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FATTY ACID COMPOSITIONS OF LIPIDS ISOLATED FROM DIFFERENT PARTS OF *CEIBA PENTANDRA, STERCULIA FOETIDA* AND *HYDNOCARPUS WIGHTIANA*

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Abstract—Lipids were isolated from different parts of *Ceiba pentandra, Sterculia foetida* and *Hydnocarpus wightiana*. Positive Halphen response indicated the presence of cyclopropene fatty acids (CFA) in the lipids of seeds and roots of *C. pentandra* and *S. foetida*. NMR and i.r. spectra showed the presence of CFA and their dihydro derivatives in the root lipids of both trees. The CFA were present in the triglyceride, the fatty acid and diglyceride fractions of the root lipids of *C. pentandra*. The cyclopentene fatty acids which characterize the seed lipids of *H. wightiana* were absent from the lipids of leaves, trunk-wood and roots. The fatty acid compositions of the lipids were determined by GLC after treatment of methyl esters with $AgNO_3$ where CFA were present. The major fatty acids in the root lipids of *C. pentandra* were the CFA, dihydrosterculic and linoleic acids. Malvalic was the main acid in the root lipids of *S. foetida* and linoleic in the lipids of buds, flowers and trunk-wood of *C. pentandra*, trunk-wood and stems of *S. foetida* and trunk-wood and roots of *H. wightiana*. Linolenic was the principal acid in the leaf lipids of *C. pentandra* and *S. foetida* and stearic acid in *H. wightiana*.

INTRODUCTION

STUDIES on plant lipids have generally been restricted to lipids of the storage parts such as seeds and tubers. Studies on lipids from other parts are not many, though these are important from the chemotaxonomic and biochemical points of view. The few such investigations are mostly confined to plants producing seed lipids of normal fatty acids.[1] We are carrying out a general survey of the occurrence of unusual fatty acids in the lipids from different parts of plants whose seed oils contain these acids. In the present communication lipids from different parts of *Ceiba pentandra* L. (Malvaceae), *Sterculia foetida* L. (Sterculiaceae) and *Hydnocarpus wightiana* Blume (Flacourtiaceae) were examined for their fatty acid compositions. The seed lipids of the first two plants are characterized by the presence of CFA[2] and of the third by cyclopentene fatty acids.[3] Preliminary studies on the lipids from different parts of *S. foetida* showed that CFA were present only in the seed and root lipids.[4]

RESULTS AND DISCUSSION

Lipids are present in significant amounts in different parts of the plant analysed, but the percentages given on dry basis are rather low compared to those in the seeds (Tables 1–3).

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[2] F. L. CARTER and V. L. FRAMPTON, *Chem. Rev.* **64**, 497 (1964).
[3] T. P. HILDITCH and P. N. WILLIAMS, *Chemical Constitution of Natural Fats*, IV edn., p. 292, Chapman & Hall, London (1964).
[4] P. SUDERSHAN and G. LAKSHMINARAYANA, *Annual Report of the Regional Research Laboratory*, p. 7, Hyderabad (1965–66).

TABLE 1. FATTY ACID COMPOSITIONS OF LIPIDS FROM DIFFERENT PARTS OF *Ceiba pentandra*

	Seeds	Buds	Flowers	Leaves	Bark	Trunk-wood	Roots
Lipid content (dry basis, %)	23·6	1·4	1·6	2·3	0·7	0·6	0·8
Fatty acids (wt. %)							
Lauric	0·0	0·0	0·0	5·1	8·9	2·1	0·0
Myristic	0·1	0·0	0·4	16·5	3·4	2·2	0·0
Palmitic	22·8	27·6	28·1	24·0	28·6	26·2	15·7
Dihydromalvalic	0·7	0·0	0·0	0·0	0·0	0·7	0·9
Stearic	0·8	1·2	1·4	8·8	0·0	2·8	1·4
Dihydrosterculic	Traces	0·0	0·0	0·0	0·0	0·0	19·4
Arachidic	0·0	0·0	1·1	0·0	0·0	0·0	0·0
Palmitoleic	0·0	3·9	2·8	1·6	0·0	2·1	0·9
Oleic	30·0	17·1	13·4	2·3	39·4	24·7	14·7
Linoleic	32·9	38·8	42·3	13·5	19·7	32·3	20·6
Linolenic	0·0	11·4	10·5	28·2	0·0	6·8	6·1
Malvalic	9·3	0·0	Traces	0·0	0·0	0·0	15·8
Sterculic	3·4	0·0	0·0	0·0	0·0	0·0	4·8

TABLE 2. FATTY ACID COMPOSITIONS OF LIPIDS FROM DIFFERENT PARTS OF *Sterculia foetida*

	Seed kernels	Leaves	Stems	Trunk-wood	Roots
Lipid content (dry basis, %)	51·8	5·4	1·0	2·0	2·9
Fatty acids, wt.%					
Decanoic	0·0	1·1	0·4	13·2	0·0
Lauric	0·0	3·6	1·4	2·7	0·0
Myristic	0·0	3·1	3·3	6·2	0·6
Palmitic	20·0	19·6	32·0	24·8	17·0
Dihydromalvalic	0·0	0·4	2·0	0·0	4·8
Stearic	1·4	1·5	1·6	0·0	6·0
Dihydrosterculic	0·0	0·0	0·0	0·0	10·4
Arachidic	0·0	0·0	1·7	0·0	0·0
Palmitoleic	0·0	1·0	0·7	4·0	0·0
Oleic	9·4	1·5	4·9	13·3	14·4
Linoleic	9·5	9·7	40·8	28·3	10·7
Linolenic	0·0	58·5	11·2	7·5	5·4
Malvalic	14·4	0·0	0·0	0·0	29·5
Sterculic	45·3	0·0	0·0	0·0	1·3

TABLE 3. FATTY ACID COMPOSITIONS OF LIPIDS FROM DIFFERENT PARTS OF *Hydnocarpus wightiana*

	Seed kernels (Ref. 14)	Leaves	Trunk-wood	Roots
Lipid content (dry basis, %)	47·4	0·7	0·7	0·5
Fatty acids (wt.%)				
Lauric	0·0	4·4	13·7	3·4
Myristic	0·0	5·6	4·4	2·2
Palmitic	4·2	23·7	24·0	27·9
Stearic	0·0	26·7	7·2	7·0
Myristoleic (?)	0·0	0·0	0·0	2·6
Oleic	3·8	0·0	13·0	9·5
Linoleic	0·0	19·2	29·2	33·7
Linolenic	0·0	20·4	8·5	13·7
Cyclopentene acids	92·0	0·0	0·0	0·0

Lipids from each part of *Ceiba pentandra* and *Sterculia foetida* were examined by the Halphen test, which is specific for CFA and sensitive up to 0·1 per cent.[5] Of these only the seed and root lipids of both plants responded strongly to the test. The flower lipids of *C. pentandra* responded faintly. The presence of both CFA and their dihydro derivatives in root lipids was shown by i.r. spectrophotometry[2] and NMR spectroscopy.[6] The i.r. spectrum showed peaks at 1000 cm^{-1} and 1865 cm^{-1} characteristic of the cyclopropene group and at 1030 cm^{-1} and 865 cm^{-1} characteristic of cyclopropane group. The NMR spectrum showed peaks for cyclopropene (τ 9·2) and cyclopropane (τ 9·4) groups. To find out in which lipid classes the CFA occur, the root lipids of *C. pentandra* were fractionated by silicic acid column chromatography and preparative TLC on silica gel G. The fractions were tested for their Halphen response. Triglyceride, free fatty acid and diglyceride fractions responded positively. Others, namely sterol esters, monoglycerides and other polar lipids including phospholipids, responded negatively.

The root lipids of *C. pentandra* contained 18% of free fatty acids (calculated as oleic acid). In this connexion, Kartha's[7] observation on the presence of a large amount of free fatty acids in the lipids from the root bark of *Ixora coccinea* is of interest. He suggested that plant tissues are capable of storing free higher fatty acids without conversion to any neutral form under special (but unspecified) circumstances. In the root lipids of *C. pentandra*, stigmasterol a β-sitosterol were identified as components of the sterol fraction by TLC[8] on silica gel G.

Fatty acid compositions of the lipids from different parts of *C. pentandra*, *S. foetida* and *Hydnocarpus wightiana* are given in Tables 1, 2, and 3, respectively. The root lipids of *C. pentandra* and *S. foetida* contained considerable proportions of CFA and their dihydro derivatives; the former contained more than even the seed fat. The ratio of malvalic to sterculic (*ca.* 3:1) is, however, approximately the same in both seed and root lipids of *C. pentandra*. The root lipids of *S. foetida*, on the other hand, contain more of malvalic acid than sterculic acid, unlike the seed lipids. The major fatty acids were CFA, dihydrosterculic and linoleic in the root lipids of *C. pentandra*. The predominant acid was malvalic in the root lipids of *S. foetida*. Striking similarities were observed in the fatty acid composition of the root lipids of both the plants. Palmitic, oleic and linolenic acids were present almost to the same extent in both. Similarly, the ratio of dihydrosterculic acid to linoleic acid (*ca.* 1:1) was the same in both.

The precursors and the enzyme system for the biosynthesis of CFA may be the same in roots and seeds, since it is known[9] that seed fat synthesis takes place from water-soluble precursors translocated from other parts of the plant. The immediate precursors of CFA may have been their dihydro derivatives as observed in the seedlings of *Hibiscus syriacus*[10] and the developing seeds of some species of the Order Malvales.[11] The function of these unusual acids in the roots, however, is not clear. Unusual acids have also been reported in the root lipids of mangel (*Beta rapa vulgaris*)[12] and *Exocarpus cupressiformis*[13] which contained erucic and octadeca-*trans*-13-ene-9,11-diynoic acids, respectively.

[5] F. C. MAGNE, *J. Am. Oil Chemists' Soc.* **42**, 332 (1965).
[6] C. Y. HOPKINS, *J. Am. Oil Chemists' Soc.* **38**, 664 (1961).
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Cyclopentene acids, namely hydnocarpic, chaulmoogric and gorlic acids, which together constitute 92% of the fatty acids in the seed lipids,[14] were absent from the root lipids of *Hydnocarpus wightiana*. The main acids were linoleic, palmitic and linolenic.

The major fatty acid was linoleic in the bud lipids of *C. pentandra*, followed by palmitic, oleic and linolenic acids in that order. The flower lipids of *C. pentandra* responded faintly to the Halphen test. GLC of their methyl esters showed the presence of only traces of CFA. Significant differences were not observed between the fatty acid compositions of lipids from these two parts of the tree.

The predominant fatty acid in the leaf lipids of *C. pentandra* and *S. foetida* was linolenic, the proportion of which was much more in the latter. Data from literature[1] also show that this acid predominates in the leaf lipids of most plants. The leaf lipids of *H. wightiana*, however, showed an unusual fatty acid composition in that it contained saturated acids to the extent of 60%, stearic acid being the major component (26·7%). A similar deviation was also observed by Hilditch and Meara[15] in the leaf lipids of nettle (*Urtica dioica*) where oleic acid (82–86%) formed the predominant fatty caid. In all, the unusual acids which characterize their respective seed fats were absent. Similar observations have been made with the leaf lipids of castor[16] and rape,[17] in which the predominant fatty acids of the respective seed fats, namely ricinoleic and erucic, were absent. Both the leaf and seed lipids of some species of Malvaceae contained CFA.[18] Similarly γ-linoleic and octadecatetraenoic acids were identified in both the leaf[19] and seed[20,21] lipids of some plants belonging to the Boraginaceae.

Linoleic formed the major fatty acid in the stem lipids of *S. foetida* and the trunk-wood lipids of all the three plants. Small amounts of lauric and myristic acids were present in the trunk-wood lipids of *C. pentandra* and appreciable amounts of decanoic and lauric acids, respectively, in those from *S. foetida* and *H. wightiana*. Unusual acids were absent from all these tissues. In the bark lipids of *C. pentandra* oleic acid predominated followed by palmitic and linolenic acids.

These data and those available from literature show that the fatty acid compositions of lipids from different parts of the tree are very different from those of the respective seed fats. These data also show considerable similarities in the fatty acid compositions of the lipids from the same part of plants of widely differing species or families. This similarity is attributed by Iskhakov and Vereshchagin[22] to a certain unity in the structure of the cell protoplasm throughout the plant kingdom. However, differences are encountered in the fatty acid compositions of the lipids derived from different parts of the same plant. These may be attributed to the different functions of these lipids in different parts of the plant.

EXPERIMENTAL

Different parts of the plants were collected from a single tree in each case. Moisture contents were determined by heating at 110° to constant weight.

The material was weighed, ground and extracted five times with $CHCl_3$–MeOH (2:1, v/v) at room temperature in the dark. The extract was concentrated *in vacuo* below 40°, diluted with water and re-extracted with

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[15] T. P. HILDITCH and M. L. MEARA, *J. Soc. Chem. Ind.* **63**, 112 (1944).
[16] A. T. JAMES, *Biochim. Biophys. Acta* **70**, 9 (1963).
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light petroleum (40–60°). The petroleum ether extract was washed with 10% aq. Na_6SO_4, dried and concentrated. After making up to a known volume, the lipid content was determined by evaporating an aliquot. Lipids from each part of *Ceiba pentandra* and *Sterculia foetida* were examined by the Halphen test[5] on a semi-micro scale. I.r. spectra were recorded as a thin film and NMR spectra were taken in CCl_4.

Determination of Fatty Acid Composition

The lipids which did not contain CFA, including those from *Hydnocarpus wightiana*, were saponified with 0·5 N alcoholic KOH and the free fatty acids were liberated after removal of unsaponifiable matter by extraction of the soaps with diethyl ether. The fatty acids were isolated and esterified with CH_2N_2. Since acidification of soaps may partly destroy CFA, lipids containing CFA were first treated with CH_2N_2 to esterify the free fatty acids, when present, and transesterified with methanol containing 1% NaOMe. The methyl esters were treated with a saturated solution of $AgNO_3$[23] in absolute MeOH to convert the CFA to stable products. The methyl esters were analysed by GLC using an F & M model 720 dual column programmed temperature gas chromatograph equipped with a thermal conductivity detector and a bronze column (8 ft × ¼ in.) of 15 per cent DEGS on Chromosorb W (60–80 mesh) maintained at 210°. H_2 (60 ml/min) was the carrier gas. Peak areas were estimated by triangulation. From the peak areas fatty acid composition (wt.%) was obtained. Peaks were identified using reference esters.

Silicic Acid Chromatography of Root Lipids of C. pentandra

The root lipids of *C. pentandra* were fractionated on a silicic acid column using mixtures of light petroleum and ether and finally MeOH. The eluted fractions were characterized by TLC on silica gel G using reference samples. Fractions of similar TLC behaviour were pooled and tested for their Halphen response and Liebermann–Burchard reaction. Sterol fraction was analysed by TLC on silica gel G using reference standards according to Shepherd *et al.*[8]

Since free fatty acids and triglycerides could not be obtained in pure form by column chromatography, these were isolated by preparative TLC on silica gel G (500 μ thick). To isolate the free fatty acids, the total lipids were treated with CH_2N_2 and the methyl esters formed were separated using petroleum ether (60–80°)–Et_2O–HOAc (97:3:1, v/v). The triglycerides were separated using the petroleum ether–Et_2O–HOAc (85:15:1, v/v). Each of these lipid classes was tested for its Halphen response.

[23] E. L. Schneider, S. P. Loke and D. T. Hopkins, *J. Am. Oil Chemists' Soc.* **45**, 585 (1968).