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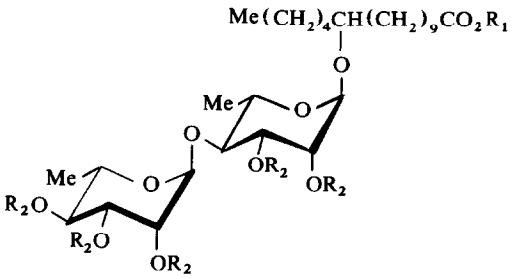
7 $+Me(CH_2)_4CHOH(CH_2)_9CO_2Me \rightarrow$

8



	R_1	R_2
9	Me	Ac
10	H	H

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EPICUTICULAR WAXES OF TWO SORGHUM VARIETIES

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Abstract—The epicuticular waxes of the two sorghum varieties Alliance A and SD 102 have been analyzed, after separation of the leaf blades from the sheaths. The major constituents were found to be free fatty acids but small amounts of esters, aldehydes, alcohols, *n*-alkanes and sterols were also detected. The typical chain lengths of aldehydes, free alcohols and free fatty acids were C_{28} and C_{30}.

INTRODUCTION

In a previous communication [1] we reported preliminary results on the composition of the leaf waxes of sorghum varieties, Alliance A and SD 102. The investigation has now been completed, analyzing separately the waxes from the leaf blades and the leaf sheaths.

RESULTS AND DISCUSSION

In sorghum, wax is concentrated mainly on the leaf sheath and the lower part of the leaf blade. Surface wax, a white soft material, wears off readily by the brushing of the leaf sheaths against neighbouring plants so that on older leaves the sheaths appear less wax covered and at the tasseling stage the most glaucous part of the plant is leaf sheath. As a practical and reliable test for detecting the wax on the different part of the plant, we made use of the property that waxy leaf surfaces reflect light strongly when dipped in water [2].

As can be seen in Table 1, there was a marked similarity in the amount of wax per plant of the two sorghum lines and, in contrast to what was apparent to the naked eye, the blades yielded a larger amount of wax than the sheaths, although the surface area of the two parts were not taken into account. A comparison was also made of the composition of the wax obtained from the blades and the sheaths of the two varieties. Free fatty acids were found as the major wax components of the leaf sheaths of both varieties and of the blades of SD 102 sorghum (Table 1). However, in the case of Alliance A leaf blades, esters constituted the major constituents, comprising 44.2% of the wax and fatty acids only 23.3%. Amounts of *n*-alkanes and free alcohols were relatively small in all wax fractions. The data in Table 1 also indicate that there is an inverse relationship between the amounts of esters and free fatty acids; thus the percentage of the latter class of compounds is low when that of esters is high.

Table 2 shows the chain length distribution in the wax components. *n*-Alkanes have the usual composition with major C_{27}, C_{29} and C_{31} homologues. The acids and alcohols obtained by acid methanolysis of esters, have a wide chain length range without any long chain major components. The alcohol composition is unusual in this respect since wax esters from other normal cereal lines generally give alcohols with major homologue, usually C_{26} or C_{28} for Gramineae [3, 4], C_{32} for maize [5]. The mass spectra of the esters showed that the major homologues were in the range C_{40}–C_{50}.

The free alcohols of sorghum are also unusual in that there are two major components, the dominant C_{28} and substantial amounts of C_{30}. The alcoholic fraction of the wax from the leaf sheaths of SD 102 contains in addition to the alcohols 37.5% of sterols. The sterol fraction was comprised of at least three components, each characterized by the three ions 426 M^+, 411 $(M\text{-}Me)^+$ and 393 $(M\text{-}Me\text{—}H_2O)^+$. These spectra are similar to those obtained for various plant sterols by other workers

Table 1. Composition and yield (%) of epicuticular wax from Alliance A and SD 102 sorghum lines

Components	Alliance A				SD 102			
	%		mg/plant		%		mg/plant	
	Blades	Sheaths	Blades	Sheaths	Blades	Sheaths	Blades	Sheaths
n-Alkanes	5.6	1.1	1.5	0.2	1.4	1.2	0.4	0.2
Esters	44.2	5.7	11.8	0.9	18.9	3.6	5.6	0.5
Aldehydes	15.6	18.0	4.2	2.9	18.6	3.7	5.4	0.5
Free alcohols	11.3	6.0	3.0	1.0	6.5	1.5	1.8	0.2
Free fatty acids	23.3	69.2	6.1	11.4	54.6	90.0	16.1	13.8
Total			26.6	16.4			29.3	15.2
Total (blades and sheaths)			43.0				44.5	

[6]. The aldehydes and free fatty acids were mainly composed of the C_{28} and C_{30} chain lengths.

The presence, in almost comparable amounts, of the two chain lengths C_{28} and C_{30} in the free fatty acids, aldehydes and free alcohols is the most characteristic property of the two sorghum lines studied. In fact, in most other plant species studied, the waxes are characterized by the presence of one dominant chain length (Table 3). Thus, the free alcohols of various wheat species have a chain length of C_{28} which is also the dominant chain length of *Triticale* wax components. Waxes of oat, rye, *Agropyron intermedium* and Bonus barley all contain a C_{26} alcohol and in *Zea mays* C_{32} is the principal chain length.

The biosynthetic significance of the presence of two major chain lengths in sorghum waxes is not yet clear. However, it is pertinent to compare some of our results with the effects of mutations on epicuticular wax composition reported in the literature. Mutations have been described that both reduce the amount of wax and change the percentage and composition of the various classes of compounds on the leaves of maize [5, 7], barley [8, 9], pea [10] and cabbage [11]. In the majority of the cases studied, the homologous constitution of the individual classes of compounds present in the leaf wax has been dramatically changed by mutations which are thought to have caused biosynthetic blocks in the elongation process with the result that the waxes have more complex composition. Percentages of the homologues are spread over a wider number of chain lengths and the major constituents are often those with a smaller number of carbon atoms compared with those of normal plants waxes. On the basis of the foregoing chemical data we might consider the composition of sorghum waxes as being determined by genetic factors similar to those operating in some plant mutations. Thus, instead of single major components typical of a normal plant, sorghum plants synthesize classes of compound with the two dominant chains C_{28} and C_{30}.

EXPERIMENTAL

Sorghum genotypes and growth of plants. The lines of sorghum studied, Alliance A and SD 102 were grown in field conditions (1975–76) near Rome. The plants were collected at tasseling stage in July 1976.

Wax collection and analysis. Wax was collected separately from the leaf blades and the corresponding leaf sheaths by immersion in $CHCl_3$ for *ca* 1 min. The waxes were fractionated by column chromatography by gradient elution on Si gel H (Merck) using hexane to elute the *n*-alkanes, CCl_4 for first esters and then aldehydes, $CHCl_3$ for alcohols and finally $CHCl_3$ containing 1% HOAc afforded the free fatty acids. The

Table 2. Composition* of fractions from Alliance A (A) and SD 102 (S) sorghum varieties

Carbon number	*n*-Alkanes				Aldehydes				Free alcohols				Free fatty acids†				Esterified fatty acids				Esterified alcohols			
	Blades		Sheaths		Blades		Sheaths		Blades		Sheaths		Blades		Sheaths		Blades		Sheaths		Blades		Sheaths	
	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)‡	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)	(A)	(S)
16																	8 1	14 3	10 7	17 3	9 0	3 9	2 7	5 7
17																								
18																	tr.		2 8	7 7	3 8	4 7	15 5	7 4	3 5	7 5
19	tr	tr	4 3	3 2																				
20	tr	tr	3 3	2 8													tr.	tr	20.0	36 4	34 5	35 0	tr	2 7	9 3	2 5
21	tr	tr	3 5	2 6																				
22	tr	tr	10 6	3 7						tr		tr	2 4	tr.	tr	tr	28 1	23 9	21 0	22 0	28 7	25 0	30 9	26 6
23	0 5	tr	3 5	7 4																				
24	0.3	tr	4 7	4 8						tr		3 4	5 2	3 2	tr	tr	33 7	17.7	22 0	21 0	21 2	13.1	20 6	26 2
25	2 2	4 3	9 5	7.4																				
26	1 8	3 1	6 6	6 4	3 1		3 5	3 4	4 4	tr.		5.1	3 4	2 6	tr	tr	3.6	tr	3 1	tr	12 8	8 8	17 4	17 9
27	16 6	14 8	15 6	17 5	tr		tr		tr		tr													
28	5 1	5 5	5 0	6 4	32 1	31 9	55 7	45 4	73 0	59 0	91 5	76.0	49 1	54 0	65.2	63 3	3.7	tr	3 7	tr	12 8	35 2	14 3	13 6
29	48 3	43 8	27 7	26 5	tr		tr		tr		tr													
30	0 4	3 8	tr	4 2	55 5	68 1	39 4	46 0	22 6	36 1	8 5	15.5	36 1	40 2	34 8	36 7	tr	tr	1 2			3 9	1 3	
31	18.8	17 3	5 7	7 1	tr				tr															
32	0 2	tr		tr	9 3			5 2	tr	4 9			3 8									tr		
33	5 7	7 4		tr																				

* In weight percentage determined by GLC † Determined as methyl esters ‡Alcohol fraction contains 37 5% of sterols

Table 3. Major chain length components of aldehydes, free alcohols and free fatty acids found in cereals and wheatgrasses waxes: a survey*

Plant	Aldehydes	Free alcohols	Free fatty acids
Zea mays [5]	32	32	—
(WF9, normal)	(99)	(99)	
Sorghum vulgare [this work]	28, 30	28, 30	28, 30
(var. Alliance A)	(44) (47)	(82) (16)	(57) (35)
(var. SD 102)	(37) (57)	(67) (26)	(58) (38)
Triticum compactum [12]	—	28	16, 22, 24
(var. Little Club)		(94)	(35) (21)† (15)†
Triticum aestivum [14]	—	28	22, 24, 28
(var. Selkirk)		(91)	(29)† (28)† (15)
Triticum aestivum [3]	28	28	—
(var. Demar 4)‡	(100)	(99)	
Triticum durum [13]	—	28	22, 24, 26, 28, 30
(var. Stewart 63)		(78)	(10) (11) (14) (26) (11)
Hordeum vulgare [8]	26, 28	26	22, 24, 26, 28, 30, 32
(var. Bonus)	(49) (36)	(87)	(14) (12) (17) (16) (17) (12)
Avena sativa [15]	—	26	26, 28
(var. Kelsey)		(96)	(39) (11)
Secale cereale [4]	—	26	16, 24, 26, 28
(var. Prolific exaploide)		(91)	(14) (17) (24) (13)
Triticale [4]	—	26, 28	16, 26, 28, 30
(strain 6A190)		(11) (80)	(15) (15) (28) (14)
Agropyron intermedium§ [16]	—	26	—
		(91)	
Agropyron Smithii [16]	—	—	24, 28, 30
			(15) (10) (27)

* Rounded percentages in brackets.
† Includes unsaturated compounds.
‡ Data refer to wax collected at 30 days after germination.
§ Data refer to wax collected from leaves and stems.

fractions obtained were analyzed by TLC, GLC and MS. Combined acids and alcohols of esters were determined after acid methanolysis. The free acids were analyzed as their methyl esters.

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