ANALYSIS OF CYCLOPROPENOID ACIDS BY GAS–LIQUID CHROMATOGRAPHY

J. H. RECOURT, G. JURRIENS† AND MRS. M. SCHMITZ
Unilever Research Laboratory, Vlaardingen (The Netherlands)
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INTRODUCTION

The cyclopropenoid acid (*e.g.* malvalic acid C_8H_{17}-C(=CH_2)=C-$(CH_2)_6COOH$, sterculic acid C_8H_{17}-C(=CH_2)=C-$(CH_2)_7COOH$) content of oils is usually determined by HCl and HBr titration methods[1–4] and by I.R. absorption measurements[5–7]. Gas chromatography (GLC) was tried by several investigators; their results were not reliable since cyclopropenoid acids tend to isomerize and decompose during passage through the GLC column[8–14].

WOLFF AND MIWA[12] found that the amount of cyclopropenoid acid obtained depends on the amount of immobile phase (Resoflex 446), column temperature and sample size. When columns packed with a large amount of immobile phase were used, less cyclopropenoid acid was obtained than on using a small amount.

Recently, RAJU AND REISER[15] described a very promising GLC-method for the determination of cyclopropenoid acids as their methyl mercaptan derivatives.

Since, however, a reliable direct GLC method would be very useful, we have studied the factors which might influence the determination of cyclopropenoid acids.

EXPERIMENTAL

Extraction and esterification of the oils

Seeds and nuts were ground and extracted with freshly distilled, optically pure light petroleum (b.p. 40–60°) in a top-drive macerater. After extraction and centrifugation, the solvent was removed by means of a Rotavapor at room temperature under nitrogen.

200 mg oil were esterified with 5 ml sodium methoxide (obtained from 0.5 g sodium and 100 ml methanol) at room temperature for 3 h using a magnetic stirrer. After dilution with water, the methyl esters were extracted from the reaction mixture by freshly distilled, optically pure light petroleum.

Apparatus and techniques

The following gas chromatographic equipment was used:

Carlo Erba, model A.I.D./f with flame ionization detector, silver injection system and glass columns.

F & M, model 400 with flame ionization detector, on-column injection and glass columns.

F & M, model 810 with flame ionization detector, stainless steel injection system and stainless steel columns.

Thin-layer chromatography (TLC) was carried out on 20 × 20 cm glass plates coated with a 0.25 mm thick layer of silica gel (ex Macherey, Nagel & Co.). The plates were developed with a mixture of isooctane–diethyl ether (60:40, v/v).

RESULTS

Influence of support

Since 1,2-dioctylcyclopropene (sterculene) isomerizes on alumina[11] we were of the opinion that the support might play an important role in the GLC analysis of cyclopropenoid acids. To confirm this view, four different supports were compared:

Diatoport S, 80–100 mesh (F & M Scientific, Avondale, U.S.A.).

Gaschrom Q, 80–100 mesh (Applied Science Labs. Inc., State College, Pa., U.S.A.).

Celite 545, alkali and acid washed, 80–100 mesh (Johns Manville Products Corp., Celite Division, New York, U.S.A.).

Chromosorb W, NAW, 60–100 mesh (Johns Manville).

With these supports column packings were prepared using 2 % Silicone Oil MS 550 (Midland Silicones Ltd., London). The methyl esters of *Sterculia Foetida* were analysed on the Carlo Erba apparatus at 165° using a glass column measuring 100 × 0.4 cm.

The Celite 545 and Chromosorb W packings gave asymmetrical peaks caused by isomerization (Fig. 1a). Diatoport S and Gaschrom Q yielded symmetrical cyclopropenoid acid peaks (Fig. 1b). Even larger amounts of immobile phase (20 % Silicone Oil MS 550 on Celite 545) gave asymmetrical isomerization peaks, whereas Diatoport S gave symmetrical peaks.

Influence of immobile phase

Most investigators in this field used polar (polyester) immobile phases for the analysis of cyclopropenoid acids. In all these cases partial or complete isomerization or decomposition was observed. Since none of these authors used Diatoport S or Gaschrom Q, we investigated whether a polyester liquid phase used in combination with one of these supports would give symmetrical cyclopropenoid acid peaks. A polyester of ethylene glycol and adipic acid (PEGA)—prepared in our laboratory—was used as immobile phase. In two cases (packings: 3 % PEGA and 10 % PEGA), the methyl esters of *Sterculia Foetida* were analyzed on the Carlo Erba. In both cases, a methyl sterculate peak (equivalent chain length (E.C.L.) 20) was observed. Apart from this peak, the typical isomerization or decomposition pattern could also be seen (Fig. 2). With the 10 % PEGA packing, more isomerization occurred than with the 3 % PEGA packing, which is in agreement with the observations of WOLFF AND MIWA[12]. The same results were obtained with a commercially available polyester phase.

From these data it can be concluded that PEGA and analogous compounds are not suitable for the analysis of cyclopropenoid acids.

A silicone fluid GE-SF 96 (Applied Science Labs. Inc.) used in combination with

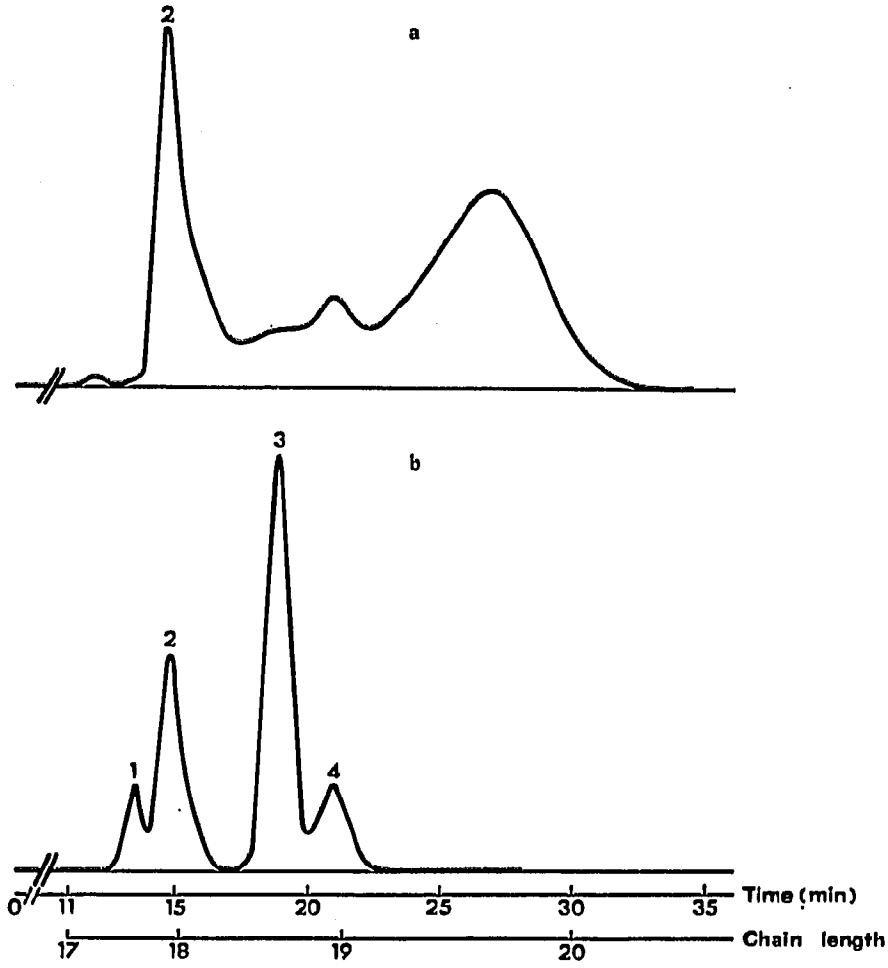

Fig. 1. GLC of methyl esters of acids of *Sterculia Foetida* on Carlo Erba, model A.I.D./f at 165°. (a) Packings: Celite 545 and Chromosorb W. NAW. (b) Packings: Gaschrom Q and Diatoport S. 1 = Malvalic acid; 2 = linoleic acid + oleic acid + stearic acid; 3 = sterculic acid; 4 = dihydrosterculic acid.

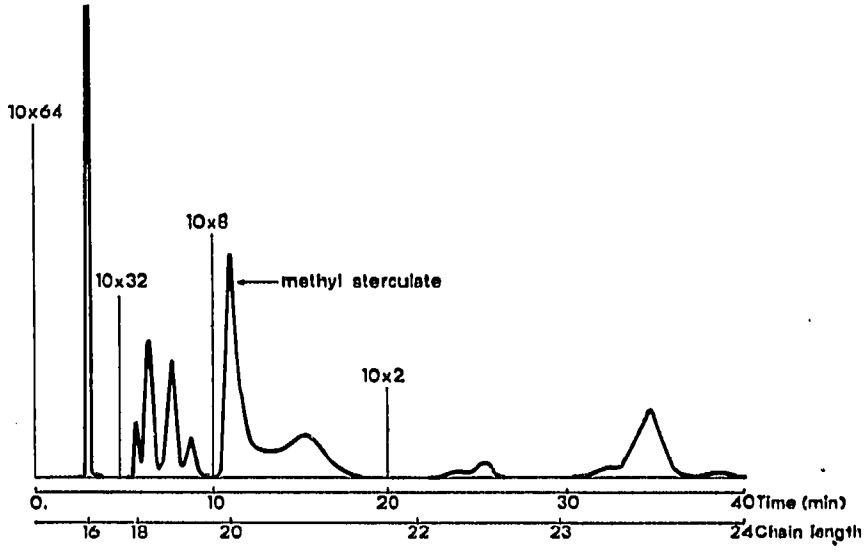

Fig. 2. GLC of methyl esters of acids of *Sterculia Foetida* on Carlo Erba, model A.I.D./f at 165° with 3% PEGA on Diatoport S.

Diatoport S and Gaschrom Q gave the same symmetrical peaks for the cyclopropenoid acids as obtained with MS 550 (Fig. 1). The advantage of SF 96 over MS 550 is that even at an immobile phase concentration of 1 %, a better separation between saturated and unsaturated acids is obtained.

Influence of injection system

To study the influence of the injection system, the methyl esters of *Sterculia Foetida* were analysed on the F & M, model 400 and on the Carlo Erba apparatus; a column measuring 120 × 0.4 cm was packed with 2 % Silicone Oil MS550 on Diatoport S. With the on-column injection method and the silver injection system, symmetrical cyclopropenoid acid peaks were obtained. We found, however, that the ratio methyl sterculate/methyl dihydrosterculate determined with the F and M 400 was higher than that obtained with the Carlo Erba (Fig. 3). It is highly probable that the greater part of the dihydrosterculate found with the Carlo Erba is in fact sterculate, which isomerized in the injection system.

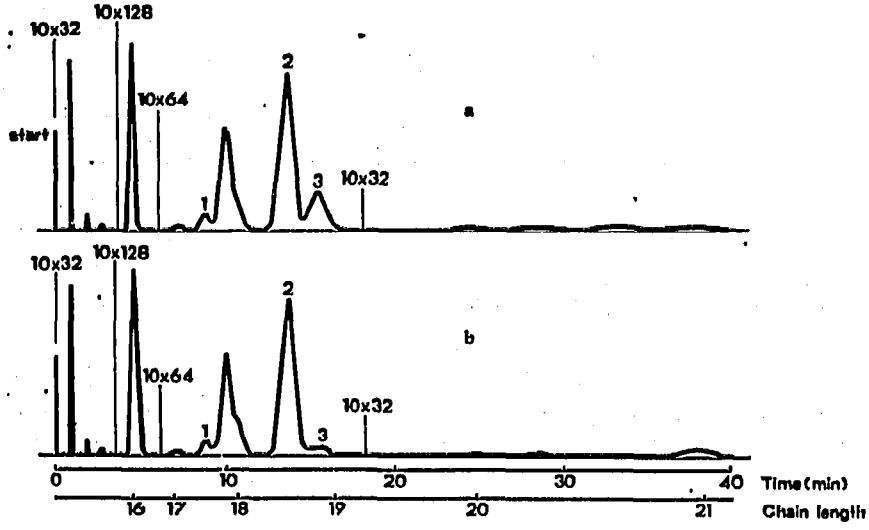

Fig. 3. GLC of methyl esters of acids of *Sterculia Foetida* on (a) Carlo Erba, model A.I.D./f; (b) F and M, model 400. 1 = Malvalic acid; 2 = sterculic acid; 3 = dihydrosterculic acid.

Using the apparatus just mentioned, the methyl esters of *Pachira Aquatica* were analyzed under identical conditions. Also in this case a higher sterculate/dihydrosterculate ratio was obtained with on-column injection. Furthermore, a peak with ECL 20 was found with the F & M while with the Carlo Erba a double peak with ECL 19.9 and 20.2 was found (Fig. 4). This component could be isolated by TLC and identified as 2-hydroxysterculic acid by I.R., m.s. and n.m.r.-analysis.

The 2-hydroxysterculic acid was analyzed on the F & M 400, the Carlo Erba and on the F & M 810 (see Fig. 5). This figure shows that only on-column injection causes no isomerization or decomposition.

Quantitative analyses

The foregoing shows that for GLC analysis of cyclopropenoid acids, a correct choice of support, immobile phase and apparatus (injection system) is important. For

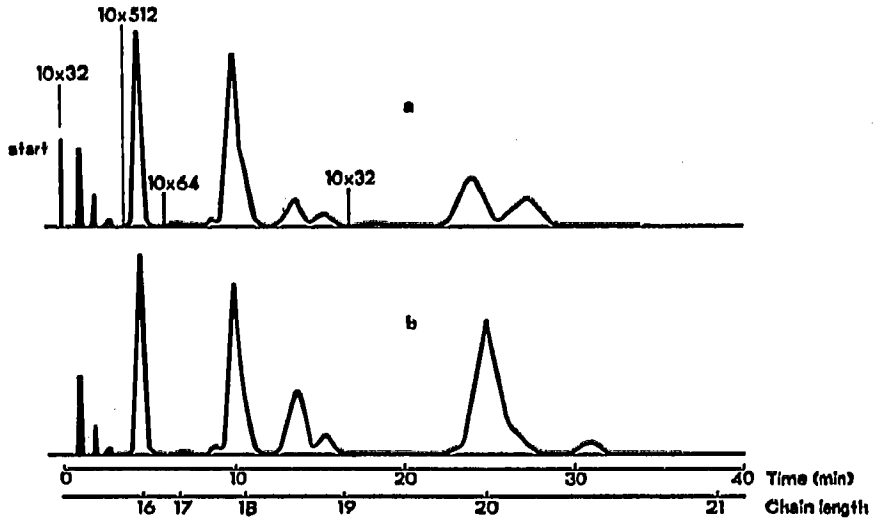

Fig. 4. GLC of methyl esters of acids of *Pachira Aquatica* on (a) Carlo Erba, model A.I.D./f; (b) F and M, model 400.

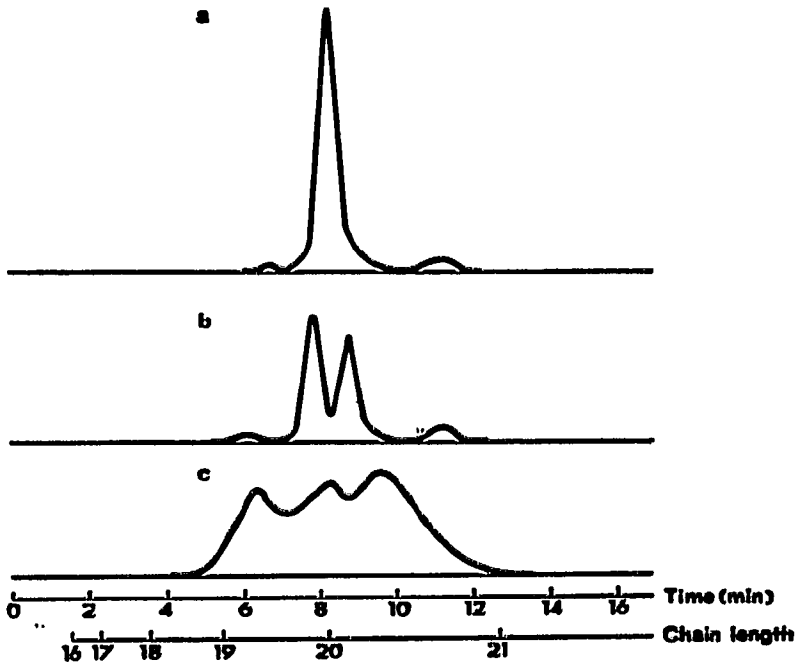

Fig. 5. GLC of 2-hydroxysterculic acid methyl ester on (a) F and M, model 400; (b) Carlo Erba model A.I.D./f; (c) F and M, model 810. Support Diatoport S with 2% MS 550.

TABLE I

QUANTITATIVE ANALYSIS (WT. %) OF OILS CONTAINING CYCLOPROPENOID ACIDS

Seeds from	C_{16}	$C_{16:1}$	C_{18} *unsat.*	C_{18}	*Dihydro-sterculic*	*Cyclopropenoid acids*				
						Malvalic	*Sterculic*	*2-Hydroxy-sterculic*	*Total obtained by*	
									GLC	*HBr-titration*
Sterculia Foetida	26		12.5	3	0.5	6.5	51		57.5	
Sterculia Oblonga	17.5	6.5	45	2.5	9.5		19		19	21.5
Pachira Aquatica	66		11.5	2.5	0.5	1	7	11	19	19
Bombacopsis Glabra										
from source (a)	51.5		11	2	1	2.5	28.5	3	34	32
(b)	55		11.5	3	1	1.5	25	2.5	29	28.5
(c)	50.5		19	4.5	1.5	0.5	22.5	1.5	24.5	
Eriodendron Anfractuosum	22.5		62.5	3	1.5	7	2.5		9.5	8.5

the quantitative analysis we used the F & M, model 400 and a glass column (120 × 0.4 cm) packed with 1 % GE-SF 96 on Gaschrom Q. The temperature of the column, injection system and detector was 130°, 145° and 145°, respectively.

In this way the methyl esters of the following seeds* were analyzed:

Bombacopsis Glabra[a, b, c]
Eriodendron Anfractuosum (from Nigerian kapok)
Pachira Aquatica[d]
Sterculia Foetida
Sterculia Oblonga[c]

The results of this analysis are set out in Table I. In a few cases HBr-titrations[3] have been carried out for comparison.

The data in Table I show a reasonable agreement between the values obtained by GLC and HBr-titration. It can now be concluded that under the conditions applied direct GLC analysis of cyclopropenoid acids is possible.

Identification of 2-hydroxysterculic acid

During analysis of the cyclopropenoid acids it was found that the seed of *Bombacopsis Glabra, Pachira Insignis* and *Pachira Aquatica* contained a compound with ECL 20 on 2 % MS 550 on Diatoport S and with ECL 19.45 on 1 % GE-SF 96 on Gaschrom Q. The presence of a hydroxycyclopropenoid acid in *Pachira Aquatica* has already been suggested[10]. As the retention time of the unknown component might be that of a sterculic acid with a hydroxy group, we separated the methyl esters of the above-mentioned oils by TLC. The spots with an R_F value equal to that of hydroxystearic acid were isolated and analyzed by GLC. In all cases a peak with ECL 19.45 (GE-SF 96 on Gaschrom Q) was found. Acetylation of the isolated compounds and GLC analysis of these products gave peaks with increased retention time. The Halphen test carried out on the isolated material gave a positive reaction. These results point to the presence of a hydroxycyclopropenoid acid.

As the highest concentration of this compound occurred in *Pachira Aquatica* further identifications were carried out on this oil. Enough material could be isolated by TLC for investigations by m.s., I.R. and n.m.r. analysis.

The molecular weight (determined by m.s.) was 324 (methyl ester), which agrees with the molecular weight of a hydroxysterculic acid methyl ester.

After ozonization of the substance and reduction of the oxidation products to the oxo-compounds, it was found by m.s. that the oxo groups occupied the 9- and 11-positions, which places the cyclopropene ring in the same position as in sterculic acid. The hydroxy group was found in the 2-position. I.R. and n.m.r. analysis confirmed that the hydroxy group occupies the 2-position.

These data confirm the results recently published by MORRIS AND HALL[17].

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* Obtained from: (a) Dr. J. A. CORNELIUS, Tropical Products Institute, London, Great Britain; (b) Dr. J. ANTON SMITH, Ministry of Agriculture, Mount Makulu Research Station, Chilanga, Republic of Zambia; (c) Mr. M. R. MILLS, Unilever Ltd., Albion Wharf, Erith, Kent, Great Britain; (d) Mr. J. E. HEESTERMAN, Koninklijk Instituut voor de Tropen, Amsterdam, The Netherlands.

SUMMARY

The possibility of analyzing cyclopropenoid acids by means of gas–liquid chromatography (GLC) was investigated using three different gas chromatographic apparatus, equipped with on-column injection, a silver injection system and a stainless steel injection system, respectively. Four different supports were compared as well as two different silicone immobile phases and two polyester immobile phases. Reliable results could be obtained with on-column injection using silicone immobile phases and Diatoport S or Gaschrom Q as support.

In the methyl esters of *Pachira Aquatica*, a new cyclopropenoid acid was found. By means of mass spectrometry, infra red and nuclear magnetic resonance analysis, the structure could be identified as that of 2-hydroxysterculic acid. The same acid could be detected in *Pachira Insignis* and *Bombacopsis Glabra* based on retention time (GLC) and R_F value (TLC).

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