Phytochemistry, 1979, Vol. 18, pp. 267–271. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0267 $02.00/0

EPICUTICULAR WAXES OF *ANDROPOGON HALLII* AND *A. SCOPARIUS**

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(*Received* 23 *June* 1978)

Key Word Index—*Andropogon hallii*; sand bluestem; *Andropogon scoparius*; little bluestem; Gramineae; epicuticular wax; composition: hentriacontane-10,12-dione; tritriacontane-12,14-dione; (5*R*)5-hydroxytritriacontane-12,14-dione.

Abstract—Leaf waxes of *Andropogon hallii* and *A. scoparius* contain hydrocarbons (2%, 2%), esters (4%, 2%), free acids (3%, 4%), free alcohols (1%, 0.2%, major component dotriacontanol) β-diketones (67%, 80%) and hydroxy β-diketones (16%, 5%). β-Diketones of *A. hallii* consist mainly of tritriacontane-12,14-dione and hentriacontane-12,14-dione (86:8) and of *A. scoparius* of tritriacontane-12,14-dione and hentriacontane-10,12-dione (67:29). Hydroxy β-diketones of *A. hallii* are composed mainly of 5-hydroxytritriacontane-12,14-dione and 5-hydroxyhentriacontane-12,14-dione (90:8); wax of *A. scoparius* contains only 5-hydroxytritriacontane-12,14-dione. The hydroxyl group of the major hydroxy β-diketone has the *R*-configuration opposite to that of all previously described hydroxy β-diketones.

INTRODUCTION

Compositions of epicuticular waxes from a number of grass species have been investigated previously [1, 2] to examine possible relationships between composition, function and classification, and also to determine whether grasses could be developed as convenient sources of plant waxes. Since the family Gramineae is very large it is desirable to consider members from the more widely different tribes. Earlier investigations were concerned with waxes of genera from 3 large tribes Festuceae, Triticeae and Aveneae [1, 2] which are believed to be fairly close from an evolutionary point of view [3]. Apart from analyses of wax of *Zea mays* [4], sugar cane [5, 6], and sorghum waxes [7], waxes from members of the sub family Panicoideae, tribes Paniceae and Andropogoneae, have not been studied. Waxes from genera of these tribes might be expected to show some differences from those of genera of the 3 tribes mentioned above.

To extend the investigation of waxes to a genus of the tribe Andropogoneae, waxes from two species of Andropogon, a large genus with probably more than 100 species, have now been analysed. The two species, *Andropogon hallii* Hack. (sand bluestem) and *A. scoparius* Michx. (little bluestem) are native to North America; they are major range grasses and occur over much of the area east of the Rocky Mountains.

Table 1. Composition* and yield of epicuticular wax from *Andropogon* species

Components	*A. hallii*	*A. scoparius*
Hydrocarbons	2	2
Esters	4	2
Free acids	3	4
Free alcohols	1	0.2
β-Diketones	67	80
Hydroxy β-diketones	16	5
Unidentified fractions		
Eluted between β-diketones and acids	3	4
Eluted after acids or lost on column	4	2.8
Yield (% dry wt)	1.0	0.6
$E^{1\%}_{1\,cm}$ at 273 nm (isooctane)	205	199

*In weight % determined by column chromotography.

RESULTS

The yield of wax from *A. scoparius* was similar to that obtained from other grasses [2] but that from *A. hallii* was appreciably greater (1%, Table 1), only *Festuca ovina* was previously found to be more waxy [2]. The major wax components are β-diketones (Table 1) which cause the high UV absorption at 273 nm. The amounts of hydrocarbons, long chain esters and free alcohols are very small. Hydrocarbons of *A. hallii* (Table 2) contain a large number of saturated unidentified components which are probably branched hydrocarbons. The normal hydrocarbons differ from those of the Triticeae and other grasses [1, 2] in not having major C_{29} or C_{31}

Table 2. Composition of hydrocarbons from wax of *Andropogon* species

Carbon No.	*A. hallii*	*A. scoparius*
23	3	6
25	4	17
27	11	20
29	14	26
31	13	27
33	13	4
Unidentified*	42(17)	—

*Number of components in parentheses.

*NRCC number 17107.

Table 3. Composition of long-chain esters from wax of *Andropogon* species

Carbon No.	*A. hallii*	*A. scoparius*
32	—	2
34	2	3
36	6	5
38	3	4
40	7	6
42	16	20
44	15	21
46	13	16
48	6	8
50	3	2
52	16	4
54	2	1
56	1	1
58	1	1
Unidentified*	9(12)	6(9)

*Number of components in parentheses.

hydrocarbons and the relatively large C_{25} and C_{27} components of the hydrocarbons of *A. scoparius* are also unusual.

Esters (Table 3) resemble those of other grasses [1, 2] in the wide chain length range and the major C_{42}–C_{46} components. Combined acids (Table 4) are similar to those of other grass waxes with major C_{20} to C_{24} components [1, 2] though the relatively large C_{18} component in *A. hallii* is unusual. Combined alcohols also show a wide chain length range without a major component. These alcohols usually have a single major component [1, 2] but as was observed with wax of *Agropyron smithii* [8] when there is a very high β-diketone content and a very small amount of free alcohols there may be no major component in the combined alcohols. In addition, the ester alcohols contained appreciable amounts of triterpenes as was also found for *A. smithii* [8] and to a lesser extent for *Poa ampla* [9]. Waxes of both species contain a small amount (3–4%) of free acids (Table 1) with the wide chain length range (Table 5) that is frequently observed [1, 2]. Compared to most grass waxes the amount of free alcohols is, due to the large β-diketone content, extremely small; the principal component is the C_{32} alcohol, dotriacontanol.

Table 4. Composition of acids and alcohols obtained by hydrolysis of esters from wax of *Andropogon* species

Carbon No.	*A. hallii*		*A. scoparius*	
	Acids	Alcohols	Acids	Alcohols
14	—	—	2	—
16	2	2	7	—
18	28	9	14	11
20	24	5	25	8
22	11	15	21	18
24	10	8	20	11
26	7	7	4	11
28	5	10	4	4
30	2	5	2	2
32	2	4	1	2
Triterpenes*	9(2)	35	—	33
Unidentified†	9(2)	—	—	—

*Probably mostly amyrins.
†Number of components in parentheses.

Table 5. Composition of free acids and alcohols from wax of *Andropogon* species

Carbon No.	*A. hallii*		*A. scoparius*	
	Acids	Alcohols	Acids	Alcohols
18	4	—	3	—
20	26	—	22	—
22	3	—	1	12
24	4	—	12	14
26	9	7	16	20
28	21	27	18	17
30	13	12	20	15
32	7	54	6	32
34	4	—	—	—
Unidentified*	9(4)	—	—	—

*Number of components in parentheses

β-Diketones are the major wax components and also structurally the most interesting. The composition is shown in Table 6, wax of *A. hallii* contains 10% of C_{31} components, mainly hentriacontane-12,14-dione, and 90% of C_{33} components, mostly tritriacontane-12,14-dione, but wax of *A. scoparius* contains 32% of C_{31} components, mainly the 10,12-dione and 68% of C_{33} components, consisting almost entirely of the same C_{33} 12,14-dione as is present in wax of *A. hallii*. Compositions were determined by GLC and structures by GLC analysis of acids produced by alkali hydrolysis [10, 11]; structures were also confirmed by GC–MS analysis of TMSi enol ethers. It has recently been shown that MS of the TMSi derivative of β-diketones gives a much simpler fragmentation pattern than that given by the parent compounds [12]. Fragmentation of derivatives of *Andropogon* β-diketones is shown in Fig. 1, the position of the β-diketone grouping in each compound is indicated by just two ions.

Hydroxy β-diketones are also present in the waxes, 16% in that of *A. hallii* and 5% in that of *A. scoparius*. The hydroxy β-diketone from *A. hallii* is principally 5-hydroxytritriacontane-12,14-dione but a small amount of 5-hydroxyhentriacontane-12,14-dione is also present (Table 6); the hydroxy β-diketone of *A. scoparius* consists only of the C_{33} homologue. Again structures were derived from the products of alkali hydrolysis.

Table 6. Composition of β-diketones and hydroxy β-diketones from wax of *Andropogon* species

	A. hallii	*A. scoparius*
β-Diketones		
Hentriacontane-10,12-dione	2	29
Hentriacontane-12,14-dione	8	3
Tritriacontane-12,14-dione	86	67
Tritriacontane-14,16-dione	4	1
Hydroxy β-diketones		
5-Hydroxyhentriacontane-12,14-dione	8	—
5-Hydroxytritriacontane-12,14-dione	90	100
5-Hydroxytritriacontane-14,16-dione	2	—

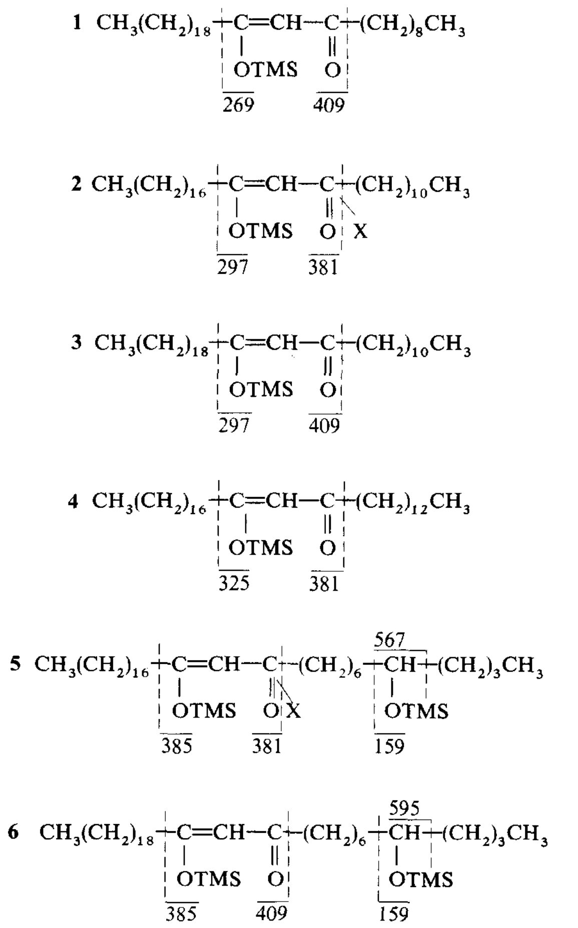

Fig. 1. MS fragmentation of TMSi ethers of β-diketones and hydroxy β-diketones from wax of *Andropogon* species. **1** hentriacontane-10,12-dione TMSi enol ether, **2** Hentriacontane-12,14-dione TMSi enol ether, **3** Tritriacontane-12,14-dione TMSi enol ether, **4** Tritriacontane-14,16-dione TMSi enol ether, **5** 5-hydroxyhentriacontane-12,14-dione di TMSi ether, **6** 5-hydroxytritriacontane-12,14-dione di TMSi ether. TMSi groups are shown attached to only one of the two possible enolic oxygens.

Those from the principal C_{33} component were henicosan-2-one and acids, identified as methyl esters, methyl icosanoate and methyl 8-hydroxy dodecanoate (structure was established by ^{13}C NMR and GC–MS); the expected hydroxy tridecanone was not characterized. Structures were confirmed by MS obtained by GC–MS of TMSi derivatives **5** and **6** (Fig. 1). 5-Hydroxytritriacontane-12,14-dione and methyl 8-hydroxydodecanoate obtained on hydrolysis both had small negative rotations showing that the hydroxyl group has the *R* configuration.

DISCUSSION

The results show that *A. hallii*, because of its relatively high wax content, might be a useful source of plant wax particularly if plants were selected for waxiness. Waxes from *Andropogon* species differ from those of other grasses [1, 2] principally in the composition of the free alcohols and of the β-diketones. Since the β-diketone content is very high and the free alcohol content correspondingly very low, the alcohol composition may not be typical of the genus. However it was found previously during an investigation of wheat waxes [13], that though alcohols of high β-diketone waxes sometimes contained components of several chain lengths, the longest chain component was octacosanol, which is typical of the genus *Triticum* [1] .Thus though dotriacontanol forms only 54 and 32% of free alcohols from waxes of *A. hallii* and *A. scoparius* respectively, it may still be a typical free alcohol for *Andropogon* species. This result is of interest since the major component of alcohols from a number of other waxes is hexacosanol or octacosanol [1, 2] but dotriacontanol is a major component of wax of *Zea mays* [4] and the genus *Andropogon* is believed to be phylogenetically closer to *Zea* than to genera of the tribe Triticeae [3]. Waxes of two varieties of *Sorghum*, also in the tribe Andropogoneae, contain C_{28} and C_{30} alcohols but these waxes were unusual in containing major C_{28} and C_{30} free fatty acids and the composition of the free alcohols appeared closely related to that of the free acids [7]. Wax of a Cuban sugar cane contained 7% free alcohols with composition C_{26} (7%), C_{28} (71%), C_{30} (10%) and C_{32} (9%) [6]; dotriacontanol is present but is not a major component.

The finding that β-diketones of waxes of *Andropogon* species consist of mixtures of two chain lengths is quite unusual for grasses since β-diketones of other grass waxes are almost entirely of one chain length [1] though waxes from dicotyledons contain mixtures of β-diketones [10, 14, 15]. In almost all grass species examined hitherto the β-diketone is hentriacontane-14, 16-dione, only in wax from *Festuca* species was tritriacontane-12,14-dione found [10, 16, 17]. Of the other β-diketones, hentriacontane-10,12-dione has been found in *Rhododendron* wax [15] and the C_{31}-12,14-dione in carnation wax [10] but the minor component tritriacontane-14,16-dione does not seem to have been reported previously. These results suggest that there are larger and possibly more useful differences between species in the genus *Andropogon* than there are between species of genera in the tribe Triticeae.

The hydroxy β-diketone of both species is 5-hydroxytritriacontane-12,14-dione but *A. hallii* wax contains a minor C_{31} component as well. There was no evidence for the presence of isomers with hydroxyl groups at other positions; in these species differences are thus in chain length and in position of the β-diketone group not in hydroxyl position. 5-Hydroxyhentriacontane-12,14-dione has not been reported before but a 5-hydroxytritriacontane-12,14-dione was found as a very minor constituent of wax of *F. ovina* [16]. The hydroxy β-diketone from *F. ovina* is dextrorotatory, like all the hydroxyhentriacontane-14,16-diones from other grass waxes [9, 11, 18–21], and presumably has the *S*-configuration. The 5-hydroxytritriacontane-12,14-dione isolated in the present investigation, however, is laevorotatory and therefore has the *R*-configuration. The formation of a racemate, with a sharp mp *ca* 4° lower than that of the hydroxy β-diketones, on mixing equal weights of the two compounds confirmed these conclusions.

Since it seems likely that hydroxy β-diketones are derived from β-diketones by enzymatic hydroxylation [18] the laevorotatory component is probably formed by replacement of a hydrogen with the *R*-configuration [22] whereas the other hydroxy β-diketones are formed by replacement of a hydrogen having the *S*-configuration. A number of hydroxy fatty acids produced by micro-organisms by hydroxylation also have the *S*-configuration [23], but most hydroxy acids from the seed oils of plants have the *R* configuration [24]. Thus the hydroxy β-ketone of *Andropogon* species has the same configuration as many of the hydroxylated lipids of plants. The fact that the hydroxy β-diketone of *Andropogon* species and hydroxy β-diketones from other grasses have opposite configurations is a further illustration of the expected difference between the Andropogoneae and the Triticeae, Festuceae and Aveneae.

EXPERIMENTAL

Grass species and wax isolation. A. hallii cv Goldstrike and *A. scoparius* cv Early Blue were grown outside from seed supplied by L. C. Newell, Department of Agronomy, University of Nebraska; the cultivars were based on collections from natural grasslands in Nebraska. Plants were cut, after flowering in the second year of growth (they did not flower the first year), the spikes were removed and wax was extracted from the leaves and stems by a 10 sec immersion in redistilled hexane.

Wax separation. Wax was chromatographed on a Si gel column using hexane containing increasing proportions of Et_2O as eluant. Fractions were examined and components identified by TLC (Si gel in $CHCl_3$–EtOH, 99:1) and GLC [17]. GLC was carried out with dual FID, the steel column was 1 m × 3 mm packed with 1.5% Dexsil 300 on Chromosorb W, and the temp. was programmed from 100° to 400° at 3°/min. GC–MS was performed using a quadrupole instrument with a data system. TMSi derivatives were prepared by treatment with a 100 fold excess of *bis*-(trimethylsilyl)-acetamide for 18 hr and dilution with CH_2Cl_2.

Hydrocarbons. The unidentified components of hydrocarbons of *A. hallii* remained unchanged after treatment with the permanganate–periodate reagent [25].

Esters. Purified esters were obtained after removal of admixed β-diketones, first as the Cu complex [26] and then as the semicarbazone [21]. After acid methanolysis, the Me esters and alcohols obtained were separated by column chromatography [27] and analysed by GLC. Alcohols were analysed as acetates and for GC–MS as TMSi ethers

β-Diketones. β-Diketones were recovered from the Cu complex, after separation from long chain esters,andanalysed by GLC: portions were hydrolysed with NaOH and acidic products separated [11] and analysed by GLC as Me esters. The molar composition of Me esters from β-diketones of *A. hallii* was C_{10}, 0.5; C_{12}, 49; C_{14}, 2; C_{18}, 4.5; C_{20}, 44%; and of Me esters from β-diketones of *A. scoparius* was C_{10}, 15; C_{12}, 35; C_{14}, 0.5; C_{18}, 2.5; C_{20}, 47%. GC–MS analysis [70 eV *m/e* (rel. int.)] of mixed β-diketones from *A. scoparius*, after trimethylsilylation, showed the TMSi enol ether of hentriacontane-10,12-dione (**1**): 536 M^+(0.5), 521 (3), 409 (32), 269 (78), 194 (15), 185 (26), 172 (18) 169 (23), 157 (20), 138 (16), 73 (100) [a minor ion at *m/e* 297 indicated the presence of the hentriacontane-12,14-dione derivative (**2**)]; and the tritriacontane-12,14-dione derivative (**3**): 564 M^+ (0.5), 549, (4), 409 (28), 297 (56), 222 (14), 185 (17), 172 (18), 169 (19), 157 (21), 138 (23), 73 (100) [minor ions at *m/e* 381 and 325 showed the presence of the tritriacontane-14,16-dione derivative (**4**)]. GC–MS of TMSi derivatives of β-diketones from wax of *A. hallii* showed that the same components were present but in different proportions. Recrystallization of the β-diketones from *A. hallii* gave tritriacontane-12,14-dione mp 63°; (Found: C, 80.1; H, 12.9. $C_{33}H_{64}O_2$ requires: C, 80.4; H, 13.1%).

Hydroxy β-diketones. After trimethylsilylation hydroxy β-diketone fractions were analysed by GC–MS [70 eV *m/e* (rel. int.)] and showed (for *A. hallii*) the presence of 5-hydroxyhentriacontane-12,14-dione *bis* TMSi ether (**5**): 624 M^+ (0.1), 609 (2), 567 (4), 385 (3), 381 (18), 295 (6), 169 (10), 159 (23), 73 (100); and of 5-hydroxytritriacontane-12,14-dione *bis* TMSi ether (**6**): 652 M^+ (0.3), 637 (3), 595 (6), 409 (23), 385 (5), 295 (10), 185 (11), 169 (14), 159 (30), 73 (100) (presence of the 5-hydroxytritriacontane-14,16-dione derivative was indicated by a small peak at *m/e* 381). The trimethylsilylated hydroxy β-diketone fraction of *A. scoparius* appeared to contain only one component with the same GC–MS peaks as **6** above. Purification of hydroxy β-diketones of *A. hallii* as the Cu complex and crystallization from EtOAc gave 5-hydroxytritriacontane-12,14-dione, mp 78.5°; $[\alpha]_D^{25}$ −0.9°, $[\alpha]_{546}^{25}$ −1.0°, $[\alpha]_{436}^{25}$ −1.9°, $[\alpha]_{365}^{25}$ −3.2° ($CHCl_3$; *c* 2.8); (Found: C, 77.9; H, 12.6. $C_{33}H_{64}O_3$ requires: C, 77.9; H, 12.7%). A mixture of equal wts of this compound and the 5-hydroxytritriacontane-12,14-dione from *Festuca ovina* [16] (mp 79.5–80° after recrystalllzation) had mp 75–75.5°. The hydroxy β-diketone was hydrolysed (NaOH) and neutral and acidic products separated. Column chromatographic separation of the acids, as Me esters [11], gave Me icosanoate (identified by GLC), mp 45° undepressed by authentic ester, and Me 8-hydroxydodecanoate. The hydroxy ester was further purified by TLC and by distillation (bp 0.1 mm, 100°,) and had $[\alpha]_D^{25}$ −1.3°, $[\alpha]_{436}^{25}$ −2.5°, $[\alpha]_{365}^{25}$ −3.6°, ($CHCl_3$; *c* 2.5); ^{13}C NMR ($CDCl_3$): 14.06 (C-12), 22.77 (C-11), 24.89 (C-3), 25.46 (C-6), 27.85 (C-10), 29.11 (C-4), 29.30 (C-5), 34.06 (C-2), 37.19 (C-9), 37.37 (C-7), 51.38 (OCH_3), 71.85 (C-8), 174.00 (C-1) in ppm from TMS, chemical shifts were assigned (and were also very close to the expected values) from shifts of the isomeric hydroxyoctadecanoates [28]: GC–MS of TMSi ether [70 eV *m/e* (rel. int.)] M^+ missing, 287 M^+-15 (1), 245 8,9 cleavage (36), 159 7,8 cleavage (100). The Me ketones were also separated by column chromatography giving henicosan-2-one, mp 61° (lit. [29] 61°) and a hydroxytridecan-2-one which was not characterized. The hydroxy β-diketone fraction from *A. scoparius* was purified and had mp 78.5–79° and mmp with the hydroxy β-diketone from *A. hallii* 78.3–78.8°; the optical rotation of the former hydroxy β-diketone was the same as that of the latter.

Acknowledgements—The authors are indebted to L.R. Hogge for GC–MS and to M. Mazurek for ^{13}C NMR.

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