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COMPOSITION OF CUTIN FROM COFFEE LEAVES

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Abstract—The constituents of the cutin of coffee leaves have been identified using TLC, GLC and GLC–MS. Dihydroxyhexadecanoic acids comprise more than 60% of the total acids. Other compounds identified include C_{16}–C_{34} monobasic acids, C_{14} and C_{15} monohydroxymonobasic acids, 16-hydroxyhexadecanoic acid and monohydroxyhexadecane-1,16-dioic acids. All acids having a secondary hydroxyl group exhibit positional isomerism with the group predominantly at both 9- and 10-positions. No major differences occur in the composition of cutin from young and mature leaves or from the adaxial and abaxial surfaces

INTRODUCTION

THE EXAMINATION of the chemical composition of cutin from coffee was undertaken as part of a general investigation of the physical and chemical properties of the leaf surface layer in relation to the penetration of foliar sprays. The coffee leaf has not been previously studied in detail. This paper describes results obtained from analyses of cutin isolated from adaxial and abaxial surfaces of young and mature coffee leaves.

RESULTS

No significant differences were found in the qualitative composition of the cutins from young and mature coffee leaves or from either adaxial or abaxial surfaces. Small quantitative variations were found but there was no correlation between them and the various samples examined. The various methods used to isolate the cuticular membranes from the leaves also had no effect upon the cutin composition. The cutin content of the membranes was always at least 60% by weight; young membranes had a slightly higher content than mature membranes.

The composition of the cutin from a sample of young coffee leaves is summarized in Table 1. Dihydroxyhexadecanoic acids comprise at least 60% of the total acids. Other C_{16} hydroxy-fatty acids also occur but C_{14}, C_{15} and C_{18} hydroxy-fatty acids are present. Up to 15% of the total acids may be C_{14} and C_{15} compounds. Small amounts of monobasic acids and alkan-1-ols are also present. Coffee cutin resembles several other angiosperm cutins, for example, those of the leaves of *Citrus aurantifolia*[1] and *Ribes* species,[2] and the fruits of *Lycopersicon esculentum*,[1,3] *Rosa canina*[1] and *Bryonia dioica*.[1]

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[1] E. A. BAKER and P. J. HOLLOWAY, *Phytochem.* **9**, 1557 (1970).
[2] E. A. BAKER and J. T. MARTIN, *Ann. Appl. Biol.* **60**, 313 (1967).
[3] C. H. BRIESKORN and H. REINARTZ, *Z. Lebensmittelunters. u-Forsch* **135**, 55 (1967).

TABLE 1. COMPOSITION OF CUTIN FROM YOUNG COFFEE LEAVES

Compound(s)	% composition*
Monobasic acids (C_{16}–C_{34})	2·7
Monohydroxytetradecenoic acids	8·2
Monohydroxypentadecanoic acids	1·7
16-Hydroxyhexadecanoic acid	2·7
Monohydroxyhexadecane-1,16-dioic acids	6·0
Dihydroxypentadecanoic acids	1·7
Dihydroxyhexadecanoic acids	64·5
9,10,18-Trihydroxyoctadecanoic acid	1·0
Alkan-1-ols (C_{28}–C_{36})	0·6
Unidentified compounds	10·9

* Relative peak areas determined from total methyl ester TMS ether GLC chromatogram.

Monobasic Acids

The acids were identified by GLC of their methyl esters using co-injection of known acid methyl esters and from their MS.[4] The composition of the fraction is shown in Table 2. The major constituents are hexadecanoic, triacontanoic and dotriacontanoic acids. Hexadecanoic acid occurs commonly in plant cutins[1] but the higher homologues are more usually found as constituents of plant surface waxes.[5]

TABLE 2. COMPOSITION OF THE MONOBASIC ACID AND ALKAN-1-OL FRACTIONS FROM CUTIN OF YOUNG COFFEE LEAVES

Homologues	% composition monobasic acids	% composition alkan-1-ols
C_{16}	21·9	—
$C_{18}:1\Delta$	4·3	—
C_{18}	5·2	—
C_{28}	1·9	5·8
C_{29}	1·5	1·7
C_{30}	17·2	52·9
C_{31}	4·9	2·5
C_{32}	25·5	24·0
C_{33}	4·6	0·8
C_{34}	8·6	8·3
C_{36}	—	4·1
Unidentified	4·4	—

Monohydroxymonobasic Acids

Monounsaturated C_{14} and saturated C_{15} homologues were identified by GLC–MS of their methyl ester TMS ether and TMS ester TMS ether derivatives. The MS are similar to those obtained by other workers[6,7] who have studied this class of hydroxy-acid. The

[4] R. RYHAGE and E. STENHAGEN, *Mass Spectrometry of Organic Ions*, p. 399, Academic Press, New York (1963).
[5] J. T. MARTIN and B. E. JUNIPER, *The Cuticles of Plants*, Edward Arnold, London (1970).
[6] C. J. WYATT, R. L. PEREIRA and E. A. DAY, *Lipids* **2**, 208 (1967).
[7] W. J. RICHTER and A. L. BURLINGAME, *Chem. Commun.* 1158 (1968).

MS of methyl-TMS-tetradecanoate is shown in Fig. 1. Positional isomers of both acids occur and these are shown in Table 3. The predominant isomers are the 9- and 10-hydroxy compounds; smaller amounts of the 7- and 8-hydroxy isomers are present. The position of the double bond in the C_{14} acid was not precisely determined but was located in the alkyl TMS ether fragment ions in the MS. The quantities of monohydroxytetradecenoic acid were variable in the samples examined and ranged from 0·7 to 10% of the total acids.

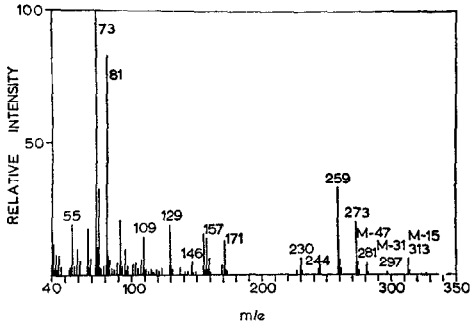

FIG. 1.

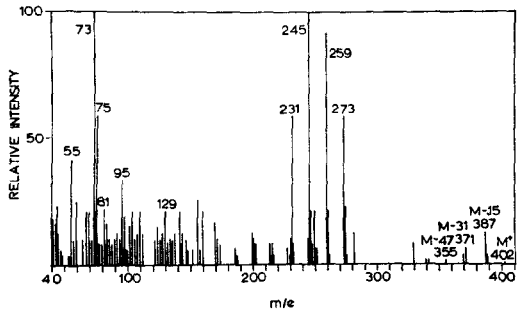

FIG. 2.

FIG. 1. MASS SPECTRUM OF COMPOUND IDENTIFIED AS METHYL TMS-TETRADECENOATE.
FIG. 2. MASS SPECTRUM OF COMPOUND IDENTIFIED AS METHYL TMS-HEXADECANE-1,16-DIOATE.

ω-Hydroxymonobasic Acids

16-Hydroxyhexadecanoic acid was identified by GLC using authentic material and from its MS.[8] The acid is a common minor constituent of many angiosperm cutins.[1] A very small amount of 16-hydroxyhexadecenoic acid was also found.

Monohydroxydibasic Acids

The methyl esters of the compounds co-eluted with the methyl esters of ω-hydroxy-monobasic acids on TLC. The 7(10)- and 8(9)-positional isomers of monohydroxyhexa-decane-1,16-dioic acid were identified by GLC–MS of their methyl ester TMS ether and TMS ester TMS ether derivatives (Table 3). The compound co-elutes with 18-hydroxy-octadecenoic acid using GLC on SE-30 columns but is resolved using either OV-210 or OV-225 phases. The MS of methyl-TMS-hexadecane-1,16-dioate is shown in Fig. 2. The overall appearance of the spectrum was similar to that obtained from an authentic sample of methyl 9-TMS-octadecane-1,18-dioate. The amounts of monohydroxyhexadecane-1,16-dioate in different samples of coffee cutin ranged from 5 to 14 %.

Dihydroxymonobasic Acids

The major component of the fraction consisted of dihydroxyhexadecanoic acids which were identified by TLC,[1] GLC[9] and GLC–MS.[8,9] Much smaller amounts of the C_{15} homologue were also identified from GLC–MS of the methyl ester TMS ether and TMS ester TMS ether derivatives. Positional isomers of both acids were found which showed a similar hydroxylation pattern, closely resembling that found in the monohydroxymonobasic

[8] G. EGLINTON, D. H. HUNNEMAN and A. MCCORMICK, *Org. Mass Spectro.* **1**, 593 (1968).
[9] P. J. HOLLOWAY and A. H. B. DEAS, *Phytochem.* **10**, 2781 (1971).

TABLE 3. POSITIONAL ISOMERS OF MONOHYDROXYMONOBASIC, DIHYDROXYMONOBASIC AND MONOHYDROXYDIBASIC ACID CONSTITUENTS FROM CUTIN OF YOUNG COFFEE LEAVES

Acid	Positional isomers present	Approx. content of each* isomer (%)
Monohydroxytetradecenoic	7-OH	3
	8-OH	12
	9-OH	55
	10-OH	30
Monohydroxypentadecanoic	7-OH	3
	8-OH	6
	9-OH	56
	10-OH	35
Dihydroxypentadecanoic	7,15-OH	5
	8,15-OH	10
	9,15-OH	53
	10,15-OH	32
Dihydroxyhexadecanoic	7,16-OH	3
	8,16-OH	12
	9,16-OH	55
	10,16-OH	30
Monohydroxyhexadecane-1,16-dioic	7(10)-OH	38
	8(9)-OH	62

* Determined from MS taken at apex of GLC peak.

acid fraction (Table 3). A partial separation between methyl 9,16- and methyl 10,16-dihydroxyhexadecanoate was obtained by preparative TLC

Trihydroxymonobasic Acids

9,10,18-Trihydroxyoctadecanoic was identified by TLC and GLC using authentic material and also from its MS.[8] The acid occurs widely in other plant cutins.[1]

Alkan-1-ols

A small amount of alkan-1-ols was found in the monohydroxymonobasic acid methyl ester fraction obtained by preparative-TLC. They were identified by mass spectrometry; the spectra were similar to those obtained by Sharkey *et al.*[10] The composition of the fraction is shown in Table 2. The major constituents are triacontan-1-ol and dotriacontan-1-ol more commonly found as consituents of plant waxes.[5]

DISCUSSION

The occurrence of C_{14} and C_{15} hydroxy-fatty acids in an angiosperm cutin has not been previously reported. However, they have been reported in some cutins of lower plants; 10-hydroxytetradec-12-enoic acid is a major constituent of cutin from *Welwitschia mirabilis* and 9-hydroxypentadecanoic and 9,15-dihydroxypentadecanoic acids minor components of

[10] A. G. SHARKEY, R. A. FRIEDEL and S. H. LANGER, *Analyt. Chem.* **29**, 770 (1957).

Araucaria imbricata cutin.[11] 7- and 8-Hydroxyhexadecane-1,16-dioic acids, also reported in cutins from several lower plants and recently in *Malus* fruit cutin,[11] are found in coffee cutin. Subsequently we have confirmed that 7- and 8-hydroxyhexadecane-1,16-dioic acids are common minor constituents of many angiosperm cutins, for example, *Avena sativa*, *Zea mays*, *Malus* leaves, *Citrus aurantifolia* and *Betula pendula*. The compounds are probably derived from the corresponding dihydroxyhexadecanoic acids by terminal oxidation.

The presence of positional isomers of hydroxy-fatty acids having single secondary alcohol groups, first reported in dihydroxyhexadecanoic acid from cutins and suberins,[9] has been confirmed in other classes of acids occurring in coffee cutin. Hydroxylation of coffee acids occurs predominantly in both 9- and 10- positions and to a much smaller extent in the 7- and 8- positions. This hydroxylation pattern also appears to hold for monohydroxyhexadecane-1,16-dioic acid although precise identification of the positional isomers by mass spectrometry is complicated by the symmetry of the molecule. The MS fragmentation of the 7- and 10-hydroxy isomers, and the 8- and 9-hydroxy isomers, is identical with the result that the MS shows the presence of only two positional isomers. The similar positional isomer contents of the various coffee cutin acids strongly suggest that they are formed by a common direct hydroxylation synthesis[12] in the plant from fatty or hydroxy-fatty acid precursors.

The presence of long-chain fatty acids and alkan-1-ols in the hydrolysis products of coffee cutin, although in small amounts, suggest that wax constituents may also be involved in the cutin polyester. The compounds are unlikely to be derived from the cuticular wax because the membranes after isolation were exhaustively extracted with solvents before hydrolysis. Polarizing microscope studies also indicate that some of the cuticular wax is chemically bound to the cutin.[13] Long chain alkan-1-ols have also been reported in cutins by Hunneman.[11]

EXPERIMENTAL

Isolation of cuticular membranes and preparation of cutin acids. Young (3rd leaf pair) and mature (6th leaf pair) leaves were collected from field grown coffee (*Coffea arabica* L. cv. SL 28) in Kenya. Cuticular membranes were isolated separately from abaxial and adaxial leaf surfaces using ammonium oxalate–oxalic acid solution,[14] pectinase[15] and $ZnCl_2$–HCl solution,[16] and exhaustively extracted with $CHCl_3$–MeOH (1:1) to remove any uncombined lipids. One hundred 1 cm^2 leaf discs yielded about 60 mg of dried cutin.

The cutin acids were obtained by alkaline hydrolysis as described earlier.[1,9] Methyl esters were prepared from CH_2N_2 using the method of Schlenk and Gellerman[17] and TMS derivatives from N,O-bis-(trimethylsilyl) acetamide[8] using anhydrous pyridine as solvent.

GLC and MS analysis. The isolated cutin acids were examined by analytical TLC and GLC as described previously.[1,9] The total methyl esters were fractionated by preparative-TLC on silica gel using the solvent system $CHCl_3$–EtOAc (8:2). Five major fractions were obtained corresponding with, (i) monobasic acids, (ii) monohydroxymonobasic acids and alkan-1-ols, (iii) ω-hydroxymonobasic and monohydroxydibasic acids, (iv) dihydroxymonobasic acids, and (v) trihydroxymonobasic acids. GLC–MS analyses were carried out using OV-1 and OV-225 columns under the operating conditions previously described.[9]

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[11] D. H. Hunneman, Ph.D. Thesis, University of Bristol (1970).
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[13] P. A. Roelofsen and A. L. Houwink, *Protoplasma* **40**, 1 (1951).
[14] F. E. Huelin and R. A. Gallop, *Austral. J. Sci. Res.* **B4**, 526 (1951).
[15] W. H. Orgell, *Plant Physiol.* **30**, 78 (1955).
[16] P. J. Holloway and E. A. Baker, *Plant Physiol.* **43**, 1878 (1968).
[17] H. Schlenk and J. L. Gellerman, *Analyt. Chem.* **32**, 1412 (1960).

Key Word Index—Coffea arabica; Rubiaceae; leaf cuticle; cutin isomerism of hydroxy-fatty acids.