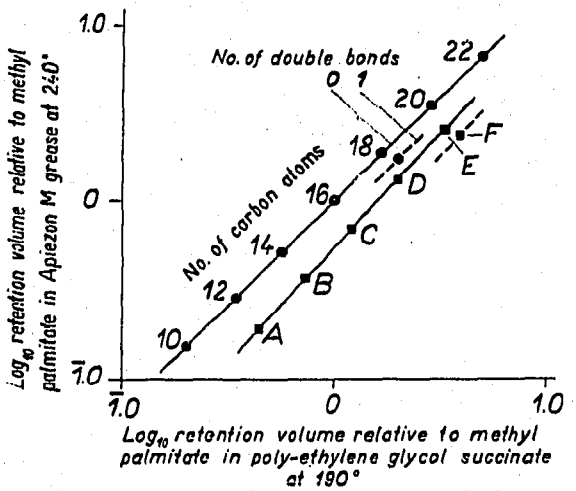

Fig. 3. Log_{10} relative retention volumes of chaulmoogra oil fatty acid methyl esters on Apiezon M plotted against log_{10} relative retention volumes on polyester. Completed by acids not occurring in chaulmoogra oil: decanoic (10:0), lauric (12:0) and behenic (22:0). Straight-chain fatty acids ●; Cyclopentenyl fatty acids ■. A = aleprestic; B = aleprylic; C = alepric; D = hydnocarpic; E = chaulmoogric; F = gorlic.

DISCUSSION

All the cyclopentenyl acids detected in chaulmoogra oil were identified on assumptions in agreement with the known behaviour of homologous series in analysis by gas chromatography[6]. The evidence of this behaviour is a linear relationship of the logarithm of relative retention volumes and the number of carbon atoms in the series, and in particular, the linear relationship obtained by comparison of the logarithm of relative retention volumes obtained on two stationary phases of different polarities. The latter relation is very important as it enables reliable identification of individual members of a homologous series. The same method was successfully applied *e.g.* for the identification of branched-chain fatty acids and less common unsaturated fatty acids[5] because the components of different structures are then situated on different lines on the graph.

The relation between the components assumed to be gorlic and chaulmoogric acids are in good agreement with that of stearic and oleic acids, *i.e.* that oleic acid precedes stearic acid on non-polar stationary phases, while the unsaturated acid follows the saturated acid on polar phases. The percentages of the thus indirectly identified cyclopentenyl acids agree very well with those reported by COLE AND CARDOSO for the oil from *Hydnocarpus Wightiana*[3]. This comparison is given in Table II.

The behaviour of cyclopentenyl and straight-chain fatty acids may be easily compared on the basis of the separation factors calculated from the retention volumes according to JAMES[5,7]; these factors are summarized in Table III. It may be concluded from the position of the line of the homologous series on the JAMES' diagram (Fig. 3) and from the separation factors that the presence of a cyclopentenyl group in the molecule causes a retardation in analysis by gas chromatography on both types of stationary phases. The retardation is greater in the case of the more polar (polyester) phase as a result of an increase of polarity by comparison with normal fatty acids. This increase of polarity is probably caused not only by the presence of a ring in the molecule, but also by the presence of a double bond in the ring.

TABLE II

FATTY ACID COMPOSITION OF CHAULMOOGRA OIL FROM *Hydnocarpus Wightiana*

Fatty acid	*Percentage in fatty acids (wt. %)*	
	Cole and Cardoso[3]	*Authors*
Myristic	—	0.2
Palmitic	1.8	3.0
Stearic	—	0.7
Eicosanoic	—	0.7
Oleic	6.5	2.6
Aleprolic	3.4	not found
Aleprestic		0.1
Aleprylic		0.2
Alepric		0.3
Hydnocarpic	48.9	52.4
Chaulmoogric	27.1	28.4
Gorlic	12.3	11.4

The line of the homologous series of cyclopentenyl fatty acids (Fig. 3) converges to that of the normal fatty acids. The separation factor for the $-CH_2-$ group of cyclopentenyl fatty acids also is smaller on polyester phase than the same factor of straight-chain fatty acids, whilst the $-CH_2-$ separation factors of both straight-chain and cyclopentenyl fatty acids on Apiezon M have the same value. Therefore, the relative polarity of cyclopentenyl fatty acids decreases gradually with increasing molecular weight since the cyclopentenyl group is situated at the end opposite to the carboxyl or ester group and the non-polar part of the molecule (*i.e.* the normal hydrocarbon chain) becomes gradually more important.

Thus, it is evident, that the chromatographic behaviour of cyclopentenyl fatty acids is affected by their structure and depends on the proportion of polar and non-polar parts of the molecule.

TABLE III

SEPARATION FACTORS CALCULATED ON THE BASIS OF THE RETENTION VOLUMES (TABLE 1)

Separation factor	*Stationary phase*	
	Apiezon M 240°	*Polyethylene glycol succinate 190°*
For the $-CH_2-$ group of normal fatty acids	1.37	1.31
For the $-CH_2-$ group of cyclopentenyl fatty acids	1.37	1.29
For the introduction of a double bond in the 9-position of normal fatty acids	0.92	1.20
For the introduction of a double bond in the 6-position of cyclopentenyl fatty acids	0.93	1.16
For the formation of a cyclopentenyl group in the molecule*	1.3	2.0
For the introduction of a cyclopentenyl group at the ω-position**	6.6	7.7

* Determined by the comparison of relative retention volumes of hydnocarpic and chaulmoogric acids with those of palmitic and stearic acids.

** Determined by the comparison of relative retention volumes of hydnocarpic and chaulmoogric acids with those calculated for the acids with 5 carbon atoms less, *i.e.* hendecanoic and tridecanoic acids.

SUMMARY

The fatty acid composition of chaulmoogra oil was investigated by means of gas–liquid chromatography. Normal straight chain fatty acids were identified directly, cyclopentenyl fatty acids, characteristic for chaulmoogra oil, were identified indirectly on the basis of present knowledge of their structure and occurrence in the oil and on the relations of the retention volumes on two stationary phases of different polarities. The chromatographic behaviour of cyclopentenyl fatty acids differs from that of normal fatty acids and depends on their structure.

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