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THE THIN-LAYER CHROMATOGRAPHIC BEHAVIOUR OF CYCLOPENTANYL AND CYCLOPENTENYL FATTY ACID METHYL ESTERS

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SUMMARY

On silicone oil-impregnated and argentated Silica Gel G layers, methyl hydnocarpate and its dihydro derivative were found to be more polar than expected from the number of carbon atoms and degree of unsaturation. The solvent systems used were acetonitrile–water–acetic acid (70:20:10) and light petroleum–ether–acetic acid (94:6:1) respectively. By comparison with straight-chain esters, it is inferred that in reversed-phase systems, this greater polarity resides in the cyclopentane ring and not the terminal double bond in the ring, while the opposite condition prevails in the argentation system.

INTRODUCTION

Information on the chromatographic behaviour of alicyclic fatty compounds is meagre. In silicic acid column chromatography, alicyclic hydrocarbons are eluted after straight-chain hydrocarbons, due to the greater polarity of the former[1,2]. Such behaviour may also be expected from fatty acids or esters carrying a ring. ZEMAN AND POKORNY[3] demonstrated the longer retention time of the cyclopentenyl fatty acid esters compared to straight-chain esters. However, no information is available concerning the behaviour of the cyclopentanyl and cyclopentenyl esters in thin-layer chromatography (TLC). Such information is reported here.

EXPERIMENTAL

Preparation of methyl hydnocarpate and chaulmoograte

The mixed fatty acids of *Hydnocarpus wightiana* oil were subjected to two urea adduct separations; the first in the ratio of 1:1.3:8 (fatty acid–urea–methanol), and the next on the filtrate in the ratio of 1:2.6:10. The fatty acids isolated from the second filtrate were methylated. The esters were fractionated in an electrically heated and packed column, and the fractions boiling at 170–172° and 174–176° at 2.5 mm/Hg were collected. The second fraction contained mainly chaulmoograte and very little gorlate, as shown by the argentation-TLC described later. The lower boiling fraction was refractionated in a Piros–Glover spinning-band column. The fraction collected

at 167° at 2.5 mm/Hg was judged as highly pure hydnocarpate from the following data: Iodine value: found, 95.1; theory, 95.3, saponification value: found, 208.9; theory, 210.6. A single spot in reversed-phase and argentation-TLC systems, described later, and a single peak in GLC, using both a diethylene glycol succinate at 205° and a SE-30 column at 240°, were found.

Preparation of dihydrohydnocarpate

Methyl hydnocarpate was hydrogenated fully in ethanol solution using palladium on carbon at 27°. TLC systems showed the absence of hydnocarpate.

Other fatty acid esters

The methyl esters of myristic, palmitic, stearic, oleic, linoleic and linolenic acids were prepared from either technical-grade acids or from vegetable fats rich in these, using a combination of crystallization, urea adduction and ester fractionation techniques. Their purity except for that of linolenate, which contained traces of linoleate, was confirmed from the appearance of single peaks in GLC using a polyester column as described for hydnocarpate and single spots in reversed-phase and argentation-TLC systems.

Preparation of Silica Gel G plates

Glass plates (20 × 20 cm) were coated with a slurry of Silica Gel G (E. Merck) in water (1:2) to a thickness of 250 μ, using a 'Camag' applicator. The plates were dried at 110° for 1 h and cooled in an empty desiccator.

Reversed-phase TLC

Impregnation was carried out by ascending development with a 5% solution of silicone oil in light petroleum. The fatty acid esters (2–3 μg) were spotted and developed with acetonitrile–water–acetic acid (70:20:10) for 90 min. The spots were visualized by spraying with 20% ethanolic phosphomolybdic acid solution and heating at 180° for 10 min. The straight-chain esters appeared as dark spots, and the cyclopentanyl and cyclopentenyl esters as dark blue spots against a yellow background. Fig. 1 shows the separations.

Argentation-TLC

Impregnation with silver nitrate was carried out by spraying with 10 ml of a 6.25% solution in 50% aqueous ethanol. The plates were dried at ambient temperature for 10 min, and at 110° for 15 min, and cooled in an empty desiccator. After spotting, the plate was developed with light petroleum–ether–acetic acid (94:6:1) for 80 min in a filter paper-lined chamber. The spots were visualized by charring with phosphomolybdic acid as before. Fig. 2 shows the separations.

DISCUSSION

No studies were made with direct TLC, since it is not effective compared with reversed-phase and argentation-TLC, in detecting contaminants differing only slightly in chain length and unsaturation. However, cyclopentenyl esters could be expected to migrate to a lesser extent than straight-chain esters.

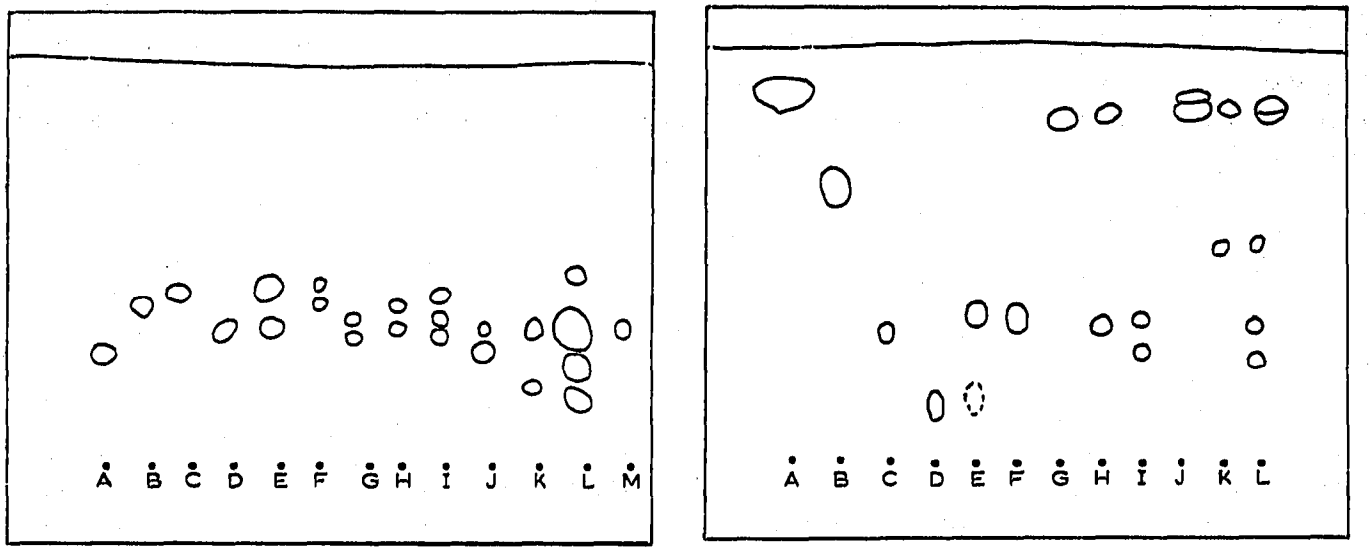

Fig. 1. Chromatogram of methyl esters on silicone oil-impregnated Silica Gel G. Developer: acetonitrile–water–acetic acid (70:20:10). Indicator: phosphomolybdic acid. (A) = Oleate; (B) = linoleate; (C) = hydnocarpate; (D) = dihydrohydnocarpate; (E) = (C) + (D) + chaulmoograte; (F) = (B) + (C); (G) = (A) + (D); (H) = (B) + (D); (I) = (A) + (B) + (D); (J) = (D) + palmitate; (K) = (D) + stearate; (L) = (D) + myristate + palmitate + stearate; (M) = (D) + chaulmoograte.

Fig. 2. Chromatogram of methyl esters on argentated plate. Developer: light petroleum–ether–acetic acid (94:6:1). Indicator: phosphomolybdic acid. (A) = Palmitate; (B) = oleate; (C) = linoleate; (D) = linolenate; (E) = hydnocarpate + chaulmoograte (+ gorlate, indicated by broken line); (F) = hydnocarpate; (G) = dihydrohydnocarpate; (H) = (F) + (G); (I) = (C) + (F); (J) = (A) + (G); (K) = (B) + (G); (L) = (A) + (B) + (C) + (F) + (G).

Reversed-phase TLC

Separation occurs in reversed-phase TLC on the basis of polarity, which in turn depends on the number of carbon atoms and double bonds. Critical pairs, however, arise because of the equivalent effects of one double bond and two methylene groups. Accordingly, hydnocarpate should move along with linoleate (myristate)*, and dihydrohydnocarpate (chaulmoograte) with oleate (palmitate). As seen in Fig. 1, the cyclopentenyl and cyclopentanyl esters migrated to a greater extent than expected from the number of carbon atoms and double bonds present in these. This increased polarity can be attributed to the presence of the ring structure as well as to the double bond in it. ZEMAN AND POKORNY[3] gave a similar explanation for the longer retention times of these esters in GLC. Almost the same differences are seen in the R_F values of oleate–linoleate on the one hand and dihydrohydnocarpate (chaulmoograte)–hydnocarpate on the other, suggesting that the double bond in the ring behaves like one in a straight-chain. The appearance of dihydrohydnocarpate and chaulmoograte as critical pairs also supports this assumption. The higher polarity of the cyclopentanyl and cyclopentenyl esters than expected from the number of carbon atoms and double bonds in these is, therefore, mainly due to the presence of the ring. The cyclopentanyl ring has higher energy content, because of the strain produced by deviation of the bond angles from the normal tetrahedral angle[4]. From the greater distances travelled

* The ester within parentheses and the preceding ester are a critical pair.

by hydnocarpate than by linoleate, by dihydrohydnocarpate than by oleate and by palmitate than by stearate, it may be estimated that the effect of a cyclopentane ring is approximately equivalent to that of one methylene group in reversed-phase TLC.

Argentation-TLC

Silver nitrate is widely used to separate fatty acid esters on the basis of number and configuration of double bonds[5]. Accordingly hydnocarpate and chaulmoograte, having one double bond each, were expected to move along with or to a slightly lesser extent than oleate, since the presence of the ring has only a small effect in increasing the polarity, as observed from the minor differences in the R_F values of palmitate and dihydrohydnocarpate. But Fig. 2 shows that hydnocarpate and chaulmoograte are well below oleate and have only a slightly higher R_F value than linoleate, while gorlate moves with linolenate. This increased polarity of hydnocarpate, chaulmoograte and gorlate is therefore caused by the presence of the double bond in the ring. WINSTEIN AND LUCAS[6] showed that cyclohexene has a greater complexing capacity than 2-pentene, in which the double bond is buried in the chain. They also mentioned that a terminal double bond complexes more easily because of reduced steric hindrance. In the case of cyclopentenyl esters, the greater polarity than expected from the number of carbon atoms and double bonds may, therefore, be explained as the effect of least steric hindrance at the terminal double bond in the ring.

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