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CHANGES IN THE COMPOSITION OF COFFEE LEAF WAX WITH DEVELOPMENT

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Key Word Index—*Coffea arabica*; Rubiaceae; coffee leaf wax; chemical composition; developmental changes.

Abstract—Coffee leaf wax contains alkanes, free primary alcohols and free acids, together with unidentified substances. The relative amounts of each fraction varied with age: alkanes from 22–35%, alcohols from 28–25%, and free acids from 22–14%. The major homologues of the alkane fraction were C_{29} and C_{31}, of the alcohol fraction C_{30} and C_{32} and of the acid fraction C_{28}, C_{30} and C_{32}. The ratio of both C_{29}:C_{31} alkanes and C_{30}:C_{32} alcohols changed from 1:1 to 1:2 during development, although their combined sum in each case remained constant at 90% (for alkanes) and 73% (for alcohols) of the weight of the respective fractions.

INTRODUCTION

It has long been known that the yield of coffee plantations diminishes if the same soil is cultivated over a long period, and this phenomenon may be caused by the accumulation of lipids in the soil [1, 2]. Two possible sources of these lipids are the cutin and the waxes of coffee leaves, but until now only the cutin has been studied in detail [3]. This paper describes results obtained from analyses of coffee leaf wax of different ages.

RESULTS AND DISCUSSION

The waxes were extracted from the oldest leaves of coffee plants having 1, 2 and 5 pairs of leaves, respectively, and then fractionated (Table 1). TLC separation gave three fractions (alkanes, primary alcohols and acids), which together made up *ca* 70% of the total waxes, irrespective of the leaf age. Coffee leaf wax thus resembles several other angiosperm waxes, for example, *Euphorbia cerifera* [4] and Sultana grape [5]. However, the proportions of the different fractions changed during leaf development; the relative amounts of alkanes increased, the alcohols changed little, while the acids decreased. Similar changes occur in other leaf waxes at different stages of maturity [6, 7].

The different fractions were analysed by GLC and the relative amount of each fraction was determined (Table 2). The main homologues of the alkane fraction were C_{29} and C_{31}. The relative amount of these two compounds changed from 1:1 in the 66-day-old leaves to 3:5 and 1:2 in the leaves at 81 and 112 days, respectively, although their combined sum remained constant at 90% of the total alkane fraction. The main homologues of the alcohol fraction were C_{30} and C_{32}. Like the alkane fraction, the ratio of

Table 1. Composition (%)* of leaf waxes of *Coffea arabica* on leaf pair no. 1†

Fraction	Development stage of plant indicated by number of leaf pairs and age of oldest leaf pair (as days after germination)		
	1(66)	2(81)	5(112)
Alkanes	22	31	35
Free alcohols	28	27	25
Free acids	22	17	14
Unidentified‡	28	25	26

* The wax content, determined by weighing the residue after $CHCl_3$ evaporation, amounting to about 0·4% of leaf dry wt TLC fractionation.

† Leaf no. 1 is the oldest leaf pair of the plant. Its age is characterized by the number of days from germination and the number of the leaf pairs of the plant.

‡ The main component of this fraction has been identified as ursolic acid (Ph.D. thesis by A. M. Kabaara, Univ. of Bristol, 1973; personal communication by Dr. P. J. Holloway, Long Ashton).

Table 2. Composition (%)* of the fractions of coffee leaf waxes from leaf pair no. 1

Chain length	Alkanes 1†	Alkanes 2	Alkanes 5	Primary alcohols 1	Primary alcohols 2	Primary alcohols 5	Acids 1	Acids 2	Acids 5
16							5·8	4·0	0·3
18							7·8	4·5	0·2
20							1·3	0·4	0·2
22							6·7	0·5	0·9
24							1·6	0·4	0·6
25							0·2	0·3	0·3
26				1·6	2·4	0·6	2·3	1·7	2·2
27	2·0	0·8	0·5	0·2	0·3	0·3	0·4	0·9	2·1
28	1·4	0·5	0·5	9·2	4·8	3·3	23·4	19·2	19·4
29	45·3	35·1	30·2	1·3	0·7	0·3	2·2	2·1	0·4
30	2·4	3·2	2·6	35·4	30·3	25·1	19·8	19·1	27·1
31	42·7	54·6	58·7	1·8	2·3	2·3	1·3	1·7	2·4
32	0·4	1·0	1·6	37·6	43·6	45·5	10·8	30·7	21·4
33	3·3	3·9	4·3	0·7	2·2	3·8	0·1	1·0	1·8
Unidentified	2·5	0·6	1·6	1·2	2·4	2·2	13·0	8·4	12·0

* Relative peak areas determined from GLC.
† For characterization of the three stages see Table 1.

C_{30}:C_{32} alcohols changed from 1:1 to 3:4 and 1:2 with increasing age, always accounting for 73% of the total alcohols. The major acids were C_{28}, C_{30} and C_{32} but the ratio of the C_{30}:C_{32} acids was not constant during development.

From a biosynthetic point of view the occurrence of C_{30} and C_{32} components in the alcohol and acid fractions, and the C_{29} and C_{31} components in the alkane fraction, is of considerable interest. The relationship between alcohols and alkanes is especially striking. The ratio of C_{30}:C_{32} alcohols and C_{29}:C_{31} alkanes both changed in exactly the same manner during development, resulting in identical GLC profiles at all three stages examined. C_{30} and C_{32} acids are also present, but not in a corresponding ratio. The alkanes may be regarded as decarboxylation products of their $n + 1$ acids, while the alcohols could be direct reduction products of the acids with equal chain length [8, 9]. Octacosanic acid, another main component of the acid fraction, cannot be integrated in this biosynthetic hypothesis, because the C_{27} alkane and C_{28} alcohol are not present in sufficient amounts.

EXPERIMENTAL

Coffee plants (*Coffea arabica* L. cv Bourbon) were cultivated in the greenhouse from January to May 1974. As soon as primordial leaves appeared, they were picked from a number of the seedlings and waxes were extracted with $CHCl_3$ [10] (waxes of stage 1). After the 2nd leaf pair appeared on the remaining plants, older leaf pairs were picked from other seedlings and waxes again extracted (waxes of stage 2). The same procedure was applied to obtain waxes of the 5th leaf pair (waxes of stage 5).

Separation of the components. Waxes were fractionated by preparative-TLC on 0·25 mm layers of Si gel-G with $CHCl_3$ as the developing solvent. The spray reagent was 0·1% Rhodamine 6G in EtOH [11] followed by examination in UV light. To obtain pure monobasic acids, the methylated acidic fraction was separated using a second development [12] (Et_2O–hexane–MeOH 40:10:1). TLC and GLC fractions were identified by co-chromatography with authentic compounds. GLC analyses were carried out on a FID instrument using N_2 as carrier gas. The columns contained 1% SE 52 on Chromosorb G and the temp was programmed from 120–280° at 7°/min. Alkanes and alcohols were dissolved in petrol and directly injected. The fatty acids were methylated with CH_2N_2 and chromatographed as their Me esters.

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