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Note

High-performance liquid chromatography of triglycerides of Flacourtiaceae seed oils containing cyclopentenyl fatty acids (chaulmoogric oils)

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The seeds of a great number of tropical shrubs and trees belonging to the plant family Flacourtiaceae contain oils that exhibit exceptional dextro-rotatory powers owing to their constituent cyclopentenyl fatty acids[1–4]. These unusual fatty acids are chiefly found as constituents of triacylglycerols, together with straight-chain fatty acids.

During the course of our studies directed towards the elucidation of the biosynthesis of cyclopentenyl fatty acids[5,6], we have developed methods for the identification of straight-chain fatty acids and cyclopentenyl fatty acids in a natural mixture[3,7,8].

Recently we have described a simple and direct high-performance liquid chromatographic (HPLC) method that provides a quick estimate of the triglyceride composition of different oils and fats[9–11]. This paper reports the application of HPLC to the analysis of triglycerides of Flacourtiaceae seed oils.

EXPERIMENTAL

Materials

Reference compounds were obtained from NU-CHEK-Prep (Elysian, MN, U.S.A.) and Larodan Chemicals (Malmø, Sweden).

Seeds of *Caloncoba echinata* and *Hydnocarpus anthelminthica* were obtained from Honolulu Botanical Gardens and from the Harold L. Lyon Arboretum of the University of Hawaii (Honolulu, HI, U.S.A.).

Oil extraction and the fractionation of triglycerides by thin-layer chromatography (TLC) were performed as reported earlier[3].

Analytical techniques

The HPLC system consisted of a Spectra Physics (SP 8000) chromatograph, coupled with a Pye Unicam LC-UV detector at 220 nm, and a Rheodyne loop (10 μl) injector (model 7120). A Knauer differential refractometer model 98,00 was used

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for refractive index measurements. An SP 8000 electronic integrator was used to obtain accurate retention times at a chart speed of 0.25 cm/min. The columns used for the separations consisted of two 150 × 4.5 mm I.D. Spherisorb S3 ODS 2 (Phase Sep UK) arranged in series and packed with 3 μm C_{18} bonded phase particles. The columns were maintained at 20°C by coupling the column oven with Hetofrig cooling system (type 03 PF 623 CB 11).

The mobile phase consisted of acetonitrile–tetrahydrofuran (68:32, v/v), both of "HPLC Grade" (Rathburn, Walkerburn, U.K.). Tetrahydrofuran was used without an inhibitor. The mobile phase was prepared by adding appropriate known volumes of each solvent to a flask and was degassed ultrasonically. The compositions reported are volume per cents. The two columns in series produced *ca.* $3.5 \cdot 10^3$ theoretical plates as measured for CCC triglyceride with a total system pressure of $2.5 \cdot 10^3$ p.s.i. at 1.5 ml/min, the usual flow-rate used. The column void volume was *ca.* 3.0 ml.

The sample size was 5–10 μl of *ca.* 10% solutions of triglycerides of *H. anthelminthica* and *C. echinata* in tetrahydrofuran.

Triglyceride peaks were collected from the HPLC effluent and analysed as their methyl esters by gas chromatography (GC). The methyl esters were prepared as described earlier[12]. Analysis of the methyl esters were carried out on a Chrompack fused-silica Silar 10 CP (0.2 μm) wall-coated open-tubular column (50 m × 0.22 mm I.D.). The instrument used was a Perkin-Elmer Sigma 2 gas chromatograph equipped with a flame ionisation detector. The data presented were recorded isothermally at 200°C with helium as carrier gas. The injector (split) and detector temperatures were maintained at 250°C. Quantitation was achieved by integration with a Hewlett-Packard Model 3390 A reporting integrator and comparison with standard mixtures. Triglyceride compositions of the eluted peaks were determined by evaluation of the

TABLE I

CONSTITUENT FATTY ACIDS OF TRIACYLGLYCEROLS FROM SEED OILS OF FLACOURTIACEAE

Analysed as methyl esters by capillary GC; traces below 0.1 wt% are omitted; "cy" denotes the cyclopentene structure of the acid.

Chain length: No. of double bonds	*Caloncoba echinata (weight %)*	*Hydnocarpus anthelminthica (weight %)*
14:1	0.2	0.2
14:1 cy	1.0	0.2
16:0	11.0	5.3
16:1	0.2	1.3
16:1 cy	1.4	57.1
18:0	0.4	0.5
18:1	2.5	3.5
18:2	1.4	0.9
18:1 cy	61.7	26.3
18:2 cy	19.2	3.6
20:1 cy	0.2	0.4
20:2 cy	0.4	0.3

methyl ester composition of each eluted peak. The triglyceride abbreviation CCG means that it is composed of two molecules of chaulmoogric acid (18:1 cy) and one molecule of gorlic acid (18:2 cy).

RESULTS AND DISCUSSION

The results from the fatty acid methyl esters of the two oils studied are presented in Table I. They show a slight deviation compared with the earlier publications[3,13]. This change in the fatty acid pattern is due to the different times of harvest and maturation. However, the characteristics of the oils *i.e.* rather high amounts of gorlic acid in *C. echinata* and high proportions of hydnocarpic acid in *H. anthelminthica* are clearly evident from the values shown in the Table I.

The complete separations of triglycerides of *C. echinata* and *H. anthelminthica* are shown in Figs. 1 and 2, respectively. The total analysis time until the elution of

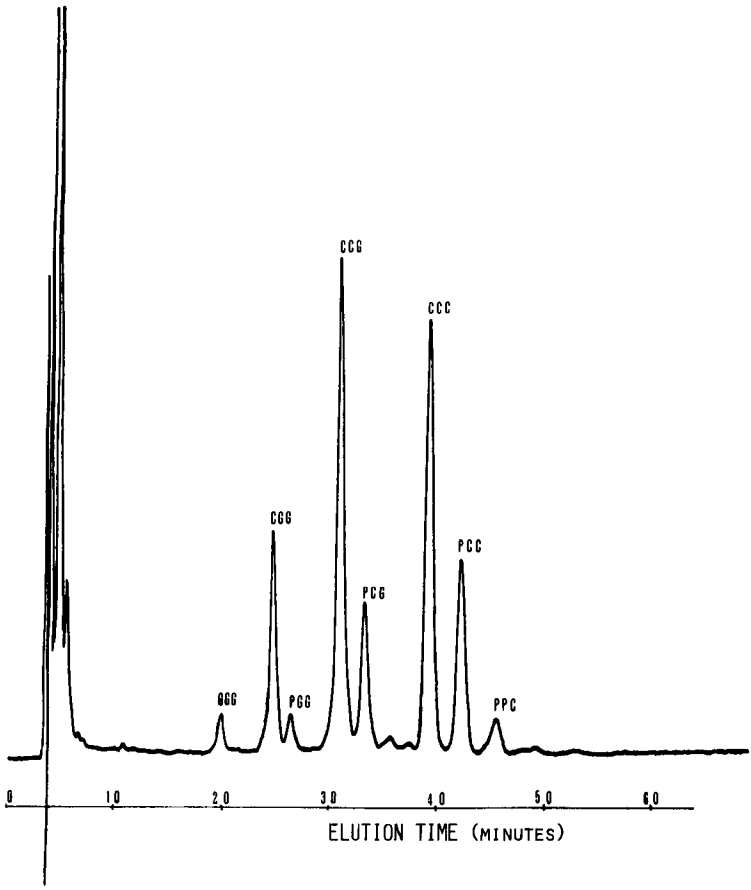

Fig. 1. Separation of triglycerides in *Caloncoba echinata* using two 150 × 4.5 mm I.D. Spherisorb S 3 ODS 2 columns and acetonitrile–tetrahydrofuran (68:32, v/v) as mobile phase at 1.5 ml/min; inlet pressure, 2500 p.s.i. at 20°C; UV detection at 220 nm. Abbreviations: P = palmitic acid; O = oleic acid; H = hydnocarpic acid 16:1 cy; C = chaulmoogric acid 18:1 cy; G = gorlic acid 18:2 cy.

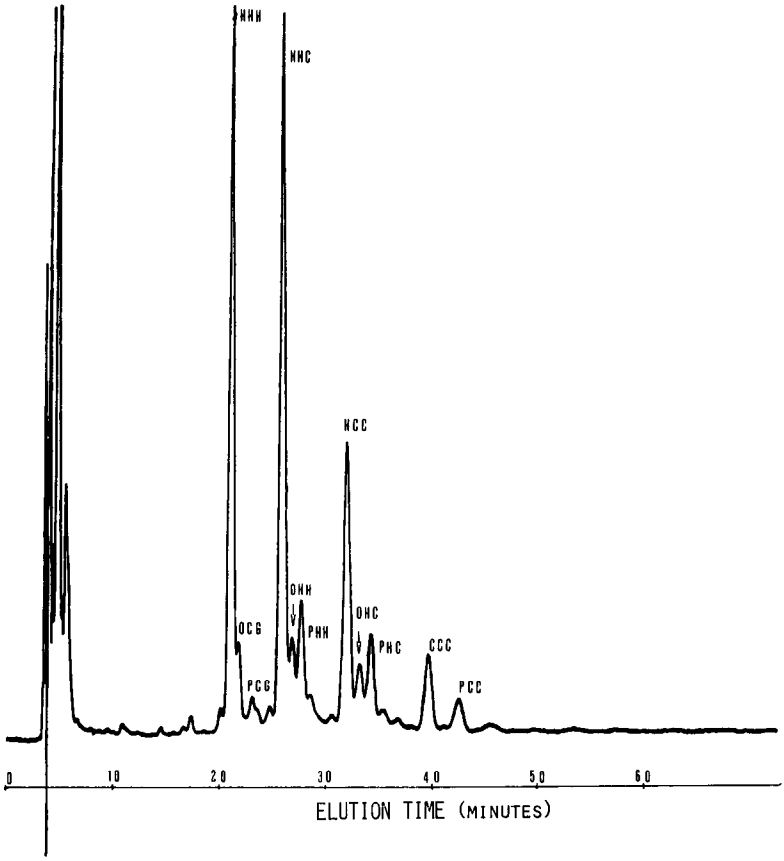

Fig. 2. Separation of triglycerides in *Hydnocarpus anthelminthica*; conditions and abbreviations as in Fig. 1.

PPC is *ca.* 45 min. The chromatograms of the purified triglycerides as presented here are much cleaner than those of the original oils owing to the removal of the early-appearing polar components with higher molar absorptivities. The structural elucidation of triglycerides was achieved by collecting each peak and analysing the respective methyl esters by GC.

In the case of *H. anthelminthica* the collection of sufficient material for analysis of OCG, PCG and OHH was not possible owing to the small amounts available; they were thus tentatively identified by linear logarithming.

The triglyceride compositions of the two oils are shown in Table II. Although Table I shows the presence of a total of 11 straight chain and cyclic fatty acids in each of the two oils only eight different triglyceride species were detected above a level of 1%. However, the present method thus far does not differentiate between different triglyceride isomers such as CCG, GCC and CGC: therefore individual peaks represent mixture of isomers.

The prevalence of C_{18} cyclic fatty acids in *C. echinata* is reflected by the exclusive contribution to the major triglyceride species CCG, CCC and CGG of this

TABLE II

MOLECULAR SPECIES OF TRIACYLGLYCEROLS FROM FLACOURTIACEAE SEED OILS

Traces (below 0.5%) are omitted; abbreviations as in Fig. 1.

Triacyl-glycerol (or their isomers)	*Relative retention time*	*Caloncoba echinata (mole %)*	*Hydnocarpus anthelminthica (mole %)*
GGG	0.97	2.9	
HHH	1.00		39.0
OCG	1.05		0.7
PCG	1.12		0.7
CGG	1.20	17.1	
HHC	1.24		27.1
PGG	1.29	1.4	
OHH	1.30		0.6
PHH	1.34		2.3
CCG	1.51	30.2	
HCC	1.55		15.1
OHC	1.62		3.2
PCG	1.64	7.2	
PHC	1.67		4.1
CCC	1.94	26.2	4.1
PCC	2.11	10.8	1.5
PPC	2.29	1.7	

oil. In *H. anthelminthica* the predominance of the C_{16} cyclic fatty acid is shown by its occurrence in the major triglycerides HHH, HHC and HCC. The triglycerides containing C_{20} cyclic fatty acids were not detected because of the presence of very low concentrations (Table I). The triglycerides containing only straight-chain fatty acids as well as those containing two straight-chain fatty acids were absent from both the oils.

A comparison of retention volumes of triglycerides containing cyclopentenyl fatty acids with those of straight-chain fatty acids show that CCC is equal to OOO, PCC is equal to POO and PPC is equal to PPO, respectively. Thus the chromatographic behaviour of triglycerides containing cyclopentenyl fatty acids and straight-chain fatty acids with the same number of double bonds is quite similar.

The triglyceride composition of *H. wightiana* oil, a plant related to *H. anthelminthica*, has been studied by lipolytic methods[14] and by GC of the hydrogenated oil[15]. Both methods show the presence of HHC and HHH as major triglycerides.

The major advantage of non-aqueous reversed-phase HPLC lies in the fact that it separates the triglyceride critical pairs that are not separated in TLC[16] and GLC[15] techniques. The one-step HPLC method offers simplicity, speed and ease of operation. Since HPLC is a non-destructive analytical technique it allows the collection of pure triglycerides containing cyclopentenyl structures to be used as standards for further identification purposes by complementary methods.

This appears to be the first report in the literature describing the separation of triglycerides of cyclopentenyl fatty acids by HPLC. An early appearance of these triglycerides will be of great help in identifying contaminations of edible oils with

chaulmoogric oils. The latter appear to be poisonous when ingested[1]; however, these oils and their constituent fatty acids have been used for the treatment of leprosy, tuberculosis and other skin diseases[17].

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