

A GLYCOSIDIC GERMACRADIENOLIDE FROM *EUPATORIUM ALTISSIMUM**

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Key Word Index—*Eupatorium altissimum*; Compositae; sesquiterpene lactone glycoside; germacradienolide glucoside; eupatorin; 5-hydroxy-3',4',6,7-tetramethoxyflavone.

Abstract—The isolation and structure determination of a glycosidic germacradienolide from *Eupatorium altissimum* are reported. Eupatorin and 5-hydroxy-3',4',6,7-tetramethoxyflavone were also found.

INTRODUCTION

The isolation of two flavones, eupatorin (1a) and 5-hydroxy-3',4',6,7-tetramethoxyflavone (1b), from an extract of *Eupatorium altissimum* which showed confirmed activity in the P-388 lymphocytic leukemia assay, has been reported [1]. As part of our continuing study [2-5] of *Eupatorium sensu stricto* which elaborates a number of sesquiterpene lactones with cytotoxic and antitumor activity we have independently examined the same species and now report isolation and structure determination of an interesting new glycosidic germacradienolide 2a in addition to 1a and 1b.

RESULTS AND DISCUSSION

The new substance, $C_{21}H_{32}O_8$, which was obtained from the more polar eluates of the usual chromatogram was soluble in water and could not be induced to crystallize. The molecular weight was deduced by chemical ionization MS; the fragment of highest m/e in the electron impact MS corresponded to $C_{15}H_{20}O_2$. That the loss of 180 mass units resulted from elimination of a hexose unit attached to a sesquiterpene lactone moiety by a glycosidic linkage was apparent from the chemical behavior (positive Molisch test, formation of a tetraacetate and a tetra-trimethylsilyl ether) and the PMR spectra (see below). Hydrolysis with Kiliani mixture permitted the identification of glucose by paper chromatography.

While the PMR spectrum of 2a in $CDCl_3$ and DMSO- d_6 did not lend itself to analysis by spin decoupling, the various signals were well separated in the PMR spectrum of the TMS derivative (2b). The sharp doublet ($J = 7$) of the anomeric proton (H-1') was located at 4.11 ppm. Irradiation at this frequency collapsed a triplet at 3.25 ppm to a doublet ($J = 7$), thus identifying it as the signal of H-2'. Irradiation at the frequency of H-2 collapsed the signal of H-1' and affected a two proton multiplet at 3.35 ppm. Irradiation at this frequency affected not only the signal of H-2', but also a broadened doublet of doublets at 3.05 ppm. Conversely irradiation at this frequency affected the two proton multiplet and converted

the AB part (H-6') of an ABX system centered at 3.65 ppm into an AB system ($J_{AB} = 12$). Consequently the two proton multiplet represented H-3' and H-4' and the signal at 3.05 ppm represented H-5'. The six carbon atoms of the glucose moiety were readily identifiable in the ^{13}C NMR spectrum of 2a as doublets at 98.8 (C-1'), 74.6 (C-2'), 80.1 (C-3'), 71.8 (C-5') and as a triplet at 63.0 ppm (C-6').

As for the aglycone part, only 14 of its 15 carbon atoms were clearly visible in the ^{13}C NMR spectrum which indicated the presence of two trisubstituted double bonds (singlets at 139.4 and 132.0 for C-4 and C-10, doublets at 130.9 and 130.4 ppm for C-9 and C-1 or the reverse), 3 methyls (quartets at 16.9, 18.2 and 25.7 ppm for C-1, C-4 and C-11) two of which were vinylic (1H signals for 2b at 1.47 and 1.52 ppm), three methylenes (triplets at 48.2, 41.8 and 39.4 ppm for C-9, C-2 and C-6) and a γ -lactone already suggested by an IR band at 1750 cm^{-1} (carbonyl carbon at 178.9, α -carbon doublet at 58.7, γ -carbon doublet at 75.0 or 77.5 ppm, with the second of these representing the point of attachment of the glucose unit). The expected C-7 doublet near 40 ppm was not discernible, possibly because of overlap. Consequently, the aglycone was a germacradienolide. The gross structure shown in formula 2a was deduced by extensive spin decoupling experiments on 2b beginning with irradiation of the C-11 methyl signal (Table 1) which permitted identification of H-11 and, in sequence, H-7

Table 1. NMR Spectrum of compound 2b*

H-1	4.81 <i>ddbr</i> (10,3)	H-13	1.40 <i>d</i> (7)†
H-2	1.88–2.28 <i>c</i>	H-14	1.52 <i>br†</i>
H-3	4.56 <i>ddbr</i> (11, 9)	H-15	1.47 <i>br†</i>
H-5	4.62 <i>ddbr</i> (12, 8)	H-1'	4.11 <i>d</i> (7)
H-6	1.88–2.28 <i>c</i>	H-2'	3.25 <i>t</i> (7)
H-7	2.36 <i>m†</i>	H-3'	3.35 <i>m</i>
H-8	3.98 <i>tbr</i> (9)	H-4'	3.35 <i>m</i>
H-9	1.88–2.28 <i>c</i>	H-5'	3.05 <i>m</i>
H-11	2.72 <i>dq</i> (10, 7)	H-6'	3.65 (12, 6)‡

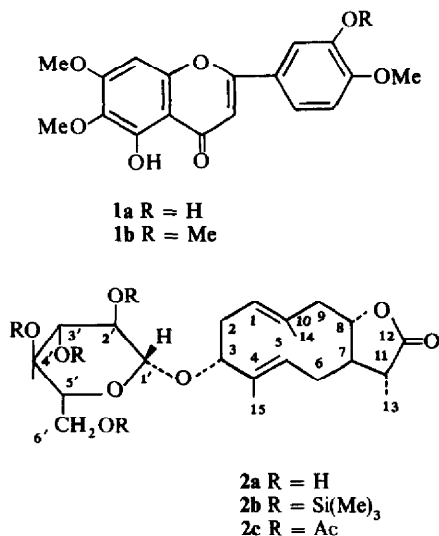
* Run in $CDCl_3$ at 270 MHz TMS as internal standard. Values in parentheses are coupling constants in hertz.

† Intensity three protons.

‡ $J_{7,8} = 9$; $J_{7,11} = 10$.

¶ Intensity two protons, AB of ABX.

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and H-8. The latter two were shown to be adjacent to methylene protons in the range 1.88–2.28 ppm, thus requiring lactone ring closure to C-8. The proton on the carbon linked to the glucose unit (broadened doublet of doublets at 4.56 ppm) was coupled only to methylene protons, hence attached to C-3 and not coupled to the vinylic protons. These in turn were coupled only to methylene protons, with H-1 also allylically coupled to H-14 and H-5 coupled allylically to H-15.

The stereochemistry was deduced in the following manner. The magnitude of $J_{1,2}$ showed that the substance was a β -glucoside. The absence of NOEs involving H-1 and H-14 on the one hand and H-5 and H-15 on the other demonstrated that the double bonds were *trans*. From this and the value of $J_{7,8}$ (9 Hz) it followed that the lactone ring is *trans*-fused, cf. laurenobiolide and dihydrolaurenobiolide [6–9], chamissarin [10]. The magnitude of $J_{7,11}$ (10 Hz) and likewise the large values of $J_{2a,3}$ and $J_{2b,3}$ (10 and 9 Hz) required that the C-11 methyl group and the glucose unit on C-3 be orientated *alpha* (models, see also chamissarin [10]) if the molecule possessed the absolute configuration (H-7 α) characteristic of all other sesquiterpene lactones of established absolute configuration. That this was so is shown by the CD curve; the sign and magnitude of the Cotton effect ($[\theta]_{213}$ 123 600 [MeOH]) corresponded to that of dihydrolaurenobiolide [10] and also indicated that the molecule adopts a conformation in which the two double bonds are 'crossed' [11].

The new substance is the first glycoside of a germanolide. Paucin [12–16] and hymenoxynin [13, 14], two other sesquiterpene lactone glycosides described earlier, are derivatives of a helenanolide and a seco-helenanolide, respectively.

EXPERIMENTAL

Above ground parts of *Eupatorium altissimum* L. (6.8 kg) collected by S. McDaniel and Michael Brooks on October 14, 1976, ca 0.5 miles SSE of Osborn, Oktibbeha Co., Mississippi (McDaniel and Brooks voucher # 20934 on deposit in herbarium of Mississippi State University), [17] was extracted with CHCl_3 and worked up in the usual manner. The dark brown gum (130 g) was pre-adsorbed on 200 g of Si gel, chromatographed over 1.2

kg Si gel (Mallinckrodt 100 mesh) packed in C_6H_6 and eluted with solvents of increasing polarity, 11 fractions were collected. Fractions 1–6 (C_6H_6), 7–10 (C_6H_6 – CHCl_3 1:1) and 11–14 (CHCl_3) were discarded. Fractions 15–17 gave a yellowish sticky mass which on trituration with Et_2O and recrystallization from EtOH afforded 2.53 g of 1b, mp 187°, reported mp 188–189 [1]. Fractions 18–19 (CHCl_3) yielded 9.4 g of a mixture of 1a and 1b, rich in 1a. Fractions 20–25 (CHCl_3) after recrystallization from C_6H_6 – CHCl_3 yielded 0.295 g of 1a, mp 192°, reported mp 191–192° [1].

Fractions 26–33 (CHCl_3 – MeOH 19:1) gave a highly coloured mixture containing little or no sesquiterpene lactones. Fractions 34–38 (CHCl_3 – MeOH 9:1) gave a gum which on TLC showed one prominent spot and a number of minor constituents. Subsequent fractions eluted with CHCl_3 – MeOH (9:1) contained nothing. The gum from fractions 34–38 was subjected 2 \times to PLC using CHCl_3 – MeOH (17:3). The predominant band of higher R_f was eluted with the same solvent mixture. Evapn of the solvents and drying *in vacuo* yielded 2a as a brittle gum (0.475 g), IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (+ OH), 1750 (γ -lactone), 1440, 1290, 1215, 1083 and 765, UV end absorption, $[\alpha]_{\text{D}}^{25} + 23.54$, CD curve (MeOH) $[\theta]_{213}^{25}$ 123 600. The elementary analysis for H and O was within permissible limits, but the C value remained low. The CI MS (isobutane) exhibited the peak of highest m/e at 413 mass units. The peak of highest m/e in the high resolution MS appeared at m/e 237 (13.3%). Calc. for $\text{C}_{15}\text{H}_{20}\text{O}_2$: MW, 232.1463. Found: MW 232.1476. Other significant peaks were at m/e (rel. int.) 217 ($\text{C}_{14}\text{H}_{17}\text{O}_2$, 10.3), 204 ($\text{C}_{14}\text{H}_{20}\text{O}$, 6.1), 189 ($\text{C}_{13}\text{H}_{17}\text{O}$, 4.4), 176 ($\text{C}_{12}\text{H}_{16}\text{O}$, 9.0), 169 ($\text{C}_8\text{H}_{13}\text{O}_3$, 23.1), 159 ($\text{C}_{12}\text{H}_{15}$, 13.3), 145 ($\text{C}_{11}\text{H}_{13}$, 23.6), 143 ($\text{C}_{11}\text{H}_{11}$, 21.0), 119 (C_7H_{11} , 38.8), 95 (C_7H_{11} , 27.2), 93 (C_7H_9 , 46.4) and 91 (C_7H_7 , 77).

The TMS derivative 2b was prepared by dissolving 0.03 g 2a in 1 ml dry Py and adding 0.5 ml each of hexamethyldisilazane and trimethylchlorosilane. The mixture was allowed to stand overnight under dry conditions and evapd *in vacuo*. The NMR spectrum is given in Table 1. The peak of highest m/e in the electron impact MS appeared at m/e 232 (loss of glucose tetramethylsilyl ether).

The acetate 2c was prepared from 0.05 g 1a and 0.5 ml Ac_2O in 2 ml Py. The mixture was allowed to stand overnight and evapd *in vacuo*. The residual gum was purified by PLC (Si gel, CHCl_3 – MeOH , 17:3). The product was a gum; IR ν_{max} cm^{-1} : 1765, 1750 (acetates and γ -lactone), 1675 (double bonds), 1450, 1385, 1230 and 1050; NMR (CDCl_3): δ 5.17 and 5.05 (m, H-2', H-3', H-4'); 4.86 (dbr, J = 10 Hz, 3-H-3), 4.54 (d, J = 7 Hz, H-1'), 4.44 (m, H-1 and H-5), 4.15 (m, H-6'), 4.12 (partially obscured, H-8), 3.59 (m, H-5'), 2.78 (m, H-9a, H-11), 2.51–2.03 (c, H-2, H-6, H-9b), 2.06, 2.00 (4 acetates), 1.61 and 1.52 (br, H-14 and H-15), 1.32 (d, J = 7 Hz, H-13). The elementary analysis for H and O was satisfactory, but the C values were again low. The electron impact MS did not show the molecular ion; the peak of highest m/e again corresponded to $\text{C}_{15}\text{H}_{20}\text{O}_2$ (loss of glucose tetraacetate).

After hydrolysis of 5 mg 2a with Kiliani reagent (HCl – HOAc – H_2O , 1:4:1) for 2 hr, the mixture was spotted on Whatman paper No. 1 together with a number of representative hexoses and developed with n -BuOH–Py– H_2O (6:4:3) for 40 hr. The paper was dried, sprayed with aniline phthalate and heated for 10 min at 110°. The spot from the hydrolysis mixture corresponded to that of glucose. The procedure was repeated \times 3 reproducible results.

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