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EPICUTICULAR WAXES OF FOUR ERAGROSTOID GRASSES*

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Key Word Index—*Bouteloua curtipendula*; *Muhlenbergia wrightii*; *Eragrostis trichoides*; *Sporobolus airoides*; Gramineae; epicuticular wax composition; hydrocarbons; triterpenol esters; alkanols; octacosanol; dotriacontanol; aldehydes.

Abstract—The major components of *Sporobolus airoides* wax were hydrocarbons (37%, C_{27}–C_{33}), those of *Bouteloua curtipendula* and *Eragrostis trichoides* waxes esters (28% and 31%, respectively) and those of *Muhlenbergia wrightii* wax free alcohols (57%, almost entirely C_{28}). Free alcohols formed 22% of the wax from *B. curtipendula*, 19% of the wax from *E. trichoides* and 10% of the wax from *S. airoides*; the compositions ranged from C_{26} to C_{32} with C_{32} the major component. These alcohol compositions are similar to those found for other species in the subfamily Eragrostoideae. The esters contain 32–46% of acylated triterpenols, principally α- and β-amyrins. Aldehydes were present in all the waxes except for that from *S. airoides*.

INTRODUCTION

Most of the North American members of the family Gramineae have been divided among three subfamilies: Festucoideae, Panicoideae and Eragrostoideae [1]. Compositions of waxes from about 157 species in the Festucoideae [2, 3] and from about 15 species in the Panicoideae [4, 5] have been determined. Waxes from only eight members of the Eragrostoideae have been analysed: *Calamovilfa longifolia*, *Muhlenbergia cuspidata*, *Sporobolus cryptandrus* [4] and *Eragrostis curvula* [6], all in the tribe Eragrosteae, *Distichlis stricta* in the tribe Aeluropodeae and *Bouteloua gracilis*, *Spartina gracilis* [4] and *Leptochloa digitata* [7], all in the tribe *Chlorideae*. These analyses were not complete, only the principal components in the major groups of wax constituents were identified, except in the case of *E. curvula* (but this was an unusual wax with a very high β-diketone content [6]).

The earlier investigation [4] showed that free alcohol composition was, to a considerable extent, characteristic of a subfamily. Thus free alcohols of waxes from the Festucoideae were principally either C_{26} or C_{28} and from Panicoideae were mixtures of C_{26} to C_{32}. In the Eragrostoideae, however, alcohols from two genera, *Calamovilfa* and *Muhlenbergia* contained mostly C_{28} [4] but those from the four other genera [4] and from *E. curvula* [6] consisted of the range of chain lengths C_{26} to C_{32}. Results concerning wax from *L. digitata* were incomplete but a range of chain lengths up to C_{32} was indicated [7].

Since wax from *E. curvula* was the only one which had been completely analysed [6] it was useful to confirm and extend the study of waxes from the subfamily Eragrostoideae by complete analysis of wax from other species. Four species, for which seed was available, and which could be grown as perennials on the Canadian prairie were selected. These were *Bouteloua curtipendula* (sideoats grama), *Muhlenbergia wrightii* (spike muhly), *Eragrostis trichoides* (sand lovegrass) and *Sporobolus airoides* (alkali sacaton). All four are caespitose grasses, native to North America, which have been developed as forage crops.

RESULTS AND DISCUSSION

Wax contents were relatively low, ranging from 0.2 to 0.34% (Table 1). This may be related to the absence of β-diketones; waxes with β-diketones as prominent constituents are generally found in larger amounts [8, 9]. Hydrocarbon contents of *B. curtipendula* and *M. wrightii* waxes were considerably lower than those found previously in waxes of *B. gracilis* and *M. cuspidata* (both 20% [4]). *E. trichoides* wax had a higher hydrocarbon content than that of *E. curvula* and that of *S. airoides* was nearly the same as that of *S. cryptandrus* [4]. Ester contents were all appreciably higher than those previously found in the other species examined [4], in fact esters are the major components of waxes from *B. curtipendula* and *E. trichoides*.

Aldehydes (5–10%) were present in three of the waxes but absent from wax of *S. airoides*. The analytical method used in the earlier investigation [4] was not capable of estimating aldehydes. Wax from *E. trichoides* was very unusual in containing 1% of diesters, previously these components have only been found in waxes of the Triticeae [9–11]. Free acids were, as usual, minor components. Free alcohols were more prominent; in *B. curtipendula* and *S. airoides* the amounts were similar to those previously found for these species, in *M. wrightii* they were the major components (57%) (previously *M. cuspidata* contained only 15% [4]). *E. trichoides* wax had a greater free alcohol content than had *E. curvula* because the latter wax contained major amounts of β-diketones.

Nonacosane and hentriacontane, which are major constituents of hydrocarbons from many grass waxes [4],

*NRCC No. 23140.

Table 1. Composition and yield of epicuticular waxes from four eragrostoid grasses*

Components	*B. curtipendula*	*M. wrightii*	*E. trichoides*	*S. airoides*
Hydrocarbons	6	5	19	37
Esters	28	21	31	26
Aldehydes	10	5	7	—
Diesters	—	—	1	—
Free acids	8	4	3	7
Free secondary alcohols	—	—	0.5	—
Free primary alcohols	22	57	19	10
Triterpenols	2	3	3	2.5
Triacylglycerols	—	—	—	0.5
Unidentified†	24	5	16.5	13
Yield (% dry weight)	0.20	0.34	0.26	0.33

*In wt % determined by CC.
†Not a single component; includes material eluted between major fractions and lost on column.

Table 2. Composition of hydrocarbons from waxes of four eragrostoid grasses

Carbon no.	*B. curtipendula*	*M. wrightii*	*E. trichoides*	*S. airoides*
23	1	1	—	—
25	11	4	2	1
27	30	13	4	4
29	28	43	19	12
31	17	27	64	47
33	4	5	6	29
35	—	—	—	3
Unidentified*	9(7)	7(5)	5(6)	4(7)

*Number of components in parentheses; includes minor components less than 1%.

were again prominent (Table 2). The less common constituents C_{25} (11%) and C_{27} (30%) were present in the hydrocarbons of *B. curtipendula* wax and C_{33} (29%) was present in hydrocarbons of wax from *S. airoides*.

Long chain esters (Table 3) had a very wide range of chain lengths which fell into two groups. One group ranged from C_{42} to C_{50} and the other from C_{54} to C_{60}. The latter group may be triterpene esters since it is usually less prominent in esters from waxes of the Triticeae which do not contain triterpene esters [9]. Very small amounts of ethyl esters of C_{24} to C_{32} acids (totalling about 1%), were identified by their characteristic mass spectra [12], during GC/MS analysis of the ester fraction, in all the waxes except that from *S. airoides*. Methyl esters of similar chain lengths, but in about one quarter the amount, were present in all the waxes. Methyl esters of long chain acids have been found in some conifer waxes [A. P. Tulloch, unpublished work] and ethyl esters have been isolated from a liverwort [13].

The principal acids from the esters (Table 4) were in the range C_{20} to C_{24}. Triterpenols formed 32–46% of the ester alcohols and their composition is discussed below along with that of the free triterpenols. The alkanols from the esters range in chain length mainly from C_{22} to C_{28} with C_{32} also prominent in *S. airoides* and *E. trichoides*. This relatively wide range is similar to that observed previously in the combined alcohols from wax of *E. curvula* [6] and also in those of waxes from some panicoid grasses [4, 5, 8, 14, 15]. Combined alcohols from waxes of festucoid grasses, on the other hand, generally contain one major component with the same chain length as that of the major free alcohol of the wax [2–4, 9, 11].

Aldehyde composition, shown in Table 5, is usefully considered along with the compositions of free acids and alcohols (Table 6). As was noted before [5] the aldehyde composition was quite close to that of the free acids, and was mainly C_{26}–C_{32}, but free alcohols had a considerably different composition. In wax of *M. wrightii*, the major and almost the only, free alcohol was C_{28}, the same as found for free alcohols from *M. cuspidata* wax [4]. In the other three waxes the alcohols had a range of chain lengths with C_{32} prominent, though C_{28} was the major component in the alcohols of *B. curtipendula* wax. Alcohols of *B. gracilis* showed a similar composition but C_{32} was greater than C_{28} [4]. C_{32} was the principal alcohol of *E. trichoides* wax but in wax of *E. curvula* [6] the major alcohol was C_{28} and C_{32} (11%) was minor. In a similar way C_{32} (89%) was the major component in *S. airoides* alcohols but in *S. cryptandrus* alcohols, C_{28} (78%) was the principal constituent and C_{32} was only 5% [4].

About 40% of the ester fraction of these waxes consisted of esters of triterpenols, also a small amount of free triterpenols was present (Table 1); these components consist largely of α- and β-amyrins. Only wax of *S. airoides* contained an unusual component which accounted for *ca* 20% of both free and esterified triterpenols (in fact most

Table 3. Composition of esters from waxes of four eragrostoid grasses

Carbon no.	*B. curtipendula*	*M. wrightii*	*E. trichoides*	*S. airoides*
38	—	2	—	—
40	1	5	1	1
42	5	16	7	10
44	10	14	12	13
46	13	10	7	10
48	9	15	10	8
50	6	7	7	5
52	5	5	13	5
54	2	2	11	4
56	3	10	16	20
58	20	12	5	15
60	16	—	2	5
62	8	—	2	—
Unidentified*	2(15)	2(7)	7(12)	5(16)

*Number of components in parentheses; includes minor components less than 1%.

Table 4. Composition of acids and alcohols obtained by hydrolysis of esters from waxes of four eragrostoid grasses

Carbon no.	*B. curtipendula*		*M. wrightii*		*E. trichoides*		*S. airoides*	
	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
14	—	—	3	—	—	—	—	—
16	2	—	8	—	8	—	8	—
18	4	—	6	—	8	1	10	—
20	23	1	27	2	27	1	18	2
22	30	10	19	15	24	9	21	11
24	17	8	16	4	12	11	23	5
26	9	14	3	4	6	3	4	8
28	7	19	10	35	9	4	5	3
30	5	3	6	4	3	2	5	5
32	1	1	1	—	—	22	4	10
34	—	—	—	—	—	—	2	—
Triterpenols	—	42	—	32	—	46	—	45
Unidentified	2(7)	2(8)	1(4)	4(7)	3(7)	1(7)	—	11(16)

*Number of components in parentheses; includes minor components less than 1%.

Table 5. Composition of aldehydes from waxes of three eragrostoid grasses

Carbon no.	*B. curtipendula*	*M. wrightii*	*E. trichoides*
20	—	—	3
22	—	—	4
24	1	2	6
26	7	5	12
28	37	44	19
30	38	32	19
32	9	5	25
Unidentified*	8(7)	12(6)	2(5)

*Number of components in parentheses; includes minor components less than 1%.

Table 6. Composition of free acids and free alcohols from waxes of four eragrostoid grasses

Carbon no.	*B. curtipendula*		*M. wrightii*		*E. trichoides*		*S. airoides*	
	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
16	1	—	—	—	—	—	3	—
18	1	—	—	—	—	—	1	—
20	1	—	1	—	1	—	3	—
22	6	—	2	—	4	—	3	—
24	10	—	4	—	8	—	5	—
26	11	4	4	1	13	3	2	3
28	13	50	34	95	22	5	11	3
30	34	14	36	3	21	23	23	4
32	7	30	7	—	23	62	23	89
34	2	—	1	—	—	—	7	—
Unidentified*	14(7)	2(5)	11(10)	1(3)	8(12)	7(9)	19(14)	1(4)

*Number of components in parentheses; includes minor components less than 1%.

Table 7. Composition of esterified and free triterpene alcohols from waxes of four eragrostoid grasses

Triterpenes	*B. curtipendula*		*M. wrightii*		*E. trichoides*		*S. airoides*	
	Esterified	Free	Esterified	Free	Esterified	Free	Esterified	Free
β-Amyrin	35	50	40	50	25	40	20	20
α-Amyrin	60	50	60	50	75	60	50	50
Simiarenol	—	—	—	—	—	—	2	5
Unidentified*	5(1)	—	—	—	—	—	28(8)†	25(7)†

*Number of components in parentheses; includes minor components less than 1%.
†See text.

of the unidentified fraction in Table 7). During GC/MS, as the TMSi ether, it had an emergence temperature close to that of the TMSi ether of octacosanol in contrast to that of all the other triterpenol TMSi ethers previously examined which had emergence temperatures in the region of those of the esters of C_{30} and C_{32} alcohols [5, 16]. The mass spectrum of both the TMSi ether and the free triterpenol contained an ion with m/z 206 (75% of base peak), the spectrum is thus different from that of any other pentacyclic triterpenol isolated from grasses [17] which had been examined previously [5, 6, 16, 18]. Compounds with a 7,8-double bond, such as bauerenol, had not been examined before but bauerenone and related compounds gave mass spectra with a fragment m/z 205 [19]. The mass spectrum of the TMSi ether of an authentic sample of bauerenol, however, was quite different from that of the ether of the unidentified triterpenol. The compound could not be isolated in pure form but ^{1}H NMR indicated that one double bond proton was present so that it was not an isomeric triterpenol with a tetrasubstituted double bond.

The unusual diesters from *E. trichoides* gave C_9 to C_{12} diols and C_{16} to C_{32} methyl esters, with C_{22} and C_{24} (*E*)-2-alkenoic acids as prominent components, on methanolysis, showing that they were quite similar to diesters previously found in wax from wheat and rye varieties [10, 11] and from some *Agropyron* species [9]. Like wax of *E. curvula* [6], wax from *E. trichoides* also contained a very small amount of secondary alcohols. GC/MS showed that these were C_{29} and C_{31} compounds with the hydroxyl at C-2, C-9, C-10 or C-11. 2-Alkanols of this chain length are not common but have been found as esters in a conifer wax [20]. Other minor constituents of wax from *S. airoides* were triacylglycerols, mainly 1,3-ditetradecanoyl-2-hexanoylglycerol previously found in wax from *E. curvula* and *Panicum texanum* [5, 6]. Since what are assumed to be similar triacylglycerols have also been detected in waxes from wheat, these components are fairly widespread in grass waxes.

Thus the present study of four species from four genera compared with other species of the same genera [4, 6], agrees with the conclusion that in waxes from most genera of the Eragrostoideae free alcohols have a range of chain lengths (C_{26}–C_{32}). The species from the genus *Muhlenbergia* again differed in having octacosanol as almost the sole alcohol. A further difference from the Festucoideae was observed in the compositions of the combined alcohols which, analogous to the free alcohols, had a wide range of chain lengths. There were also appreciable differences from the other species in the same genus in the proportions of different classes of wax components but these are all large genera with at least 50 species [1] and considerable interspecific variation, such as was observed within genera of the Triticeae [2], is to be expected.

EXPERIMENTAL

Plant material. Seeds of *B. curtipendula* (Michx.) cv Pierre and *S. airoides* (Torr.) Torr. were obtained from U.S. Department of

Agriculture, Bismarck, North Dakota, seeds of *E. trichoides* (Nutt.) Wood cv Nebr. 27 were from K. P. Vogel, U.S. Department of Agriculture, Lincoln, Nebraska and seeds of *Muhlenbergia wrightii* Vasey cv El Vado were from W. R. Oaks, Plant Materials Center, U.S. Department of Agriculture, Los Lunas, New Mexico. Seeds were planted outside and, 18 months later, flowering plants were cut and epicuticular wax extracted immediately by 10 sec immersion in redistilled hexane.

Wax analysis. Wax was fractionated by CC on silica gel, elution was with hexane containing increasing amounts of Et_2O [11]. Fractions were examined by GC and TLC as before [21], most were also examined by GC/MS as previously described [5].

Esters and aldehydes. To detect Me and Et esters, the ester–aldehyde mixture (from CC separation) was analysed by GC/MS before separation by prep. TLC into the two classes of components. Typical MS were: [70 eV, *m/z* (rel. int.)] Me octacosanoate 438 $[M]^+$ (14), 255 (2), 199 (6), 143 (22), 87 (78), 74 (100) and Et octacosanoate 452 $[M]^+$ (13), 367 (1), 269 (1), 213 (4), 157 (20), 101 (85), 88 (100). Esters and aldehydes were then separated by prep. TLC and aldehydes identified as previously described [5]. Esters were subjected to acid methanolysis and Me esters separated from alkanols and triterpenols by CC [22]. Me esters and the alkanol–triterpenol mixture (as TMSi ethers) were analysed by GC and GC/MS. MS of the triterpene derivatives were the same as those previously reported [5] except for that of an unidentified triterpenol from *S. airoides* which had MS (as TMSi ether): 498 $[M]^+$ (5), 393 (15), 286 (6), 257 (7), 255 (5), 243 (7), 241 (9), 206 (72), 205 (32), 191 (21), 163 (38), 137 (53), 95 (59), 73 (100) (the GC RR_t of this component, compared to the TMSi ether of octacosanol, was 1.019.); as the free triterpenol the MS was: 426 $[M]^+$ (5), 411 (8), 259 (7), 257 (5), 206 (78), 205 (28), 191 (24), 163 (40), 137 (50), 95 (58), 43 (100). GC/MS of the TMSi ethers of the triterpenols obtained by $LiAlH_4$ reduction of a portion of the esters was the same as that obtained by methanolysis showing that none of the components were artefacts.

Diesters. These were isolated only from wax of *E. trichoides* (0.079 from 6.2 g wax) and were eluted, after the ester–aldehyde mixture, with hexane–Et_2O (49:1). A portion of the diesters was refluxed with methanolic HCl (5%) for 48 hr, neutralized with Ag_2CO_3 and solvent removed giving a mixture of diol and Me esters. Components were identified by GC/MS (diols converted to TMSi ethers) as previously described [9]. The composition of the diols (GC of diacetates) was: C_9, 28%; C_{10}, 32%; C_{11}, 24%; C_{12}, 16%. The composition of the Me esters was: C_{16}, 4%; C_{18}, 6%; C_{20}, 8%; C_{22}, 11%; (*E*)-2-docosenoate, 23%; C_{24}, 9%; (*E*)-2-tetracosenoate, 18%; C_{26}, 5%; C_{28}, 7%; C_{30}, 5%; C_{32}, 4%.

Free acids and primary and secondary alcohols. Fractions containing free acids and some alcohols were eluted with hexane–Et_2O (97:3) and fractions containing mainly alcohols with hexane–Et_2O (4:1 to 23:2). All these fractions were generally combined, treated with CH_2N_2 and separated, by CC on silica gel [5], into Me esters and alkanols which were analysed by GC and identified by GC/MS (alkanols as TMSi ethers). The acid fraction from *E. trichoides* wax was separately treated with CH_2N_2 and chromatographed; after elution of Me esters with hexane–Et_2O (49:1), elution solvents in the ratio 97:3 gave a mixture of Me esters and secondary alcohols. After trimethylsilylation, GC/MS showed that they had the composition (in order of elution): 10-OH C_{29}, 15%; 11-OH C_{29}, 9%; 2-OH C_{29}, 9%; 9-OH C_{31}, 32%; 10-OH C_{31}, 32%; 2-OH C_{31}, 18%. The 2-alkanols (TMSi ethers) had MS: 2-OH C_{29}, 481 $[M-15]^+$ (2), 117 (100), 75 (16); 2-OH C_{31}, 509 $[M-15]^+$ (2), 117 (100), 75 (15). MS of the ethers of the other secondary alcohols were the same as those reported previously [6].

Triterpenols and triacylglycerols. Triterpenols were usually isolated as mixtures with the 1-alkanols and were analysed and identified by GC and GC/MS. During CC fractionation of the Me ester–alcohol mixture from *S. airoides* wax, a fraction consisting mainly of a mixture of triterpenols and triacylglycerols was obtained. After acetylation this mixture was rechromatographed giving the same crude unidentified triterpenol (as acetate) as that obtained from wax esters (see above). This compound could not be obtained free from some 1-alkanyl acetates but ^1H NMR ($CDCl_3$, 100 MHz) showed a double bond proton multiplet at δ5.44 and a triplet at δ4.46 (width at half height, 18 Hz, H-3). Bauerenol acetate (a gift from W. J. Baas, Utrecht) had ^1H NMR: δ5.33 (1H, *m*, H-7), 4.52 (1H, *t*, J = 8 Hz, H-3). Bauerenol TMSi ether had MS: 498 $[M]^+$ (3), 393 (12), 319 (22), 255 (10), 241 (21), 229 (100), 206 (11), 205 (16), 191 (5).

GC/MS of triacylglycerols eluted after the acetates, showed that the principal component was 1,3-ditetradecanoyl-2-hexanoylglycerol; the MS were the same as those of the triacylglycerols from *Eragrostis curvula* [6] and *Panicum texanum* [5].

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REFERENCES

1. Gould, F. W. (1968) *Grass Systematics.* McGraw-Hill, New York.
2. Tulloch, A. P., Baum, B. R. and Hoffman, L. L. (1980) *Can J. Botany* **58**, 2602.
3. Tulloch, A. P. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.) p. 235. Elsevier, Amsterdam.
4. Tulloch, A. P. (1981) *Can. J. Botany* **59**, 1213.
5. Tulloch, A. P. (1982) *Phytochemistry* **21**, 2251.
6. Tulloch, A. P. (1982) *Phytochemistry* **21**, 661.
7. Kranz, Z. H., Lamberton, J. A., Murray, K. E. and Redcliffe, A. H. (1961) *Aust. J. Chem.* **14**, 264.
8. Tulloch, A. P. and Hoffman, L. L. (1979) *Phytochemistry* **18**, 267.
9. Tulloch, A. P. (1983) *Phytochemistry* **22**, 1605.
10. Tulloch, A. P. (1971) *Lipids* **6**, 641.
11. Tulloch, A. P. and Hoffman, L. L. (1974) *Phytochemistry* **13**, 2535.
12. Ryhage, R. and Stenhagen, E. (1959) *Arkiv Kemi* **14**, 483.
13. Matsuo, A., Nakayama, M., Hayashi, S. and Nagai, K. (1980) *Phytochemistry* **19**, 1848.
14. Tulloch, A. P. and Hoffman, L. L. (1980) *Phytochemistry* **19**, 837.
15. Tulloch, A. P. and Bergter, L. (1980) *Phytochemistry* **19**, 145.
16. Tulloch, A. P. and Hoffman, L. L. (1982) *Phytochemistry* **21**, 1639.
17. Ohmoto, T. and Ikuse, M. (1970) *Phytochemistry* **9**, 2137.
18. Tulloch, A. P. (1977) *Lipids* **12**, 233.
19. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
20. Tulloch, A. P. and Bergter, L. (1981) *Phytochemistry* **20**, 2711.
21. Tulloch, A. P. and Hoffman, L. (1973) *Lipids* **8**, 617.
22. Tulloch, A. P. (1974) *Lipids* **9**, 664.