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NOTE

Chromatography of Lipids Containing Cyclopentenyl Fatty Acids

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Tropical plants of the family *Flacourtiaceae* produce seeds whose lipid constituents are rich in cyclopentenyl fatty acids (*1*). The major naturally occurring acids of this type are hydnocarpic (11-(2-cyclopenten-1-yl)-undecanoic), chaulmoogric (13-(2-cyclopenten-1-yl)-tridecanoic), and gorlic (13-(2-cyclopenten-1-yl)-6-tridecenoic) acids.

In the course of studies directed toward the elucidation of the biosynthesis of cyclopentenyl fatty acids, we were led to develop methods for the analysis of lipids containing the cyclopentene ring, and for distinguishing them from straight-chain compounds. The present communication records some results of this work.

We have found that mixtures of lipids containing both straight-chain and cyclopentenyl fatty acids can be resolved, by adsorption chromatography, into classes of compounds having the same type and the same number of functional groups per molecule, such as monoglycerides, diglycerides, and triglycerides. For example, triglycerides of straight-chain saturated and unsaturated fatty acids migrate together with those of cyclopentenyl fatty acids. Each lipid class can be isolated and resolved into simpler mixtures or even pure compounds by the use of argentation chromatography or chromatography of acetoxymercuri methoxy compounds or reversed-phase partition chromatography or gas chromatography. As an example, in Fig. 1 the chromatographic behavior of the methyl esters of straight-chain fatty acids, viz., methyl palmitoleate, methyl oleate, and methyl linoleate, is compared with that of the methyl esters of cyclopentenyl fatty acids hav-

ing the same number of carbon atoms and the same number of double bonds, viz., methyl hydnocarpate, methyl chaulmoograte, and methyl gorlate.

(A) Argentation chromatography (*2*) was carried out on layers of Silica Gel G, 0.25 mm in thickness, using hexane–diethyl ether, 70:30, *v/v*, as developing solvent; the various compounds were detected by charring after spraying the plates with chromic-sulfuric acid solution. (B) Acetoxymercuri methoxy compounds (*3*) were chromatographed on layers of Silica Gel G, 0.25 mm in thickness, with the solvent *n*-propanol–acetic acid, 100:1, *v/v*; a 0.1% solution of *s*-diphenylcarbazone in ethanol was used as spray reagent (*4*). (C) Reversed-phase partition chromatography was done on layers, 0.5 mm thick, of Kieselguhr G which had been impregnated with a 5% solution of liquid paraffin in hexane (*5*), using acetic acid–acetonitrile–water, 10:70:25, *v/v/v*, as developing solvent, and iodine vapors as indicator (*6*).

In argentation chromatography (A), the methyl esters of straight-chain fatty acids migrate ahead of the methyl esters of the corresponding cyclopentenyl fatty acids. Thus, methyl palmitoleate and methyl oleate, esters of straight-chain monounsaturated fatty acids, are well separated from methyl hydnocarpate and methyl chaulmoograte which contain a cyclopentene ring. Similarly, methyl linoleate having two isolated double bonds in a normal chain is separated from methyl gorlate which contains one double bond in the cyclopentene ring and one in the aliphatic chain.

Chromatography of acetoxymercuri-methoxy compounds (B) yields separations comparable to those obtained by argentation chromatography. However, considerable overlapping occurs between the derivative of methyl linoleate and the derivatives of methyl hydnocarpate and methyl chaulmoograte.

Reversed-phase partition chromatography (C) does not resolve the methyl esters of straight-chain fatty acids and the corresponding cyclopentenyl fatty acids. Thus, methyl palmitoleate overlaps with methyl hydnocarpate, methyl oleate with methyl chaulmoograte, and methyl linoleate with methyl gorlate. The rate of migration of methyl esters of straight-chain or cyclopentenyl fatty acids having one double bond is the same as that of esters having two double bonds and two more methylene groups. Thus, methyl palmitoleate, methyl hydnocarpate, methyl linoleate, and methyl gorlate all migrate together.

Obviously, argentation chromatography is most useful for fractionating complex mixtures of the methyl esters of straight-chain and

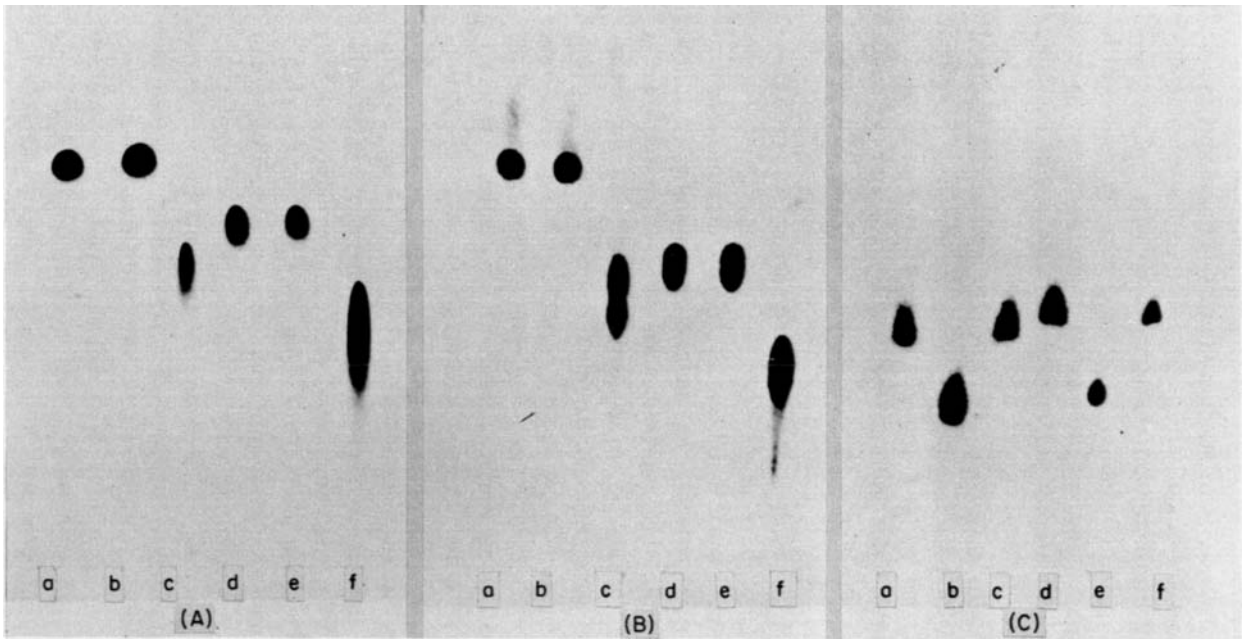

FIG. 1. Comparison, by thin-layer chromatography, of the chromatographic behavior of methyl esters of straight-chain fatty acids with that of methyl esters of cyclopentenyl fatty acids. A, argentation chromatography. B, chromatography of acetoxymercuri-methoxy compounds. C, reversed-phase partition chromatography. Esters of straight-chain fatty acids: a, methyl palmitoleate; b, methyl oleate; c, methyl linoleate. Esters of cyclopentenyl fatty acids: d, methyl hydnocarpate; e, methyl chaulmoograte; f, methyl gorlate. See text for experimental conditions.

cyclopentenyl fatty acids prior to gas chromatography. It is practical to separate the methyl esters of straight-chain saturated and monounsaturated fatty acids from all the polyunsaturated and cyclopentenyl fatty acids by thin-layer chromatography on silica gel containing silver nitrate, to elute each of the two groups of esters with water-saturated diethyl ether, and to analyze them separately by gas chromatography on a relatively polar stationary phase. For example, methyl oleate and methyl hydnocarpate, esters that overlap in gas chromatography on ethyleneglycol succinate (*7*), can thus be resolved from each other and from all the other esters.

We have found argentation chromatography suitable also for the fractionation of triglycerides containing straight-chain and cyclopentenyl fatty acids. Separations were carried out on layers, 0.25 mm in thickness, of Silica Gel G, containing 5% of silver nitrate, with hexane–diethyl ether, 50:50, *v/v*, as developing solvent; the lipid fractions were detected by charring or, when recovery was intended, by spraying the plates with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol. Mixtures of triglycerides having the same number of double bonds were eluted from the adsorbent with water-saturated diethyl ether, and resolved further by reversed-phase partition chromatography. This was done on layers of Kieselguhr G which had been impregnated with a 5% solution of liquid paraffin in hexane; acetone–acetonitrile, 70:30, *v/v*, was used as developing solvent (*8*) and iodine vapors as indicator. The molecular species of triglycerides in the various fractions were identified by gas-chromatographic analysis of their constituent fatty acids.

The fractionation, by argentation chromatography, of oils containing triglycerides of both straight-chain and cyclopentenyl fatty acids is greatly enhanced by the pronounced effect the cyclopentene ring exhibits on the rate of adsorption. Thus, complete analyses of such oils are accomplished more easily, despite their greater complexity, than are analyses of oils containing only triglycerides of straight-chain fatty acids. As an example, Fig. 2 shows the fractionation of the triglycerides of maratti (*Hydnocarpus wightiana*) seed oil into fractions of triglycerides containing a total of five, four, and three double bonds in the three cyclopentenyl fatty acid moieties. These fractions are followed by triglycerides containing two cyclopentenyl fatty acids and one straight-chain fatty acid, and by a fraction of triglycerides containing one cyclopentenyl fatty acid and two straight-chain fatty acids. Hydnocarpodipalmitin, a monounsaturated triglyceride con-

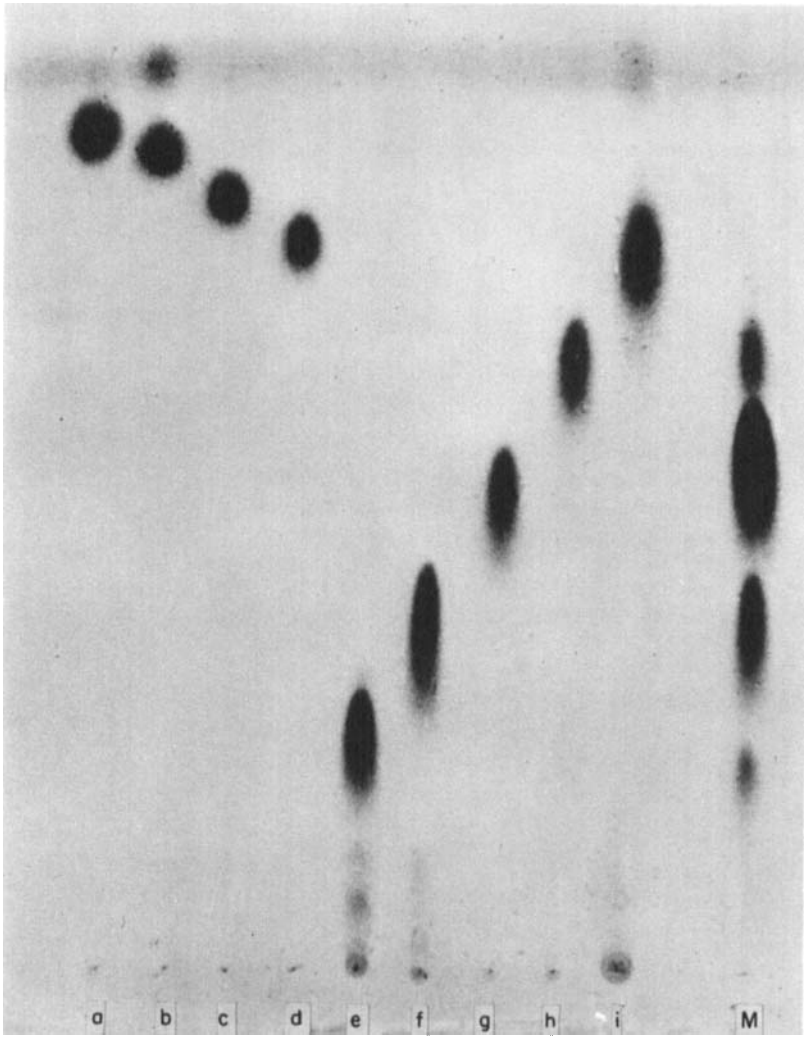

FIG. 2. Argentation chromatography of triglycerides containing straight-chain and cyclopentenyl fatty acids. Triglycerides of straight-chain fatty acids: a, tristearin; b, oleodistearin; c, stearodiolein; d, triolein. Triglyceride fractions isolated from maratti oil: e–i; triglycerides containing five, four, three, two, and one double bond in cyclopentenyl fatty acids. M, total triglycerides of maratti (*Hydnocarpus wightiana*) seed oil. See text for experimental conditions.

taining a single cyclopentenyl fatty acid, migrates at about the same rate as does triolein, a triglyceride having a total of three double bonds in straight-chain acyl moieties.

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