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STRAIGHT-CHAIN UNSATURATED FATTY ACIDS AND CYCLOPENTENYL FATTY ACIDS IN LEAF LIPIDS OF *CALONCOBA ECHINATA* AND *HYDNOCARPUS ANTHELMINTHICA*

FRIEDRICH SPENER and HELMUT K. MANGOLD

Bundesanstalt für Fettforschung, 44 Münster (Westf.), Germany

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Key Word Index—*Caloncoba echinata*; *Hydnocarpus anthelminthica*; Flacourtiaceae; leaves; lipids; unsaturated fatty acids; cyclopentenyl fatty acids.

Abstract—The total lipids in leaves of *Caloncoba echinata* and *Hydnocarpus anthelminthica* exhibited consistent fatty acid patterns with palmitic, linoleic and α-linolenic acids being the major acids. Small amounts of cyclopentenyl fatty acids were found in *C. echinata* (4·4%) and in *H. anthelminthica* (0·5–1·5%). Analyses of the constituent fatty acids of individual lipid classes showed cyclopentenyl fatty acids to be present mainly in triglycerides and as free fatty acids. The major lipids in the two species were monogalactosyl diglycerides, digalactosyl diglycerides and phosphatidyl cholines. The straight-chain monounsaturated fatty acids consisted mainly of Δ-9 isomers, whereas the polyunsaturated fatty acids belonged exclusively to the Δ-9 series. There was no structural relationship between the straight-chain unsaturated acids and the cyclopentenyl acids.

INTRODUCTION

Little is known about the distribution of the cyclopentenyl fatty acids and nothing about the biosynthesis of these unusual lipids [1]. Hydnocarpic, chaulmoogric and gorlic acids, the major cyclopentenyl fatty acids, are found in seeds of the tribes Pangieae and Oncobeae of the Flacourtiaceae [2]. Cyclopentenyl fatty acids constitute up to 85% of the total fatty acids in some of these seeds [3]. Small amounts of cyclopentenyl fatty acids occur also in tissue cultures of *Hydnocarpus anthelminthica* [4].

The present communication records analyses of the constituent fatty acids of lipids in leaves of *Caloncoba echinata* and *Hydnocarpus anthelminthica*. Straight-chain monounsaturated and polyunsaturated fatty acids were isolated and their structures were determined in an attempt to elucidate possible biogenetic relationships between these acids and the cyclopentenyl fatty acids.

RESULTS

The lipids in leaves of *C. echinata* (tribe Oncobeae) and *H. anthelminthica* (tribe Pangieae) were investigated. Fresh leaves of these plants yielded an average of 2·4% total lipids including chlorophyll and other pigments. Aliquots of these lipids were subjected to methanolysis, and the resulting methyl esters were analyzed by GLC (Table 1). The patterns of the constituent straight-chain fatty acids were quite similar. This was true also for leaves of *H. anthelminthica* collected over a period of 2 yr at different locations, and for leaves of *H. kurzii* (King) Warb. In all samples, α-linolenic acid constituted over 50% of total fatty acids, the other major acids being linoleic and palmitic acids. Among the monounsaturated fatty acids, octadecenoic acids predominated. Small amounts of cyclopentenyl fatty acids were detected in all samples investigated. Thus, in the total lipids from leaves of *C. echinata* 2·8% of hydnocarpic, 1·3% of chaul-

Table 1. Fatty acid composition of the total lipids in leaves of Flacourtiaceae*

Chain length: Number of double bonds†	*Caloncoba echinata*	*Hydnocarpus anthelminthica*
12:0	0·2	0·4
14:0	0·6	0·8
14:1	0·1	0·3
15:1	0·2	0·1
16:0	9·2	16·4
16:1	2·0	1·1
16:1cy	2·8	0·8
17:0	0·2	0·4
18:0	6·0	7·4
18:1	3·6	3·4
18:2	12·4	12·9
18:3	57·8	54·0
18:1cy	1·3	0·1
18:2cy	0·3	tr
20:0	1·4	0·2
20:1	0·3	0·1
22:0	1·2	0·1

* Expressed in wt %; tr = trace (<0·1%); the following acids were also found in trace amounts: 13:0, 15:0, 17:1, 19:0, 20:2, 21:0, 22:1, 23:0, 24:0, 24:1.

† The affix "cy" denotes the cyclopentene structure of the acid.

moogric and 0·3% of gorlic acids were found. The occurrence of these unusual fatty acids prompted us to isolate individual lipid classes and to determine their component fatty acids.

Isolation and identification of lipid classes

Each extract was fractionated by column chromatography and TLC into four fractions, whose weights were determined gravimetrically. They were similar for the leaves of the species investigated: Fraction 1, 6·1%, containing hydrocarbons, steryl esters and wax esters, aldehydes and pigments; Fraction 2, 0·6%, containing triglycerides and pigments; Fraction 3, 8·6%, containing sterols, alcohols, fatty acids, diglycerides and pigments; Fraction 4, 84·7%, containing phospholipids, glycolipids and chlorophyll.

Fraction 1 contained most of the non-polar lipids from surface waxes. From Fractions 2 and 3, only TG* and FFA were isolated, respectively. In Fraction 4, PC, PE, PA, PI and PG were identified by two-dimensional TLC in all samples, however, PE and PA were barely detectable in lipid extracts of *C. echinata* leaves. In addition, PS and some lyso compounds may have been present in minute amounts. With the exception of CER, glycolipids were identified by comparison with reference samples and specific stains; however, no effort was made to determine the nature of the sugar and sterol moieties. Thus, CER, MGDG, DGDG, SQDG, ESG and SG were identified in all samples.

Viewing the chromatoplates after spraying with chromic–sulphuric acid and charring showed that MGDG and DGDG were the major polar lipids present in leaf tissues, followed by PC, which was the major phospholipid. In two-dimensional TLC, a few sugar-positive spots were visible near PC, PI and the origin. These lipids may be polygalactosyl diglycerides [5] and/or sterylglycolipids [6].

Fatty acids of different lipid classes

The fatty acid composition of various lipid classes from leaves of *C. echinata* and *H. anthelminthica* was determined. The results are presented in Tables 2 and 3. Due to lack of material, the constituent fatty acids of PE and PA in the lipid extract of *C. echinata* leaves could not be analyzed. It is evident from Tables 2 and 3 that cyclopentenyl fatty acids occurred mainly as constituents of TG and FFA; only very small proportions were found in phospholipids and glycolipids. As the proportions of TG and FFA were low in comparison to those of phospholipids and glycolipids, the percentage of cyclopentenyl fatty acids in the total fatty acids was indeed small i.e. 4·4% in *C. echinata* (see Table 1).

Isomeric unsaturated fatty acids

The position of double bonds in the constituent straight-chain unsaturated fatty acids of lipids in *C. echinata* and *H. anthelminthica* leaves were determined. The results are shown in Table 4. The *cis*-hexadecenoic acids consisted mainly of the Δ-9 isomer and smaller amounts of Δ-6 and Δ-7 isomers. A similar distribution for these acids was found in tissue cultures [4]. *trans*-Δ-3- Hexadecenoic acid, whose *trans* double bond was established by the behavior of the methyl ester in

* *Abbreviations used*: TG = triglycerides; FFA = free fatty acids; PC, PE, PI, PG and PS = phosphatidyl cholines, -ethanolamines, -inositols, -glycerols and -serines, respectively; PA = phosphatidic acids; CER = cerebrosides; SG = steryl glycosides; MGDG, DGDG and SQDG = monogalactosyl-, digalactosyl- and sulfoquinovosyl diglycerides, respectively; ESG = esterified steryl glycosides.

Table 2. Constituent fatty acids of various lipid classes in leaves of *Caloncoba echinata**

Chain length: Number of double bonds†	TG	FFA	PC	PI	PG	MGDG	DGDG	SQDG	ESG
12:0	0·5	1·6					0·3	1·1	
14:0	1·2	6·6	0·7	2·6	2·2	0·4	0·3	1·5	2·4
14:1	0·2	0·5	0·1						
15:0	0·2	0·5	0·4	1·9	1·0	0·1	0·1	0·8	1·0
15:1	0·2	2·6	0·3	0·6	0·7	0·1	0·1	0·4	1·1
16:0	9·1	16·1	29·1	29·7	38·1	2·2	11·3	42·4	20·8
16:1	1·9	2·6	3·0	3·3	5·0	0·5	0·9	3·2	4·3
16:1 *trans*					6·8				
16:1cy	42·1	30·9	0·9	1·9	1·2	tr	0·3	0·4	0·9
17:0	0·2	0·3	1·0	1·9	1·2	0·2	0·8	1·0	0·9
17:1	0·3	0·5	1·0	3·1	1·7	0·2	0·3	1·3	1·4
18:0	2·8	5·9	6·2	19·4	12·0	1·6	8·6	10·5	17·3
18:1	8·8	5·6	17·2	14·9	12·7	1·5	1·2	7·3	8·4
18:2	4·8	3·9	17·2	8·1	8·0	11·8	9·6	6·3	9·6
18:3	10·8	13·8	22·3	10·4	5·3	81·3	65·4	21·9	26·0
18:1cy	13·5	4·6		tr	0·3			tr	1·9
18:2cy	2·6	1·6	tr	tr	1·5		0·1	0·5	2·0
19:1	0·1	0·3	tr		0·5		0·2	0·6	0·8
20:0	0·3	0·9	tr	0·6	0·3	tr	0·1	0·3	0·8
20:1	0·2	0·4	tr	1·6	1·5	0·1	0·4	0·5	0·4
20:1cy	0·2								
20:2cy	0·1								
22:0		0·5							
22:1		0·3							

* Composition expressed in wt%; tr = trace (<0·1%).
† The affix "cy" denotes the cyclopentene structure of the acid.

Table 3. Constituent fatty acids of various lipid classes in leaves of *Hydnocarpus anthelminthica**

Chain length: Number of double bonds†	TG	FFA	PC	PE	PA	PI	PG	MGDG	DGDG	SQDG	ESG
12:0		0·7	0·4	1·0	0·6				0·5		0·9
14:0	1·4	1·9	0·5	1·2	0·1	0·9	1·4	0·2	3·2	0·8	0·9
14:1				1·2		3·3		0·1			
15:0	0·3	0·2	0·2	0·6	0·5	0·4	0·3	tr	0·2	0·2	0·2
15:1	0·3	0·2	0·1	0·6	0·4	0·4	0·2	tr	tr	0·2	
16:0	20·2	8·9	31·8	27·0	30·8	22·4	31·1	2·0	13·6	31·5	37·0
16:1	2·6	1·1	0·9	2·7	3·1	3·9		0·4	0·5	1·9	
16:1 *trans*							18·8‖				18·7
16:1cy	2·3	1·0		0·8	1·6	1·1			0·1	0·2	
17:0	0·8	0·3	1·3	1·7	1·6	2·6	1·0	0·5	1·3	1·5	1·1
17:1	0·4	0·2	0·3	4·6	1·4	6·1	0·9	0·2	2·5	0·6	1·1
18:0	11·7	3·2	11·0	17·4	10·1	21·0	18·6	1·8	14·0	16·2	16·3
18:1	7·3	3·9	6·5	5·0	7·7	4·4	12·4	1·2	1·6	6·7	10·7
18:2	16·5	11·5	19·9	15·1	17·1	9·2	5·0	11·0	9·0	7·6	4·4
18:3	32·0	64·7	27·1	15·4	20·6	22·8	9·0	81·4	52·2	30·8	8·1
18:1cy	1·9	0·7		1·4		0·4		0·3	0·3	0·1	
18:2cy	1·0	0·8			0·9			0·3		0·5	
19:1	0·2	0·2		1·7	0·9	tr	0·5	0·3	0·3	0·8	0·6
20:0	0·6	0·1		1·1	0·9	tr	0·2	tr	0·2	0·2	tr
20:1	0·5	0·1		1·5	0·7	1·1	0·6	0·3	0·5	0·2	
20:1cy											
20:2cy											
22:0		0·2									
22:1		0·1									

* Composition expressed in wt%; tr = trace (<0·1%).
† The affix "cy" denotes the cyclopentene structure of the acid.
‖ Total 16:1 *cis* and 16:1 *trans*.

Table 4. Isomeric unsaturated fatty acids of the lipids in leaves of Flacourtiaceae*

	Positions of double bonds (Δ)								
	3	4	5	6	7	8	9	10	11
Caloncoba echinata									
16:1				9·9	8·8		75·5	1·7	4·1
16:1 *trans*	100·0								
18:1						1·1	93·0	0·5	5·4
18:2							100·0		
18:3							100·0		
Hydnocarpus anthelminthica									
16:1				10·4	7·0		78·3	0·1	4·2
16:1 *trans*	100·0								
18:1						0·7	94·5	1·2	3·6
18:2							100·0		
18:3							100·0		

* Isomer distribution expressed in mole %.
For polyunsaturated fatty acids the position of the double bond closest to the carboxyl group is given.

argentation TLC and GLC, was also found. This acid is considered to be characteristic of photosynthetic tissue [7, 8]. Oleic acid was the major octadecenoic acid, accompanied by small amounts of *cis*-vaccenic acid. Diunsaturated and triunsaturated fatty acids occurred exclusively as linoleic and α-linolenic acids, thus showing no difference to polyunsaturated fatty acids in seeds [3] and tissue cultures [4] of these plants.

DISCUSSION

The leaves investigated were collected from trees and shrubs of different species, at different times and at different locations, but no effort was made to distinguish between young and old leaves. The lipid classes present in these leaves were characteristic for photosynthetic tissues, with MDGD, DGDG and SQDG being present in high proportions. These lipids were minor components in the seeds [3]. Phospholipids, which are the major polar lipids in seeds, were less abundant in leaf tissues. The fatty acid composition of all samples showed the pattern characteristic of photosynthetic tissue of higher plants, with α-linolenic acid contributing over 50%, in each case. The fatty acid patterns were clearly distinguished from those of non-photosynthetic seed tissue [3] and tissue cultures [4], with cyclopentenyl fatty acids predominating in the former and palmitic and linoleic acids in the latter. The total lipids in leaves of *C. echinata* were found to contain 4·4% cyclopentenyl fatty acids whereas those of *H. anthelminthica* included only minute amounts. It has been reported previously that the leaf lipids of *Hydnocarpus wightiana* do not contain cyclopentenyl fatty acids [9].

Structural analyses of straight-chain unsaturated fatty acids did not reveal the presence of acids which could conceivably be precursors of cyclopentenyl fatty acids. Individual lipid classes exhibited high proportions of polyunsaturated fatty acids which belonged exclusively to the Δ-9 series. The proportions of polyunsaturated fatty acids may be even higher than shown in Table 2 because some breakdown of these acids occurred during two-dimensional TLC of the polar lipids prior to analysis.

The cyclopentenyl fatty acids were predominantly esterified in TG; however, small amounts were detected also in phospholipids and glycolipids. Another fraction containing relatively high proportions of cyclopentenyl fatty acids was found to be the FFA fraction. The origin of this very small fraction is not clear, but it may have arisen through enzymic breakdown of lipids during shipping of the plants or storing of the extracts. In seeds of the plants investigated, the bulk of the cyclopentenyl fatty acids was found esterified in TG, which constituted up to 98% of total lipids in the mature tissues [3]. In addition, here too cyclic fatty acids were found in phospholipids and glyco-

lipids. It appears that the metabolism of lipids containing cyclopentenyl fatty acids is not mediated by special enzymes. A similar conclusion was reached by those who found cyclopropane and cyclopropene fatty acids to be ubiquitous in the acyl lipids of several *Malva* species [10]. These cyclic fatty acids were also esterified preferentially in TG.

The occurrence of unusual fatty acids in both seed and leaf tissues of the same plant has only seldom been reported. Thus, in some Malvaceae total fatty acids in seeds and leaves constitute up to 28% cyclopropene fatty acids and cyclopropane fatty acids [10]. Similarly, the seeds and leaves of Boraginaceae and Caryophyllaceae contain polyunsaturated fatty acids of unusual structures, viz. Δ-6,9,12-18:3 and Δ-6,9,12,15-18:4 fatty acids [11, 12]. In contrast to the various cyclic fatty acids, the unusual polyunsaturated fatty acids found in higher plants occur predominantly in glycolipids [11–13].

EXPERIMENTAL

Leaves were collected at different locations on the island of Oahu, Hawaii, U.S.A. Reference cyclopentenyl fatty acids were prepared as described elsewhere [3]. All solvents used were analytical grade and distilled prior to use.

Lipids were extracted from leaf tissue by maceration of the leaves in *iso*-PrOH and $CHCl_3$–MeOH (2:1) [14] and purified following established procedures [15]. The total lipids extracted were separated by column chromatography on silicic acid into neutral, and ionic and other polar lipids using $CHCl_3$ and $CHCl_3$–MeOH–H_2O (3:5:2) [16] as eluants, respectively. Solvents used for TLC of neutral lipids were hexane–Et_2O (19:1, twice) [17] and hexane–Et_2O–HOAc (70:30:1) [17], for ionic and other polar lipids $CHCl_3$–MeOH–HOAc (80:25:1) [14], $CHCl_3$–MeOH–H_2O (65:25:4) [18], $CHCl_3$–MeOH–HOAc–H_2O (50:30:8:2) [14], and the two-dimensional system $CHCl_3$–MeOH–7 N NH_4OH (65:25:4) followed by $CHCl_3$–MeOH–HOAc–H_2O (170:25:25:4) [3, 14]. Individual lipid classes were identified by comparison with reference samples. The molybdate reagent was used for the detection of phospholipids [19], the α-naphthol reagent for glycolipids [20], ninhydrin for lipids containing amino groups [21] and 50% H_2SO_4 for sterols and sterol containing compounds [4].

The individual lipid classes were isolated by preparative TLC using hexane–Et_2O (9:1, twice) for neutral lipids and the aforementioned two-dimensional system for polar lipids. Zones and spots, visible under UV light after spraying with 2',7'-dichlorofluorescein solution, were scraped off and methyl esters were prepared immediately without prior elution of the lipids from the adsorbent [22]. Methyl esters were analyzed by GLC on both a polar column (15% DEGS on Anakrom D) and a non-polar column (3% OV-1 on Supelcoport) [3]. The structures of isomeric straight-chain unsaturated fatty acids were determined by ozonolysis and GLC of the fragments as described elsewhere [3, 23].

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