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EPICUTICULAR WAXES OF *PANICUM MILIACEUM*, *PANICUM TEXANUM* AND *SETARIA ITALICA**

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Key Word Index—*Panicum miliaceum*; *P. texanum* ; *Setaria italica*; Gramineae; millets; epicuticular wax composition; triterpene alcohols and esters; triacylglycerols; dotriacontanol.

Abstract—Leaf waxes from *Panicum miliaceum*, *P. texanum* and *Setaria italica* have been analysed; the principal components are hydrocarbons, esters, aldehydes and alcohols. The esters are composed partly of esters of triterpene alcohols. The major free alcohol is dotriacontanol. Free triterpene alcohols are also present, particularly in wax from *P. miliaceum*. A mixture of unusual triacylglycerols, with 1,3-ditetradecanoyl-2-hexanoylglycerol as principal component, is a minor component (5%) of wax from *P. texanum*.

INTRODUCTION

During investigations of waxes to compare the range of compositions in the Gramineae at the subfamily, tribal and generic levels, waxes from more than 120 species in the tribe Triticeae [1], from a smaller number of other festucoid species and from some panicoid and eragrostoid species have been examined [2–4]. Analyses of waxes from *Zea mays* [5], sugar cane [6], sorghum [7], two *Andropogon* species [8], *Echinochloa crusgalli* [9] and from *Panicum virgatum* [10], have shown that the free alcohol compositions differ appreciably from those of festucoid species [3, 4]. Thus alcohols are frequently major components (40%) of waxes from festucoid species and consist largely of a single component, usually either hexacosanol or octacosanol. On the other hand, alcohols are less prominent in waxes from panicoid grasses and often consist of a range of alcohols, tetracosanol to dotriacontanol, or else dotriacontanol is the major alcohol. *Panicum* is the largest grass genus with *ca* 600 species [11]; the only species previously examined is *P. virgatum* [10] but wax from this species contains more than 70% of β-diketones and consequently has a low alcohol content. It was useful, therefore, to investigate other species in this genus which did not contain β-diketones. In this study waxes from *P. miliaceum* L. (proso millet) and *P. texanum* Buckl. (Texas millet) have been examined. Both species are grown in the southern United States for forage and for seed and both have been placed in the subgenus *Eupanicum* [12]. *Setaria* is another large genus (125 species [11]) in the tribe Paniceae, and apart from partial analysis of *S. viridis* [4], no species have been studied. Wax from *S. italica* (L.) Beauv. (foxtail millet), which is also cultivated for seed and forage, has now been analysed.

RESULTS AND DISCUSSION

Wax yields and compositions of the three species are shown in Table 1. Yields were similar to those from non-β-diketone-containing festucoid grasses (usually 0.2–0.4% [3, 4]) and much better than the very low yield from *E. crusgalli* [9]. There was no single large group of components comparable to the major alcohol or β-diketone found in many other species [1, 3, 4]. Hydrocarbons, esters, aldehydes and free alcohols were the major groups in each wax.

Hentriacontane was the major hydrocarbon of the two *Panicum* waxes and nonacosane of the *Setaria* wax (Table 2); these are the two most common wax hydrocarbons [2]. A major part of the esters from *P. miliaceum* and *S. italica* and a minor part from *P. texanum* consisted of esters of triterpene alcohols (Table 1), the values being derived from the proportions of triterpenes in the alcohols isolated from methanolysed esters. Triterpene esters were also found in the β-diketone-containing wax from *P. virgatum* [10]. Ester composition could not be determined by GC due to decomposition and lack of resolution of the longer chain (C_{52}–C_{62}) components, which were presumably the triterpene esters. GC/MS analysis indicated that the shorter chain esters (C_{40}–C_{50}) were those of the normal alkanols.

The acids of the esters were quite similar for the three species with C_{20}–C_{24} as principal components and the alkanols were also fairly similar ranging from C_{24} to C_{32} with dotriacontanol the largest single component for the two *Panicum* species (Table 3).

The triterpene alcohols from the esters were identified by GC/MS, α- and β-amyrins being prominent components in the two *Panicum* species and the only component of the *Setaria* species (Table 4). In *P. miliaceum* germanicol was also a major component whereas isoarborinol was prominent in *P. texanum*; arborinol and ψ-taraxasterol were also present in this species. Small amounts of free triterpene alcohols were also found, particularly in wax of *P. miliaceum*, the principal components being the same as those of the esterified triterpenes but β-glutinol being additionally present in *P. miliaceum* and simiarenol in *P. texanum*. These two components have very similar mass spectra but ion *m/z* 231 is present only in the

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Table 1. Composition and yield of epicuticular waxes from *Panicum miliaceum*, *P. texanum* and *Setaria italica**

Components	*P. miliaceum*	*P. texanum*	*S. italica*
Hydrocarbons	28	13	13
Esters	23†	32†	29§
Aldehydes	6	11	19
Free acids	1	1	7
Free alcohols	18	20	16
Triterpene alcohols	8	2	2
Triacylglycerols	—	5	—
Unidentified fractions			
Eluted before alcohols	4	5	5
Eluted after alcohols	12	11	9
Yield (% dry weight)	0.33	0.35	0.26

*Determined from weights of components by silicic acid chromatography.
†Contains esters of triterpene alcohols (72%).
‡Contains esters of triterpene alcohols (36%).
§Contains esters of triterpene alcohols (82%).

Table 2. Composition of hydrocarbons from waxes of *Panicum miliaceum*, *P. texanum* and *S. italica**

Carbon No.	*P. miliaceum*	*P. texanum*	*S. italica*
23	—	—	2
25	—	1	3
27	2	2	8
29	8	10	55
31	44	56	26
33	36	26	1
35	4	1	—
Unidentified†	6(5)	4(4)	5(6)

*Components present in less than (0.5%) have been omitted.
†Number of components in parentheses; includes components with emergence temperatures which suggested branched-chain structures or an even number of carbons.

Table 3. Composition of acids and alcohols obtained by hydrolysis of esters from waxes of *P. miliaceum*, *P. texanum* and *S. italica**

	P. miliaceum		*P. texanum*		*S. italica*	
Carbon No.	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
16	1	—	2	—	3	—
18	3	—	2	—	7	—
20	14	—	8	—	21	1
22	43	2	32	3	35	1
24	23	3	17	5	15	3
26	6	4	11	8	2	1
28	4	1	13	5	4	4
30	2	1	5	4	5	3
32	2	9	2	27	—	4
34	—	2	—	—	—	—
Triterpenes	—	72	—	36	—	82
Unidentified†	2(4)	6(5)	8(16)	12(18)	8(10)	1(4)

*Components present in less than 0.5% are omitted.
†Number of components in parentheses; includes components with emergence temperatures which suggested branched-chain structures or an odd number of carbons.

Table 4. Composition of free and esterified triterpene alcohols from waxes of *P. miliaceum*, *P. texanum* and *S. italica*

	P. miliaceum		*P. texanum*		*S. italica*	
Triterpenes	Esterified	Free	Esterified	Free	Esterified	Free
β-Glutinol	—	12	—	—	—	—
Germanicol	42	37	—	—	—	—
β-Amyrin	18	18	27	14	43	50
α-Amyrin	40	33	33	42	57	50
Simiarenol	—	—	—	9	—	—
Arborinol	—	—	10	—	—	—
Isoarborinol	—	—	24	35	—	—
ψ-Taraxasterol	—	—	6	—	—	—

spectrum of simiarenol, due to the much easier loss of a C_3H_7 fragment from ion *m/z* 274 [13]. The presence of β-glutinol was confirmed by comparison of the methyl singlet pattern in the ^1H NMR spectrum with that of an authentic sample. Arborinol and isoarborinol have identical mass spectra but were distinguished by the relative GC emergence temperatures. Similar relative emergence times were reported for the methyl ethers [14]. These triterpene alcohols except for ψ-taraxasterol have been reported previously in extracts of grasses [14, 15].

Wax from *P. texanum* also contained triacylglycerols (5%) which had chain lengths (total of constituent acid chain lengths) ranging from C_{32} to C_{38} with C_{34} the major component. Mass spectra of these components were the same as that of the triacylglycerols, of similar composition obtained from wax of *Eragrostis curvula* [15]. The mass spectrum of the C_{34} component was the same as that of synthetic 1,3-ditetradecanoyl-2-hexanoylglycerol (C_{34}) and the ^{13}C NMR spectrum of the natural mixture was very similar to that of the synthetic compound [16]. Thus in each of the triacylglycerols, the short C_6 acid is attached to position two of glycerol; the other acids at positions one and three are: for C_{32},C_{12} and C_{14}; for C_{34}, both C_{14}; for C_{36}, C_{14} and C_{16}; for C_{38}, both C_{16} or C_{14} and C_{18}. These are the same structures as were observed in wax from *E. curvula* [15]; incompletely characterized triacyl glycerols were found earlier in wax from a variety of *Triticum aestivum* [17]. Thus these unusual components occur in a number of plant waxes including that of the thistle, *Cirsium arvense* [16].

Compositions of the aldehydes, free acids and alcohols are compared in Table 5. These groups of components were fairly similar in the three species but C_{34} compounds were present only in the *Panicum* species. The C_{32} content of aldehydes, acids and, to a lesser extent, of the alcohols is lower in *Setaria italica*. Within each species there is a close correspondence between the chain lengths of the aldehydes and the acids, i.e. major components range from C_{24} to C_{32} (or C_{34}), which would be expected if acids were non-specifically reduced to aldehydes. Alcohols, however, have a rather different composition with C_{32} the only major component. This might be due to the presence of two separate reductases responsible for alcohol formation in plants, an acid-reductase and an aldehyde-reductase [18]; the latter might be selective, reducing C_{32} aldehyde preferentially. Another explanation, however, is that ester formation might also be selective, shorter alcohols being esterified in preference to C_{32}. Comparison of free and combined acids indicates that the shorter acids may be selectively esterified.

Both these preferences would have the effect of producing shorter chain esters (mainly C_{40}–C_{52}). Esterified alcohols have been noted previously to

Table 5. Composition of aldehydes, free acids and free alcohols from waxes of *P. miliaceum*, *P. texanum* and *S. italica**

	P. miliaceum			*P. texanum*			*S. italica*		
Carbon No.	Aldehydes	Free acids	Free alcohols	Aldehydes	Free acids	Free alcohols	Aldehydes	Free acids	Free alcohols
20	1	—	—	—	—	—	—	1	—
22	2	4	—	3	3	—	—	3	—
24	6	6	—	2	4	—	9	10	2
26	16	13	4	7	8	4	21	17	3
28	13	16	6	12	16	6	24	26	6
30	24	23	6	24	27	12	27	27	20
32	26	17	80	35	26	67	9	6	60
34	10	10	3	7	6	5	—	—	—
Unidentified†	2(4)	11(17)	1(4)	10(9)	10(7)	6(7)	10(10)	10(15)	9(7)

*,†See footnotes to Table 3.

have average chain lengths shorter than those of free alcohols [8–10], particularly in the case of *Echinochloa crusgalli* [9]. Thus free alcohols could be regarded as residual alcohols, those left over after esterification of shorter alcohols. In general the difference in chain lengths of combined and free alcohols is greater in those waxes in which the major free alcohol is dotriacontanol. In the waxes under discussion, however, the situation is complicated by the presence of triterpene alcohols which may in part take the place of the longer chain alcohols during ester formation. Possibly esters of triterpene alcohols have more desirable physical properties.

The compositions of these waxes indicate further differences between panicoid grasses and many species of festucoid grasses. As indicated earlier, waxes from festucoid grasses which do not contain β-diketones, frequently contain major amounts of free alcohols (almost always consisting of either hexacosanol or octacosanol [1–4]). The panicoid grasses discussed here contained increased amounts of hydrocarbons and esters and, in the case of *S. italica*, also of aldehydes and only moderate amounts of free alcohols (principal component dotriacontanol). These results agree with those obtained in partial analyses of some panicoid species [4].

EXPERIMENTAL

Seeds of *Panicum miliaceum* cv Panhandle were obtained from High Plains Agriculture Laboratory, Sidney, Nebraska; *P. texanum* cv Artex from R.C. Roush, U.S. Department of Agriculture, Brooksville, Florida; *Setaria italica* cv Empire from W. R. Childers, Canada Agriculture, Ottawa, Ontario, and planted outside. *Ca* 100 days after germination, entire flowering plants were extracted with hexane as previously described [8].

Waxes were fractionated by CC on Si gel, which was eluted with hexane containing increasing amounts of Et_2O. Fractions were identified by TLC and GC (Dexsil 300 column) as described previously [19]; most were also examined by GC/MS using a 30 m capillary column coated with Silicone OV 101.

Esters and aldehydes. Esters were eluted from the column along with aldehydes and were separated from them by prep. TLC in hexane–$CHCl_3$(1:1). Acid methanolysis converted esters to methyl esters and alcohols which were separated by CC [20]. The amounts of triterpene alcohols in the separated alcohols were determined by GC; identification and analysis is described below. Aldehydes were identified by GC/MS analysis of the alcohols (as TMSi ethers) obtained by $NaBH_4$ reduction [3].

Free acids, triterpene alcohols, triacylglycerols and alkanols. During chromatography of wax from *P. miliaceum*, free acids were eluted along with β-glutinol and were separated after CH_2N_2 treatment. β-Glutinol, as the acetate, had MS [70 eV, *m/z* (rel. int.)] M^+ missing, 408 $[M-60]^+$ (1), 274 (13), 259 (19), 134 (87), 95 (100); ^1H NMR ($CDCl_3$) Me singlets at 0.84, 0.94, 0.98, 1.00, 1.04, 1.06, 1.10, 1.16; authentic β-glutinol acetate had the same signals. The remaining triterpene alcohols were eluted with the alkanols and were identified by GC/MS as the TMSi ethers. Chromatography of wax from *S. italica* yielded free acids as a separate fraction; triterpene alcohols were eluted with part of the alkanols and were identified by GC/MS as above. Chromatography of wax from *P. texanum* gave free acids as a mixture with triterpene alcohols and triacylglycerols. Rechromatography after CH_2N_2 treatment gave methyl esters and a mixture of triterpenes and triacylglycerols. After acetylation, acetates of triterpenes were separated from triacylglycerols by chromatography. The triterpene acetates had GC/MS [70 eV, *m/z* (rel. int.)]: β-amyrin M^+ missing, 218 (100), 203 (85), 189 (42); α-amyrin 468 $[M^+]$ (1.5), 408 (1), 218 (39), 203(27), 189(66), 107(100); simiarenol M^+ missing, 274 (22), 259(34), 231(13), 134(71), 122(100); isoarborinol 468$[M]^+$ (2), 453 (11), 301 (26), 255 (8), 241 (30), 229 (8), 95 (100); relative emergence times were: β-amyrin, 1.000; α-amyrin, 1.019; simiarenol, 1.048; isoarborinol, 1.054; dotriacontanol acetate, 1.070. Free triterpene alcohols from *P. miliaceum* and *S. italica* and triterpene alcohols obtained from esters from all three waxes, as TMSi ethers, had GC/MS [70 eV, *m/z* (rel. int.)]; germanicol 498$[M]^+$ (1), 231 (9), 204 (48), 189 (89), 177 (50), 95 (100); arborinol 498$[M]^+$ (1), 483 (4), 393 (19), 331 (8), 255 (11), 241 (38), 229 (10), 95 (86), 73 (100); isoarborinol 498$[M]^+$ (1), 483 (4), 393 (19), 331 (8), 255 (11), 241 (38), 229 (10), 95 (86), 73 (100); ψ-taraxasterol M^+ missing 408$[M-90]^+$ (1), 203 (10), 189 (100), 121 (99), 73 (71). The patterns were very similar to those of authentic compounds. The fragments from the ethers of α-and β-amyrins were almost the same as those previously reported. Relative emergence times (as ethers) were: β-amyrin, 1.000; germanicol, 1.005; α-amyrin 1.015; arborinol, 1.034; isoarborinol, 1.047; ψ-taraxasterol, 1.054; dotriacontanol, 1.099. The triacylglycerols were analysed by GC and by GC/MS; the MS of the components were the same as those previously reported for the triacylglycerols of wax of *Eragrostis curvula* [15] and the MS of the C_{34} component was the same as that of the C_{34} triacylglycerol from wax of *Cirsium arvense* [16]. The composition was: C_{32}, 8%; C_{34}, 51%; C_{36} 32%; C_{38}, 9%; the ^{13}C NMR spectrum was very similar to that of 1,3-ditetradecanoyl-2-hexanoylglycerol [16]. Alkanols were analysed by GC as acetates and structures were confirmed by GC/MS of the TMSi ethers.

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