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EPICUTICULAR WAX OF *ERAGROSTIS CURVULA**

ALEXANDER P. TULLOCH

National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada S7N OW9

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Abstract—Epicuticular wax of *Eragrostis curvula* contains hydrocarbons (6%), esters (13%), acids (3%), alkanols (4%), tritriacontane-12,14-dione (47%), 5(*S*)-5-hydroxytritriacontane-12,14-dione (14%) as major components. The esters consist of triterpenol esters (42%) as well as alkanol esters. The free alkanols consist principally of C_{26}–C_{32} components, resembling those of waxes from panicoid, and some other eragrostoid, grasses. Minor components are triterpenols (0.7%), triterpenones (0.5%), triacylglycerols (0.3%), secondary alkanols (0.1%) and 5-oxotritriacontane-12,14-dione (0.1%).

INTRODUCTION

The Gramineae has been divided into six subfamilies but most of the grasses in North America belong to the three subfamilies, Festucoideae, Panicoideae and Eragrostoideae [1]. Epicuticular waxes from only seven species in the Eragrostoideae have been partially analysed [2, 3] but none of these contained β-diketones. Since grass waxes contain a large number of different β-diketones [4–7], it was useful to make a complete analysis of a β-diketone-containing wax from this subfamily. Reports that some species of *Eragrostis*, the largest genus in the subfamily with 250 species [1], were glaucous [8, 9] suggested that β-diketones might be present in the wax; accordingly wax from *E. curvula*, weeping lovegrass, has now been completely analysed. This grass was introduced from Africa and has been used in much of the southern U.S.A. for erosion control and pasture [10].

RESULTS AND DISCUSSION

E. curvula had a relatively high wax content (0.8% dry wt), the composition is shown in Table 1 and the chain lengths of some of the components in Table 2. Hydrocarbons formed 6% of the wax and had a chain length range similar to that of hydrocarbons from other grass waxes. The wax contained 13% of esters and methanolysis gave alcohols consisting of triterpenols (42%) and alkanols; only the composition of the latter is shown in Table 2. Presumably due to the presence of triterpene esters, the GC response was low and an accurate analysis was not obtained.

Both combined and free acids had a wide chain length range with C_{20} the largest single component but a considerably larger proportion of C_{26}–C_{34} components was present in the free acids. Esterified and free alkanols showed similar differences, the former ranged from C_{18} to C_{28} with C_{26} the largest component but the latter ranged from C_{24} to C_{32} with C_{28} the major component. Thus, as was discussed previously for other wax esters [6, 7, 11], the shorter chain acids and alcohols have been selectively involved in esterification thus avoiding formation of very long chain esters. The triterpenols are discussed later.

Table 1. Composition and yield of epicuticular wax from *Eragrostis curvula**

Component	%
Hydrocarbons	6
Esters	13
Acids	3
Alkanols	4
Tritriacontane-12,14-dione	47
5(*S*)-5-Hydroxytritriacontane-12,14-dione	14
Triterpenols	0.7
Triterpenones	0.5
Triacylglycerols	0.3
Secondary alkanols	0.1
5-Oxotritriacontane-12,14-dione	0.1
Unidentified	11.3
$E^{1\%}_{1cm}$ at 273 nm (*iso*-octane)	114

*In wt % determined by column chromatography.

It was previously observed [2] that of six members of the subfamily Eragostoideae (none in the genus *Eragrostis*), the free alkanols of four contained a range of chain lengths from C_{26} to C_{32}, like those of the waxes from all the species in the Panicoideae so far examined, but those of the other two consisted almost entirely of octacosanol (like waxes from festucoid grasses [2, 4]). The free alkanols of *E. curvula*, which include dotriacontanol (11%), thus resemble those from the larger group of eragrostoid species which have a range of chain lengths.

The principal components of the wax are β-diketone and hydroxy β-diketone, which form 61% of the total. The β-diketone consisted of a single com-

*NRCC no. 19714.

ponent tritriacontane-12,14-dione and the hydroxy β-diketone of 5-hydroxytritriacontane-12,14-dione. These are structurally the same β-diketones as were obtained from *Andropogon* spp. [6], *Panicum virgatum* [7] (both panicoid) and from *Festuca ovina* [12] (festucoid). The hydroxy β-diketone was dextrotatory and, therefore, has the *S*-configuration, the same as that from *P. virgatum* and *F. ovina* [7, 13] but opposite to that from *Andropogon* spp. [6]. A very minor amount of 5-oxotritriacontane-12,14-dione was detected and identified by GC/MS, oxo-β-diketones are not found as frequently as hydroxy β-diketones but 5-oxohentriacontane-14,16-dione is present in wax from *Poa ampla* and they occur in a number of waxes from the tribe Triticeae [4].

Besides these major components quite small amounts of triterpene ketones and alcohols and also secondary alkanols and triacylglycerols were detected. The ketones were lupenone and probably α-amyrenone; lupenone has previously been reported in ouricuri wax [14].

The triterpenols, like those present as esters in the ester fraction, were mixtures of seven alcohols and were identified by GC/MS analysis (as TMSi ethers), using a capillary column. The compositions of free and esterified triterpenols are shown in Table 3 along

Table 2. Composition of wax fractions from *Eragrostis curvula*

No. of carbon atoms	Hydrocarbons	Hydrolysis products of esters		Free acid	Free alcohols
		Acids	Alcohols*		
14	—	1	—	—	—
16	—	10	—	2	—
18	—	7	3	2	—
20	—	36	3	23	—
22	—	15	16	8	—
23	2	—	—	—	—
24	—	5	19	6	2
25	9	—	2	—	1
26	—	3	33	16	29
27	10	—	2	—	2
28	—	3	22	20	50
29	14	—	—	—	—
30	—	—	—	7	4
31	33	—	—	—	—
32	—	—	—	9	11
33	27	—	—	—	—
34	—	—	—	2	—
Unidentified†	5(9)	20(17)	—	5(12)	1(4)

*Triterpenols were also obtained from ester cleavage (see Table 3).
†Number of components in parentheses.

Table 3. Composition and emergence temperatures of esterified and free triterpenols (as TMSi ethers) from wax of *Eragrostis curvula*

Triperpene	Relative emergence temperature*	Esterified	Free
Unidentified-1	0.988	4	2
β-Amyrin	0.994	9	4
β-Glutinol	0.995	—	3
α-Amyrin	1.008	22	12
Lupeol	1.014	57	29
Unidentified-2	1.017	—	29
α-Fernenol	1.026	2	—
Simiarenol	1.031	4	21
β-Fernenol	1.036	2	—

*Relative to emergence temp. of triacontanol TMSi ether as 1.000.

with their relative emergence temperatures. The order of emergence is generally similar to that previously reported for the methyl ethers [15] and was used to identify α- and β-fernenol (which have indistinguishable mass spectra).

The free and esterified triterpenols have similar compositions but an unidentified component was prominent in the unesterified fraction. The amyrins and lupeol were the major esterified alcohols and have previously been found as esters in grass waxes [6, 7, 16]. The other minor components have been isolated from extracts of whole grasses [15].

The secondary alkanol fraction had 21 components with chain lengths ranging from C_{25} to C_{33} and substitution on C-3 to C-11 but 9- and 10-hydroxyalkanes were the most common. The detection of this very small amount of secondary alkanols is of interest since these compounds are major constituents of waxes from other plant families, particularly Rosaceae [4], Papaveraceae, Ranunculaceae [4, 17] and a number of gymnosperm families [4]. In these other waxes, however, the secondary alcohol is a single component, such as 10-nonacosanol, not a range of components.

The triacylglycerol fraction consisted of C_{32}–C_{38} components which were shown by GC/MS to consist of triacyglycerols each (except for part of the C_{38} component) with one C_6 and one C_{14} acyl group and, for C_{32} with a C_{12} acyl group, for C_{34} with a second C_{14} acyl group, for C_{36} with a C_{16} acyl group and for C_{38} with a C_{18} acyl group (fragments with *m/z* 411 and 239 showed that triacylglycerols with two C_{16} acyl groups were also present). Triacylglycerols have very characteristic mass spectra which unambiguously indicate the acyl groups [18–20], thus these glycerides all had a base peak *m/z* 99 due to $Me(CH_2)_4\overset{+}{C}O$. Further studies of this type of triacylglycerol will be reported later. Acylated glycerols with this medium MW range are not common but were previously reported (but not fully characterized) in wax of spring wheat [21].

Thus, wax from this *Eragrostis* sp. does not contain any unusual major components; the free alkanol composition resembles that obtained by partial analysis of other members of this subfamily [2]. The presence of the β-diketone with the dicarbonyl grouping at C_{12} to C_{14} and the hydroxyl at C-5 shows that this type of β-diketone is widespread among grasses; it has now been found in representatives of all three major subfamilies. It appears that the β-diketone with 31 carbons and the dicarbonyl group at C_{14}–C_{16} may be limited to waxes of the Triticeae and some other festucoid tribes [2, 4, 22]. In waxes of the Triticeae hydroxylation is at C-25, or occasionally at C-8 and C-9. In other festucoid genera, such as *Avena*, *Poa* and *Alopecurus*, hentriacontane-14,16-dione is also present but hydroxylation is usually at C-5 [2, 5, 23].

EXPERIMENTAL

Seeds of *E. curvula* (Schrad) Nees were obtained from P. W. Voigt, Grassland, Soil and Water Laboratory, Temple, Texas, and were grown outside. The plants flowered 85 days after germination and were extracted with distilled hexane as previously described [6]. Wax was chromatographed on a column of Si gel and eluted with hexane containing increasing amounts of Et_2O [24]; fractions were examined as before [6, 7]. Fractions containing triterpenes, secondary alkanols, triacylglycerols, β-diketone and hydroxy β-diketone were also examined by GC/MS (after conversion to TMSi ethers as appropriate [25]). A 50 m × 0.3 mm column coated with OV-1 was employed, linear velocity of He was 40 cm/sec, samples were injected at 50°, the temp. immediately raised to 150°, programmed at 4°/min to 300° and held at 300° for 20 min.

Major components. Esters were isolated from the mixture with β-diketones as previously described [24] and subjected to acid methanolysis, methyl esters were then separated from triterpenols and alkanols by CC [26]. A portion of the alcohol components was acetylated and the ratio triterpenols : alkanols (21 : 29) was determined by GC analysis.

After isolation as the Cu complex the β-diketone (as TMSi ether) had an MS indistinguishable from that of tritriacontane-12,14-dione [6]; the methyl esters of the acids obtained by alkaline cleavage consisted of C_{12} and C_{20} esters only, in a molar ratio of 1 : 1. The hydroxy β-diketone was purified as the Cu complex and crystallized from EtOAc, mp 79.0–79.5°, the mmp with 5(*S*)-5-hydroxytritriacontane-12,14-dione from wax of *Panicum virgatum* [7] was not depressed; $[\alpha]_D^{25} + 0.63°$, $[\alpha]_{436}^{25} + 1.56°$, $[\alpha]_{365}^{25} + 2.59°$ ($CHCl_3$; *c* 2.7): the MS of the bis TMSi ether was the same as that of the hydroxy-β-diketone from *P. virgatum* wax [7].

Minor components. The two triterpenones were eluted separately, after the ester β-diketone mixture, with hexane–Et_2O, 99 : 1. The first and major ketone had the MS expected for α-amyrenone [27] [70 eV, *m/z* (rel. int.) 424 M^+ (18), 409 (9), 218 (100), 205 (50), 203 (37), 189 (34)]; and the second had a ^1H NMR spectrum ($CDCl_3$) and MS the same as that of lupenone [14].

The secondary alkanols were eluted with hexane–Et_2O, 24 : 1 and analysed as TMSi ethers by GC/MS [70 ev, *m/z* (rel. int.)]: ethers are listed in order of elution with the approx. percentages in parentheses after the designation of the structure; 7-hydroxy C_{25} (3) 425 $[M-15]^+$ (1), 355 (37), 187 (100); 6-hydroxy C_{25} (3) 425 (2), 369 (35), 173 (100); 5-hydroxy C_{25} (3) 425 (1), 383 (29), 159 (100); 9-hydroxy C_{27} (3) 453 $[M-15]^+$ (2), 355 (48), 215 (100); 9-, 10- and 11-hydroxy C_{29} (35) 481 $[M-15]^+$ (4), 383 (24), 369 (52), 335 (8), 243 (15), 229 (100), 215 (45); 9-, 10- and 11-hydroxy C_{31} (20) 509 $[M-15]^+$ (3), 411 (15) 397 (45), 383 (13), 243 (28), 229 (100), 215 (28); 6-hydroxy C_{31} (3) 509 (1), 453 (26), 173 (100); 5-hydroxy C_{31} (3) 509 (1), 467 (19), 159 (100); 4-hydroxy C_{31} (3) 509 (1), 481 (16), 145 (100); 3-hydroxy C_{31} (3) 509 (1), 497 (17), 131 (100); 10- and 11-hydroxy C_{32} (3) 523 $[M-15]^+$ (3), 411 (45), 397 (30), 243 (65), 229 (100); 10- and 11-hydroxy C_{33} (9) 537 $[M-15]^+$ (2), 425 (45), 411 (23), 243 (51), 229 (100); 6-hydroxy C_{33} (3) 537 (1), 481 (22), 173 (100); 5-hydroxy C_{33} (3) 495 (19), 159 (100); 4-hydroxy C_{33} (3) 509 (16), 145 (100).

Free acids, part of the triterpenols, triacylglycerols and the oxo-β-diketone were eluted together with hexane–Et_2O, 47 : 3. After CH_2N_2 treatment, methyl esters were separated by rechromatography [23] and oxo-β-diketone was separated as the crude Cu complex. The mixture of triterpenols, as TMSi ethers, and triacylglycerols was analysed by GC/MS (70 eV, *m/z* (rel. int.)); triterpene ethers from this fraction and from the esters, as listed in Table 3, had MS: unidentified-1 498 (M^+) (14), 483 $[M-15]^+$ (5), 408 $[M-90]^+$ (1), 393 (6), 293 (4), 279 (15), 257 (8), 229 (17), 218 (64), 206 (44), 205 (74), 204 (48), 203 (64), 191 (45), 190 (75), 189 (89), 109 (100); β-amyrin 498 (1), 218 (100), 203 (38), 190 (18), 189

(20); β-glutinol 498 (1), 408 (7), 393 (7), 274 (23), 259 (27), 134 (100); α-amyrin 498 (2), 218 (100), 203 (14), 190 (16), 189 (23); lupeol 498 (13), 369 (16), 218 (23), 204 (21), 203 (40), 191 (40), 190 (51), 189 (81), 73 (100); unidentified-2 498 (12), 483 (10), 408 (14), 341 (34), 205 (44), 203 (22), 195 (23), 189 (19), 81 (100); α-fernenol 498 (14), 483 (22), 393 (70), 331 (25), 255 (32), 241 (100); simiarenol 498 missing, 408 (3), 274 (14), 259 (18), 231 (6), 134 (100); β-fernenol 498 (16), 483 (31), 393 (88), 255 (32), 241 (100); MS of the named compounds were the same as those of authentic compounds, except for α-fernenol which was not available.

The composition (in parentheses) and MS of the triacylglycerols was: C_{32} (14) M^+ missing, 467 $[M-115]^+$ (3), 383 $[M-199]^+$ (13), 355 $[M-227]^+$ (18), 211 (18), 183 (21), 99 (100); C_{34} (43) 495 $[M-115]^+$ (3), 383 $[M-227]^+$ (37), 211 (36), 99 (100); C_{36} (29) 523 $[M-115]^+$ (2), 411 $[M-227]^+$ (13), 383 $[M-255]^+$ (18), 239 (15), 211 (16), 99 (100); C_{38} (14) 438 $[M-227]^+$ (3), 411 $[M-255]^+$ (22), 383 $[M-283]^+$ (8), 267 (6), 239 (20), 211 (8), 99 (100).

The oxo-β-diketone was partially purified as the Cu complex and GC/MS of the TMSi ether showed the presence of 5-oxotritriacontane-12,14-dione: 563 $[M-15]^+$ (4), 409 (36), 311 (44), 85 (47), 73 (66), 41 (100).

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