LIST OF PUBLICATIONS

1. Cyclopropenoid Fatty Acids in Seed Oils of *Sida acuta* and *Sida rhombifolia* (Malvaceae)
   J. Amer. Oil Chem. Soc. 53:698 (1976).

2. Studies On Herbaceous Seed Oils-III
   Fette Seifen Anstrichmittel, 80:225 (1978).

3. The characterisation and Measurement of ·OBr-reacting Acids in *Hucuna pruriens* and *Urena lobata* Seed Oils
   J. Sci. Food Agric. 29:372 (1978).

4. *Hibiscus sabdariffa* Seed Oil: A Reinvestigation
   J. Sci. Food Agric. (communicated).

5. Studies On Herbaceous Seed Oils-XII
   J. Oil Tech. Assn.(India)(communicated).

6. *Vernonia roxburghii* Seed Oil: A Rich Source of Epoxy Acids - accepted for presentation in "International Congress on Oilseeds and Oils", to be held at New Delhi, February 9-13,1979.

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*Reprints (1-3) are attached.

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Cyclopropenoid Fatty Acids in Seed Oils of *Sida acuta* and *Sida rhombifolia* (Malvaceae)

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ABSTRACT

Seed oils of *Sida acuta* and *Sida rhombifolia* were found to contain sterculic (11.0, 10.8%) and malvalic (1.7, 2.0%) acids respectively, in addition to the normal fatty acids. Co-occurrence of these acids was established by gas liquid chromatography of the silver nitrate-methanol-treated methyl esters using *Sterculia foetida* esters as a reference standard. This gas liquid chromatography technique of quantitation was found most suitable to estimate these acids in low level cyclopropenoid acid-containing seed oils.

INTRODUCTION

Recently cyclopropenoid fatty acids have been the subject of much investigation due to their profound biological effects on animals (1-3) and cocarcinogenic properties (4-5). Fatty acids containing cyclopropene ring have been found in seed oils (6-8) of the genus *Sida* (Malvaceae). In a recent report in this journal, Rao et al. (9) observed that *S. acuta* seed oil contains the usual fatty acids in its glycerides. These authors found no cyclopropene acids because they made no effort to look for these acids and used laboratory procedures which destroyed cyclopropene acids. As a part of a screening programe aimed at the search for biologically active cyclopropene acids in herbaceous seed oils, it was found that *S. acuta* seed oil gave positive test (Halphen) for cyclopropenoid acids. Seed oil from another species of this genus, *S. rhombifolia,* also responded to Halphen test. Therefore these two cyclopropenoid acid-containing seed oils were thoroughly studied and the present paper describes the results of their fatty-acid analysis.

EXPERIMENTAL PROCEDURES

Oils were extracted from crushed seeds with petroleum ether (40-60 C) in a soxhlet apparatus, and the solvent was evaporated under vacuum in a rotary evaporator. The fatty acid methyl esters were prepared by transmethylation of 1 g of oil in 50 ml of absolute methanol that contained 1% sodium methoxide. The reaction was allowed to proceed by refluxing for 20 min, and the methyl esters were extracted with ether as usual.

The methyl esters of each oil (200 mg) were treated with 60 ml of absolute methanol saturated with silver nitrate (10). The reaction was allowed to proceed at room temperature with stirring for 24 hr. The normal methyl esters and the reaction products from cyclopropenes were recovered from the reaction mixture by adding 100 ml of distilled water and extracting with ether. The extracts were dried over anhydrous sodium sulfate and the solvent evaporated in the stream of nitrogen.

Infrared (IR) spectra were determined in CCl_4 using Perkin-Elmer model 521 Spectrophotometer. Ultraviolet (UV) spectra were measured on a Beckman-DU-Spectrophotometer using a methanolic solution. Nuclear magnetic resonance (NMR) spectra were run in $CDCl_3$ on EM-360 60 MHz spectrometer with tetramethyl silane as the internal standard.

The esters of each oil were examined qualitatively by direct, reversed-phase and argentation thin layer chromatography (TLC) using *S. foetida* esters as the cyclopropenoid acid reference. Direct TLC showed only non-oxygenated acids. The reversed-phase TLC, using acetonitrile-acetic acid-water (70:10:20, v/v) as the solvent system, revealed a spot near the starting point corresponding to the spot exhibited by *S. foetida* esters. Clear spots of usual critical pairs were also obtained. Argentation TLC showed spots of saturates; monoene and diene parallel to those obtained from *S. foetida* esters resolved alongside.

Gas liquid chromatography (GLC) was done on F & M - 720 GLC unit provided with flame ionization detector using EGSS-X Column (8 ft x 3/16 in.). The separations were carried out isothermally at 200 C. The temperatures at the injection port and detector block were 300 C. Nitrogen at a flow rate of 360 ml/hr was the carrier gas, and chart speed was 15 in./hr.

Freshly prepared *S. foetida* esters were treated with silver nitrate-methanol. The esters containing sterculate and malvalate derivatives thus obtained were used in GLC analysis as reference standard. Comparison of the relative retention times of the derivatives of *S. foetida* esters as well as those of *S. acuta* and *S. rhombifolia* esters clearly established the presence of sterculic and malvalic acids in these seed oils. Peak areas were calculated by triangulation method.

RESULTS AND DISCUSSION

Light petroleum extraction of the crushed seeds yielded 12% oil in *S. acuta* and 14% in *S. rhombifolia.* Oil characteristics (Table I), iodine value, saponification value, refractive index, nitrogen (crude protein) and moisture percent-

TABLE I

Analytical Data on Seeds and Oils

Species	Seed Analysis			Oil Properties		
	Oil content %	Protein content Nx6.25, %	Moisture %	I.V.[a]	S.V.[b]	Ref. Index N_D^{28}
Sida acuta	12	26.8	6	76	184	1.4628
Sida rhombifolia	14	21.8	7.5	85	212	1.4669

[a]Iodine value (I.V.)
[b]Saponification value (S.V.)

age were determined by AOCS methods (11).

Both oils responded to Halphen test (12), thereby indicating the presence of cyclopropenoid acid. The oils showed the typical NMR signal at 9.28τ for the cyclopropene moiety. The methyl esters of each oil had the characteristic IR band for the cyclopropene moiety at 1008 cm^{-1}. There was no indication in the spectrum of a hydroxyl or terminal acetylenic group. The UV spectra indicated no conjugation in the oils. Quantitation of total cyclopropenoid fatty acid by the method of HBr titration (13) showed the presence of 11.92% and 12.51% by weight of cyclopropenoid acid in *S. acuta* and *S. rhombifolia* seed oils respectively.

GLC of methyl esters was done after treatment with silver nitrate in absolute methanol to form stable derivatives of cyclopropenoid acid according to the method of Schneider et al. (10). GLC compositional data is given in Table II.

GLC data of the cyclopropenoid acids in two seed oils were found to agree with those obtained by the method of HBr titration. As compared to the hydrogenation method (14-16) and methyl mercaptan derivatization technique (17) for the quantitation of cyclopropenoid fatty acids, the silver nitrate method produced a clear resolution of sterculate and malvalate derivatives in the GLC chromatogram. Further, this method has the additional advantage of not reacting with the other unsaturated acids present in the oil.

TABLE II

Gas Liquid Chromatography Analysis of the $AgNO_3$-Methanol Reacted Methyl Esters

Fatty Acids	*Sterculia foetida* Wt %	*Sida acuta* Wt %	*Sida rhombifolia* Wt %
Palmitic	26.0	35.8	26.6
Palmitoleic	1.0	5.7	3.5
Stearic	3.4	7.1	3.3
Oleic	9.4	20.3	38.1
Linoleic	1.3	18.4	15.7
Linolenic	0.6	–	–
Malvalic	7.1	1.7	2.0
Sterculic	51.2	11.0	10.8

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Studies on Herbaceous Seed Oils III

*By S. K. Husain, M. U. Ahmad, S. Sinha, A. A. Ansari and S. M. Osman**

Seed oils from seven species belonging to four botanical families have been analysed for their fatty acid composition by using chromatographic and spectroscopic techniques. Oils from six species are very interesting in containing high percentage (63.7–84.0 %) of C_{18} unsaturated acids. Chemical screening of seed oils reveals that the species producing highly unsaturated oils merit attention for evaluation as perspective crops.

Studien über die Samenöle der krautartigen Pflanzen III

Die Fettsäurezusammensetzung der Samenöle aus sieben Spezies, die zu vier botanischen Stämmen gehören, wurde mit Hilfe von gaschromatographischen und spektroskopischen Methoden untersucht. Die Öle aus sechs Spezies zeichnen sich durch den hohen Gehalt (63.7–84.0 %) an C_{18} ungesättigten Fettsäuren aus. Diese Untersuchungen haben gezeigt, daß einige Spezies zur Erzeugung von hochungesättigten Ölen in Betracht gezogen werden sollten.

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As related in a previous paper of this series[1], a programme is in progress to determine by chemical screening analyses what amounts and general classes of fatty acids are contained in uncultivated herbaceous seed oils. Those with suitably high oil, protein content and fatty acid composition thought to be sufficiently different from that of present commercial vegetable oils to make them of potential practical interest, are then scheduled for more intensive chemical study.

In this paper we report results obtained on seven species representing four botanical families; *Leguminosae, Labiatae, Pedaliaceae* and *Chenopodiaceae.*

Infrared and Ultraviolet absorption gave no evidence of significant amount of unusual components. Various thin layer chromatographic (t. l. c.) techniques confirmed the absence of oxygenated acids and/or unusual functional group. Argentation t. l. c.[2] of the esters gave clear spots corresponding to the saturates, monoene and diene parallel to those from authentic linseed ester resolved alongside. Esters of *T. purpurea, V. sativa, C. album* and *M. diandra* (item 1, 3, 6 and 7) indicated the presence of triene also. Reversed-phase t. l. c.[3] of the esters confirmed the presence of C_{16} and C_{18} saturated acids in all the esters and also C_{12} and C_{14} saturated acids in (item 7) and C_{14} saturated acid in (item 2) respectively.

Quantitative examination was undertaken by gas-liquid chromatography (g. l. c.) using stainless steel packed column coated with diethylene glycol succinate (DEGS, 15 % on chromosorb W, 45—60 mesh) and authentic esters run under identical conditions. Occasionally saturated, mono- and poly-ethenoid esters were separated by preparative silver-ion chromatography and re-examined by g. l. c.

Seed analysis and oil characteristics are given in Table 1 along with the chromatographic analysis of the methyl esters from the oils. Only one species (item 7) contains a high percentage (62.5 %) of protein. The calculated protein content of the rest six oil-free meal on a dry basis, 15—37 %, is lower than that of the usual oilseed meals, but adequate to be useful as a feed material. The compositional data confirm that all the oils examined, are composed of common fatty acids but in widely varying proportions.

Oils Rich in C_{18} Unsaturated Acids

Six species (item 1—6) contain high percentage (63.7—84.0 %) of C_{18} unsaturated acids (as identified by g. l. c.). Oleic and linoleic acids were found to be the predominent unsaturated acids in these oils. The highest percentages of oleic acids were found in four species; 35.5 % (item 1), 60.5 % (item 2), 34.8 % (item 6) and 37 % (item 7). Linoleic acid was found to be the predominent unsaturated acid (46.3—76.6 %) in four species; 53.7 % (item 3), 76.6 % (item 4), 61.5 % (item 5) and 46.3 % (item 6). It is noteworthy that *Labiatae* species (item 4 and 5) are the richest source of linoleic acid in all these seed oils examined. Three species (item 4—6) were unusual in having major amount (> 80 %) of oleic and linoleic acids.

Saturated Acids

The content of total saturated acids varied from 15—53 %. Among the saturated acids palmitic acid was a major acid, the maximum amount (48.2 %) being present in seed oil of family *Pedaliaceae* (item 7). The amount of stearic acid which is usually present in minor amounts, is present to the extent 16.5 % (item 2) and in trace amount in one species (item 6). Complete absence or presence of stearic acid in trace amount would be most unusual but such a result is often within the precision of the method. Other than C_{16} and C_{18} saturated

Table 1

Analytical Data on Seeds and Oils

No.	Source	Seed Analysis: Oil content [%]	Seed Analysis: Protein content N × 6.25 [%]	Seed Analysis: Moisture [%]	Oil Properties: I. V.*	Oil Properties: S. V.**	Oil Properties: Ref. Index n_D^{33}	Methyl Ester Composition [%] by GLC: 16:0	18:0	18:1	18:2	18:3	Other
1.	*Tephrosia purpurea* (Leguminosae)	11.0	30.0	6	118	184	1.4880	22.4	4.7	35.5	20.7	16.7	—
2.	*Terannus labialis* (Leguminosae)	3.8	21.8	6	70	175	1.4740	18.2	16.5	60.5	3.2	—	14:0 (1.4 %)
3.	*Vicia sativa* (Leguminosae)	1.5	15.3	7	135	186	1.4750	17.4	1.3	19.5	53.7	8.1	—
4.	*Hyptis suaveolens* (Labiatae)	12.4	22.0	9	146	216	1.4830	15.3	2.1	6.0	76.6	—	—
5.	*Anisomeles ovata* (Labiatae)	19.0	28.4	7.2	130	190	1.4769	14.7	3.0	20.8	61.5	—	—
6.	*Chenopodium album* (Chenopodiaceae)	3.0	37	10	126	224	1.4860	15.8	trace	34.8	46.3	2.9	—
7.	*Martynia diandra* (Pedaliaceae)	12.0	62.5	5	62	197	1.4847	48.2	0.5	37.0	0.8	9.2	12:0 (2.5 %) 14.0 (1.7 %)

* Iodine Value; ** Saponification Value

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acids, C_{12} and C_{14} saturated acids are also present in two species (item 2 and 7).

Reports about the fatty acid composition of three seed oils studied by us are available from the literature. *J. M. Hagemann* et al.[4] have determined the fatty acid composition of *H. suaveolens* (item 4). There appeared to be no significant difference in the fatty acid composition reported by these authors with the composition found in the present study. There was a slight difference in the fatty acid content of oil from *T. purpurea* (item 1), analysed by *F. D. Gunstone* et al.[5] from the present analysis. The seed oil of *M. diandra* (item 7) has been studied by *J. N. Tayal* and *S. Dutt*[6] and *A. V. Rage* et al.[7]. These authors have used the ester fractionation method in their analysis. Among the acids, oleic acid was the major acid (74.5 and 40.3 %), whereas the present sample showed the palmitic acid (48.2 %) as the major one. This discrepancy in composition may result from differences in the source of samples as well as the method of analysis.

The *Labiatae* (mint family) have previously been known[8,9] to contain members producing oils with high iodine values. *Perilla* is the principal representative of the family among industrial seed oils but published analyses for some 15 other species[8,9] indicate that several should produce oils of similar drying quality. Oils of the two *Labiatae* in this study are similar in composition to other *Labiatae* oils with high iodine values. The most interesting result was that for a *Leguminous* seed oil (item 3), a species producing oil with high iodine value. Oils which had over 60 % of linoleic and/or linolenic acid may be of value as drying oils.

Other important oils, such as groundnut oil and olive oil, have less than 20 % each of saturated acids and of linoleic acid and a high content of oleic acid (> 60 %). Oil of *T. labialis* (item 2) came close to this.

Finally there is an interest in, and a demand for, solid fats similar to cocoa butter, with about 60 % saturated acids, since such fats are rich in glycerides of type 1-saturated 2-unsaturated 3-saturated. Oil of *M. diandra* (item 7) approached this composition (saturated —52.9 %, monoethenoid —37 % and polyethenoid —10 %). Saturated acid was predominently palmitic (48.2 %).

In conclusion it may be added that oils with high iodine values should serve as drying oils or as raw materials for other applications in which a large amount of unsaturation is important. Further, species whose oils are rich in specific acids should be studied to ascertain whether they have advantages in quality of the oils, productivity or range of adaptability over species now in commercial use.

Experimental

IR spectra were obtained with Perkin-Elmer 621 Spectrophotometer. UV spectra of the oils/methyl esters measured on Beckman DK-2A Spectrophotometer using methanolic solution. The gas chromatographic analysis was carried out with Perkin-Elmer model 154, equipped with a thermal conductivity detector using DEGS column (temp. 200° C; H_2 flow rate — 70 ml per min). Thin-layer chromatographic plates were coated with silica gel. A 20 % aqueous solution of perchloric acid was used as the spraying agent.

Sample preparation and analytical procedures used were as previously described[1]. Methyl esters were prepared from 1 g samples of oil by methanolysis, with sodium methoxide or hydrogen chloride as catalyst. In general the esters were examined by various t. l. c. techniques prior to gas chromatographic analysis.

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The Characterisation and Measurement of HBr-reacting Acids in *Mucuna pruriens* and *Urena lobata* Seed Oils

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Seed oils of *Mucuna pruriens* (Leguminosae) and *Urena lobata* (Malvaceae) were found to contain HBr-reacting acids to the extent of 1·3 and 10·8%, respectively. Acetylation of the *M. pruriens* seed oil followed by saponification and separation of the acids gave 12,13-dihydroxyoleic acid. From this and other evidence it is concluded that 12(13)-epoxyoleic (vernolic) acid is present as a constituent of the glycerides. On the other hand, the cyclopropenoid material in *U. lobata* oil was characterised as a mixture of malvalic (4,8-) and sterculic (8,9-) acids by the gas–liquid chromatography (g.l.c.) of the silver nitrate–methanol treated methyl esters. Seed oils of *Vernonia anthelmintica* and *Sterculia foetida* were used as reference standards.

1 Introduction

Among the three naturally occurring HBr-reacting acids, conjugated dienol, epoxy-, and cyclopropenoid acids, the latter two classes of fatty acids have recently been of continuing interest to lipid chemists. Seed triglycerides of a number of plants have been found to contain conjugated dienol acids[1] viz. calendic and coriolic acids have been characterised in the seed oils of some species of the Calendulaceae and Coriariaceae families. Seed oils rich in epoxy acids are of potential interest as stabilisers for plastic formulations. Swern *et al*[3] have tested epoxy compounds for carcinogenic effects by repeated subcutaneous injections in mice during their search for functional group–biological activity relationships and have reported that weak carcinogens for the subcutaneous tissue were obtained by such compounds. Recently, cyclopropenoid acids have also attracted considerable attention due to their profound biological effects in animals[4–6] and cocarcinogenic properties[7,8]. In the early screening analyses of seed oils it was customary to report HBr-reacting acids as the constituent epoxyoleic and/or sterculic acid. In the absence of any clear-cut quantitative procedure for estimating these acids, literature reports on compositional data have often prompted re-examination of the seed oils. Results which were obtained in a continuing screening programme at this laboratory revealed that seed oils of *M. pruriens* (Hindi, *Kawach*, *Kiwach*)[9a] and *U. lobata* (Hindi, *Kumbi*)[9b] contain HBr-active acids in amounts of 1·3 and 10·8%, respectively. *M. pruriens* is cultivated in some parts of the country for the sake of its brown velvety legumes which are cooked and eaten as a vegetable. The previous work[10] on the seeds of *M. pruriens* has not shown the presence of HBr-active acids in its oil. Cornelius *et al*[1] have reported the presence of a cyclopropenoid acid (sterculic) in trace amounts in *U. lobata* oil. These authors have estimated the cyclopropenoid content on the basis of HBr titration of oil and g.l.c. of methyl esters, excluding peaks due to the cyclopropenoid and associated breakdown products. To overcome the difficulty of the estimation of individual cyclopropenoid acids, several investigators proposed hydrogenation,[12–14] methyl-mercaptan- and/or silver nitrate[15] derivation of methyl esters followed by g.l.c.

Because of biological effects attributed to these two classes of fatty acids, it was deemed essential to analyse these two seed oils with a view to characterisation of the individual HBr-reacting acids and to evaluate procedures as a basis for the estimation of minor proportions of these acids present in seed oils.

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2. Experiments and results

2.1. General methods

Esterifications and transesterifications were carried out as follows except where specified Samples were refluxed for 1 h in a large excess of anhydrous methanol containing 1% sulphuric acid (v v) In each case, the resulting mixtures were diluted to the cloud point with water, chilled in an ice bath, and then extracted repeatedly with ether Combined extracts were dried over sodium sulphate and evaporated *in vacuo*

Thin-layer chromatography (t l c) was performed on plates coated with 0.25 mm or 1 0 mm layers of silica gel G or 20% silver nitrate-impregnated silica gel G with 20 ether in hexane as the solvent For reversed phase t l c , the dried, coated plate was uniformly impregnated with silicone oil (E Merck, A. G Darmstadt) Acetonitrile–acetic acid–water (70 10 20, v/v) was used as the developing solvent Spots on analytical plates were visualised by charring with a 20% aqueous solution of perchloric acid Triglycerides containing epoxy group was revealed by the on-the-plate test with picric acid as described by Fioriti and Sims [17]

Infrared (i r) spectra were obtained with a Perkin–Elmer 621 spectrophotometer in liquid film, and ultraviolet (u.v.) measurements were made on methanolic solution with a Beckman DK-2A spectrophotometer Gas–liquid chromatography (g l c) was carried out with Perkin Elmer Model 154 instrument equipped with a thermal conductivity detector, using a stainless steel packed column (2 m × ¼ in) coated with diethylene glycol succinate (DEGS, 15% on chromosorb W, 45–60 mesh) The separations were carried out isothermally at 200 C, chart speed 30 in h^{-1} with a hydrogen flow of 70 ml mm^{-1} All g.l.c data reported are given as area percentages.

2.2. Preliminary analysis of oils

Ground seeds were exhaustively Soxhlet-extracted with petroleum ether (b p 40–60 C). The u v spectra showed no conjugation in the oils and methyl esters Although i.r of *M. pruriens* oil gave no informative spectrum for epoxy group (848–826 cm^{-1}), presumably due to its very low concentration the picric acid t l c test gave a positive indication of epoxy acid in the oil. The methyl esters of *U. lobata* oil showed the characteristic i r band for the cyclopropene moiety at 1008 cm^{-1}. Only *U. lobata* oil responded to the Halphen test [18] indicating the presence of cyclopropenoid acids. Both the oils were titrated with HBr, according to the procedure of Harris *et al.*[19] at two different temperatures (3 and 55 C)

The analytical values of oils and seeds were determined according to the procedures recommended by the AOCS methods[20] and the data are summarised in Table 1

Table 1. Analytical data on seeds and oils

	Mucuna pruriens (Leguminosae)	*Urena lobata* (Malvaceae)
Oil content of seed, %	4 3	18 0
Unsaponifiable content, %	13.6	3 1
Protein content, N × 6 25, %	35 0	24 9
Moisture, %	3 3	5 8
Iodine value (Wijs)	47	68
Saponification value	189	200
Refractive index, n_D^{30}	1 4740	1 4800
Halphen test	Negative	Positive
Oxirane oxygen, %	0 06	—
HBr equiv	1 17[a]	10 13[b]
Ultraviolet (u v)	Usual	Usual
Infrared (i.r.)	Usual	Cyclopropene (1008 cm^{-1})

[a] Expressed as % epoxyoleic.
[b] Expressed as % cyclopropenoid.

2.3. Oil of *M. pruriens*

A portion (20 g) of the oil was acetolysed with five volumes of glacial acetic acid for 7 h as described by Gunstone.[21] The resulting acids obtained after saponification with 1N-KOH/EtOH followed by acidification with HCl, were then subjected to partitioning between 80% methanol and light petroleum ether (b.p. 40–60°C). The methanol extract was re-extracted with light petroleum ether (4 × 50 ml) to remove any trace of non-oxygenated acids. Direct t.l.c. revealed a clear spot on the base line, thus showing the presence of oxygenated acid in the fraction isolated from methanolic phase. The crude dihydroxy acid isolated from the methanolic phase on successive crystallisations from acetone and petroleum ether–ethyl ether (3:1) afforded a crystalline product (yield, 0.26 g), melting at 54–55°C (lit.[21] m.p. 53–54°C) and gave the following analysis: C, 68.75; H, 10.69% (calc. for dihydroxyoctadecenoic acid, $C_{18}H_{34}O_4$: C, 68.78; H, 10.82%). It showed no depression when mixed with an authentic sample of 12,13-dihydroxyoctadecenoic acid prepared from *Vernonia anthelmintica* seed oil. Co-chromatography on a t.l.c. plate, with an authentic sample of 12,13-dihydroxyoctadecenoic acid also gave a single spot. The iodine value was 98.7 (lit.[21] 95.8), which was somewhat higher than that calculated for one double bond; there is evidently absorption of iodine due to reaction with the OH groups.

A portion (100 mg) of the dihydroxyoctadecenoic acid, on hydrogenation in methanol with Adam's catalyst, yielded a product (65 mg). On crystallisation from ethyl acetate it melted at 96–97°C (lit.[21] m.p. 95–96°C; found: C, 68.36; H, 11.27; calc. for $C_{18}H_{36}O_4$: C, 68.35; H, 11.39%). No depression of the melting point was observed on admixture with an authentic sample of 12,13-dihydroxystearic acid and it showed identical movement on direct t.l.c. plate.

The light-petroleum phase contained only non-oxygenated acids present in the oil. The concentrate obtained from petroleum extracts was esterified using methanol–sulphuric acid and the resulting methyl esters were examined by g.l.c. According to g.l.c. analysis, these esters had the composition indicated in Table 2. Examination of these esters by t.l.c. on silver nitrate-impregnated silica revealed distinct spots attributable to saturates, monoene and diene, parallel to those from authentic esters resolved alongside. Reversed-phase t.l.c. of the esters confirmed the presence of C_{14}, C_{16} and C_{18}-saturated acids.

Table 2. Component fatty acids (% wt.) of seed oils

Acid	*Mucuna pruriens* (Leguminosae)	*Urena lobata* (Malvaceae)
Myristic	1.3	9.5
Palmitic	53.7	34.7
Palmitoleic	—	5.4
Stearic	19.8	4.4
Oleic	13.6	20.2
Linoleic	10.2	14.9
Malvalic	—	4.8
Sterculic	—	6.0
12,13-Epoxyoleic	1.3	—

2.4. Oil of *U. lobata*

The methyl esters from *U. lobata* oil were prepared by transesterification of 1 g of the oil in 50 ml of anhydrous methanol containing 1% sodium methoxide as catalyst. The reaction was allowed to proceed by refluxing for 20 min; the methyl esters were extracted with ether and examined qualitatively by various t.l.c. techniques using *S. foetida* esters as the cyclopropenoid acid reference. Direct t.l.c. showed only non-oxygenated acids. The reversed-phase t.l.c. revealed a spot near the starting point corresponding to the spot exhibited by *S. foetida* esters. Clear spots of usual critical pairs were also obtained. T.l.c. of these esters on silica gel G impregnated with silver nitrate showed spots

of saturates, monoene and diene, parallel to those obtained from the *S. foetida* esters resolved alongside.

A 200 mg portion of methyl esters were treated with 60 ml of anhydrous methanol saturated with silver nitrate, following the procedure of Schneider *et al.*[10] The silver nitrate–methanol treated esters were then analysed by g.l.c using freshly prepared $AgNO_3$-derivatives of *S. foetida* esters as a reference standard. Characterisation of the individual cyclopropenoid acids, malvalic and sterculic, were carried out by a comparison of the relative retention times. The result of g.l.c. analysis is given in Table 2.

3. Discussion

Light petroleum extraction of the crushed seeds gave 4.3% oil in *M. pruriens* and 18% oil in *U. lobata*. Seed oil of *M. pruriens* gave a low saponification value in comparison with theoretical value (189 *vs* 200). A similar deviation is also apparent between the theoretical iodine values (calculated from the fatty acid composition) and the values determined for both seed oils. In oils with a very low or a very high i.v. and containing no unusual structures, theoretical and calculated values are found to be in close agreement. In other oils the agreement is not as close as might be desired. Reasons for the disparity in values may be due to a larger concentration of unsaponifiables in seed oils. Sometimes this deviation from the typical behaviour of common vegetable oils can be ascribed to structural features; such as conjugated unsaturation, high carbonyl content, the presence of apparent hydroxy acids (free acids or glyceryl hydroxyl) or the presence of essential oils. The standard procedures generally provide acceptable values when usual types of C_{18} acids are present. Conversely, in the presence of unusual constituents, such values may serve only as guides to oil that deviate from the normal.

Oil of *M. pruriens* showed 0.06% oxirane oxygen, equivalent to 1.17% epoxyoleic acid. Acetylation of the oil, followed by saponification and separation of the acids, gave 12,13-dihydroxyoleic acid (m.p. and mixed m.p. 54–55°C). The yield of dihydroxyoleic acid was 0.26 g from 20 g of oil, equivalent to 1.3% of the weight of the oil. If its precursor is epoxyoleic acid, as believed, the content of epoxyoleic is estimated to be 1.3% of the total fatty acids of the oil. Quantitative determination of the oxirane content of the oil agrees with the amount of the dihydroxyoleic acid isolated. The presence of epoxyoleic acid in *M. pruriens* oil was further confirmed by hydrogenation of the unsaturated dihydroxy acid. It yielded 12,13-dihydroxystearic acid (m.p. 96–97°C), the identity of which was subsequently confirmed by mixed m.p. with an authentic sample prepared from *V. anthelmintica* seed oil. Comparison of the mobility of saturated and unsaturated dihydroxy acids on t.l.c. plates with authentic samples also demonstrated the identity of structures. The original dihydroxy acid is therefore 12,13-dihydroxy-9-octadecenoic (12,13-dihydroxyoleic) acid. No dihydroxyoleic acid was obtained from the whole oil by the solvent partition procedure when the acetylation step was omitted. Thus the dihydroxy acid is not present in the crude oil but must be formed by acetylation of the epoxy-acid. The original component of the oil from which dihydroxy acid was obtained is therefore *cis*-12,13-epoxy-*cis*-9-octadecenoic (vernolic) acid. The proportions of the other acids were calculated from g.l.c. of the methyl esters and are shown in Table 2.

Quantitative analysis of the total cyclopropenoid material by HBr-titration showed appreciable cyclopropenoid acid content (10.13%) in *U. lobata* seed oil. The g.l.c. analysis of the silver nitrate–methanol treated methyl esters clearly established the presence of malvalic (4.8%) and sterculic (6.0%) acids by a comparison of the relative retention times of the derivatives of *S. foetida* esters. The total cyclopropenoid content agrees approximately with the HBr-titration result. The fatty acid composition and oil content differs from that reported earlier for *U. lobata*[11] (Fiji variety). In this oil (yield, 12%) linoleic acid was the predominant acid (68%) whereas the present sample (Indian variety, 18% oil) showed palmitic acid (34.7%) as the major component. Although there is difference in the fatty acid composition of Fiji and Indian varieties, the nature of fatty acids in both the varieties are similar. Noticeable differences in the fatty acid composition of a seed oil from two different sources have usually been ascribed to environmental factors—varietal, climate and soil.

In conclusion it may be mentioned that g.l.c. analysis of silver nitrate–methanol-treated esters of cyclopropenoid acid-containing oils is a method of choice both for characterising and estimating the individual (malvalic and/or sterculic) acids in seed oils. This method of analysis has been used in our laboratory for the analysis of seed oils containing low levels of cyclopropenoid material. The oxirane ring in epoxy acids is fairly reactive and is opened up either during saponification of the glycerides or during esterification of the acids. Therefore, it may be added that Gunstone's method[21] of converting epoxy acid to dihydroxy acid appears to be a convenient procedure for the analysis of oils containing minor proportions of epoxy acids.

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