Phytochemistry, 1980, Vol. 19, pp. 837–839. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0501-0837 $02.00/0

EPICUTICULAR WAX OF *PANICUM VIRGATUM**

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(*Revised received* 10 *August* 1979)

Key Word Index—*Panicum virgatum*; Gramineae; switchgrass; epicuticular wax composition; tritriacontane-12,14-dione; 5(*S*)-5-hydroxytritriacontane-12,14-dione.

Abstract—Leaf and stem wax of *Panicum virgatum* contains hydrocarbons (4%), esters (3%), free acids (2%), free alcohols (1%), triterpene alcohols (2%), β-diketones (69%) and hydroxy β-diketones (6%). Principal free alcohols range in chain length from C_{26} to C_{32}. β-Diketones consist almost entirely of tritriacontane-12,14-dione and the hydroxy β-diketone consists only of 5(*S*)-5-hydroxytritriacontane-12,14-dione. The configuration of the hydroxyl group is the same as that of hydroxy β-diketones from festucoid grasses but opposite to that of the hydroxy β-diketone from *Andropogon* species.

INTRODUCTION

Waxes of the Gramineae have been studied previously [1, 2] to compare compositions and perhaps to relate them to physiological properties such as drought resistance. Also waxes have been examined for their relationship to positions in the different tribes and subfamilies [2]. Knowledge of compositions of waxes from a number of tribes and genera could also be useful in indicating the genera or species most likely to produce commercially useful waxes. These would probably be those with highest ester content or with greatest content of longest chain components.

Most grass waxes previously examined have been obtained from festucoid grasses [1, 2] and only a few have been from panicoid grasses such as *Zea mays* [3], sorghum [4], sugar cane [5], two *Andropogon* species [6], and *Echinochloa crusgalli* [7]. Except for *E. crus-galli*, these grasses are all in the tribe Andropogoneae [8]. No species of the genus *Panicum* (from the other panicoid tribe Paniceae), which is the largest grass genus with about 600 species [8], have been examined previously.

Wax of *Panicum virgatum* L. has now been investigated. *P. virgatum*, or switchgrass, is a valuable pasture and forage grass. It occurs in most regions of the United States except the northwest and California [8] and is also found in Saskatchewan, Manitoba, Ontario and most of the eastern provinces of Canada [9].

RESULTS AND DISCUSSION

Yield of wax (*ca* 0.5%, Table 1) was similar to that previously reported for other grasses [1, 2]. Table 1 also shows wax composition, β-diketones are the major components and as has been observed before when a grass wax is largely composed of β-diketones the free alcohol content is very low [1, 2, 6, 10]. It seems that for panicoid grasses, free acid content is also relatively low since those not containing β-diketones may contain appreciable percentages of free acids [4, 5, 7]. The β-diketone content was confirmed by the high UV absorption value; wax extracted from vegetative plants at the end of the first year of growth had a similar high value (161) showing that, in this species, β-diketones are produced in both vegetative and flowering plants. Some grass species do not produce much β-diketone until the year of flowering [2].

Table 1. Composition and yield of epicuticular wax from *Panicum virgatum**

Component	%
Hydrocarbons	4
Esters	3
Free acids	2
Free alcohols	1
Triterpene alcohols	2
β-Diketones	69
Hydroxy β-Diketone	6
Unidentified	13
Yield (% dry wt)	0.45
$E^{1\%}_{1\,cm}$ at 273 nm (isooctane)	164

*In weight % determined by column chromatography.

The compositions of the fractions are listed in Table 2. A wide range of chain lengths are present in the hydrocarbons, C_{23} to C_{37}, and in the esters, C_{38} to C_{60}. Esters also contain *ca* 50% of esters of the triterpene alcohols α- and β-amyrins. It has previously been noted that waxes which contain major amounts

*NRCC number 18032.

Table 2. Composition of wax fractions from *Panicum virgatum*

No. of carbon atoms	Hydro carbons	Esters*	Hydrolysis products of esters		Free acids	Free alcohols
			Acids	Alcohols†		
16	—	—			1	—
18	—	—	3		2	—
20	—	—	20	3	38	—
22	—	—	22	17	2	—
23	3	—	—	—	—	—
24	—	—	18	15	2	3
25	7	—	—	—	—	3
26	—	—	15	18	7	29
27	14	—	—	—	—	6
28	—	—	6	4	12	7
29	27	—	—	—	—	11
30	—	—	11	2	23	6
31	26	—	—	—	—	—
32	—	—	5	1	10	30
33	3	—	—	—	—	—
34	—	—	—	—	1	3
35	2	—	—	—	—	—
37	1	—	—	—	—	—
38	—	1	—	—	—	—
40	—	1	—	—	—	—
42	—	2	—	—	—	—
44	—	11	—	—	—	—
46	—	17	—	—	—	—
48	—	9	—	—	—	—
50	—	4	—	—	—	—
52	—	1	—	—	—	—
54	—	1	—	—	—	—
56	—	1	—	—	—	—
58	—	1	—	—	—	—
60	—	1	—	—	—	—
Unidentified	17(6)	—	—	—	12(11)	2(2)

* Also contain 50% triterpene esters.
† Also contain 50% triterpene alcohols.

of β-diketones often also have appreciable quantities of triterpene esters [6, 10, 11]. Free triterpene alcohols are also present and exceed the amount of normal free alcohols; ^{1}H NMR and GC–MS (on TMSi ethers) comparisons with spectra of the authentic triterpene alcohols indicated that *ca* half of these consisted of either simiarenol [12, 13] or β-glutinol [12, 14] and the rest of α- and β-amyrins.

Free alcohols, though small in quantity, have a wide range of chain lengths with major C_{26} and C_{32} components; unusual amounts of odd carbon free alcohols (identified by GC–MS) are also present. The chain length range, and particularly the presence of dotriacontanol, is fairly similar to that observed in waxes of other panicoid grasses [3–7] and contrasts with the free alcohols of festucoid grasses which so far have always contained principally hexacosanol or octacosanol [1, 2, 10].

Combined alcohols, which are mainly C_{22} to C_{26}, consist of appreciably shorter chain components than the free alcohols. This type of difference between free and combined alcohols was also observed in waxes of sorghum [4], *Andropogon hallii* and *A. scoparius* [6], and *Echinochloa crusgalli* [7]. The compositions of free and combined alcohols from waxes of festucoid grasses, on the other hand, are generally fairly similar [1, 2, 10].

As is often observed in grass waxes [1, 2], free acids have a wide chain length range, C_{16} to C_{34}, with C_{20} and C_{30} major components. This composition is quite similar to that of the free acids from waxes of the *Andropogon* species [6].

The β-diketone fraction consists almost entirely of one component, tritriacontane-12,14-dione, and this is also the major β-diketone of waxes from *A. hallii* [6] and of *Festuca ovina* [15] and *F. glauca* [16], but wax from *A. scoparius* contains appreciable amounts of hentriacontane-10,12-dione as well.

Only one hydroxy β-diketone was found, 5-hydroxytritriacontane-12,14-dione, present to the extent of 6%. The *Andropogon* species [6], which are the only other panicoid grasses in which hydroxy β-diketones have been found, also contain a hydroxy β-diketone with the same structure. Surprisingly, however, the hydroxy β-diketone from *P. virgatum* is dextrorotatory showing that the hydroxyl group has the *S*-configuration. This is the same as the configuration of the hydroxy β-diketone, which is a very minor

constituent of wax from *F. ovina* [15], but opposite to that of the hydroxy β-diketone from wax of *Andropogon* species [6]. All other hydroxy β-diketones have been isolated from festucoid grasses and all have had the *S*-configuration [10,17–21]. Since the present species and the *Andropogon* species [6] are the only panicoid grasses from which hydroxy β-diketones have so far been isolated, no conclusions can be drawn about the taxonomic significance of this finding.

Aside from this difference, however, wax from *P. virgatum* is generally similar to waxes from other panicoid grasses. Thus the results tend to confirm the proposal that the compositions of waxes from panicoid grasses differ appreciably from those of festucoid grasses [6, 7].

EXPERIMENTAL

Panicum virgatum cv Nebraska 28 was grown outside from seed supplied by Dr. L. C. Newell, University of Nebraska, Lincoln, Nebraska. It did not flower during the first year of growth but wax was extracted as previously described [6] from the leaves in September and also from leaves and stems of flowering plants in July of the second year of growth. Wax was chromatographed on a column of Si gel and eluted with hexane containing increasing amounts of Et_2O [21]. All fractions were examined by TLC ($CHCl_3$ containing 1% EtOH) and also by GLC (Dexsil 300 column), components were identified after addition of authentic compounds [17, 21]. Triterpene alcohols and free alcohols, as TMSi ethers, were also examined by GC–MS using a 10 m × 0.25 mm SP2100 capillary column.

β-Diketones were purified as the Cu complex [16], the composition was C_{31}, 4%; C_{33}, 96%; acids obtained on alkaline hydrolysis were analysed as Me esters (GLC) and had the molar composition C_{10}, 1.5; C_{12}, 45; C_{18}, 0.5; C_{20}, 53%. After crystallization from EtOAc the mp was 64.5°. GC–MS analysis of the TMSi enol ether showed the same fragmentation pattern as that previously found for the derivative of tritriacontane-12,14-dione [6]. During column chromatography a mixture of free acids, part of the triterpene alcohols and unidentified components (0.53 g from 10 g wax) was eluted with hexane–Et_2O (19:1). After CH_2N_2 treatment, Me esters of free fatty acids (0.21 g) were separated by rechromatography (elution with hexane–Et_2O, 99:1). The crude triterpene alcohol fraction (0.18 g, elution with hexane–Et_2O, 19:1) showed ^{1}H NMR (100 MHz, CCl_4) signals at δ 3.35 (*m*, $W_{1/2}$ = 7.5 Hz, H-3) and δ 5.5 (m, H-6) and MS (70 eV, *m/e*) 274, 259, 134. The rest of the triterpene alcohols, free alcohols and unidentified components could not be completely separated but ^{1}H NMR showed a signal at δ 5.1 (*m*, H-12) in addition to signals due to long chain alcohols. During GC–MS (70 eV) analysis, the triterpene components gave fragments (*m/e*) 218, 203, 189.

Part of the hydroxy β-diketone fraction after trimethylsilylation was analysed by GC–MS (70 eV, *m/e* (rel. int.))[6, 22] and showed the presence of 5-hydroxytritriacontane-14,16-dione bis TMSi ether: 652 M^+ (0.4), 637 (10), 595 (23), 409 (85), 295 (40), 159 (91), 73 (100). After purification as the Cu complex and crystallization from EtOAc, the mp was 78.5–79.0°, mmp with hydroxy β-diketone from *F. ovina* [15] was 78.5–79.0°, mmp with hydroxy β-diketone from *A. scoparius* was 75.0–75.3°; $[\alpha]_D^{25}$ + 0.5°, $[\alpha]_{546}^{25}$ + 0.9, $[\alpha]_{436}^{25}$ + 1.5°, $[\alpha]_{365}^{25}$ + 2.4° ($CHCl_3$, *c* 3.1); (Found: C, 78.2, H, 12,7, $C_{33}H_{64}O_3$ requires: C, 77.9; H, 12.7%).

Acknowledgements—The authors thank L. R. Hogge for GC–MS and W. C. Haid for ^{1}H NMR.

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