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9α,15-DIHYDROXYGERMACRA-1(10),4-DIEN-11β,13-DIHYDRO-6α,12-OLIDE, A GERMACRANOLIDE ISOLATED FROM *CENTAUREA ASPERA* SUBSP. *STENOPHYLLA*

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Key Word Index—*Centaurea aspera* subsp. *stenophylla*; Compositae; 11,13-dihydrostenophyllolide; sesquiterpene lactone; germacranolide.

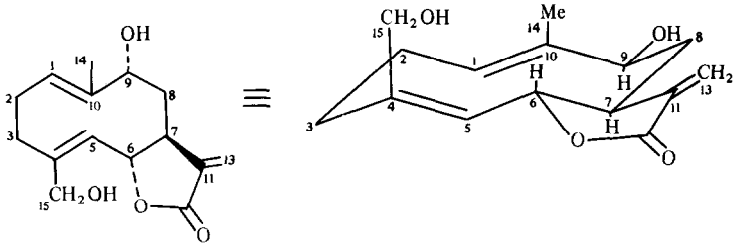
Abstract—A new germacranolide, isolated from *Centaurea aspera* subsp. *stenophylla*, was identified as 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide by spectroscopic evidence and partial synthesis.

INTRODUCTION

Centaurea aspera L. subsp. *stenophylla* (Dufour) Nyman has been examined in our laboratory and we have isolated from the hexane extract α-amyrin, β-amyrin, taraxasterol and lupeol [1] and from the alcoholic extract apigenine, 6-methoxyluteoline, ethyl-7-*O*-apigenin-glucuronate, melitensin, 11,13-dehydromelitensin and 9β,15-dihydroxygermacra-1(10),4,11-trien-6α,12-olide (stenophyllolide) [2]. The alcoholic mother liquors remaining after crystallization of stenophyllolide contained material which displayed an IR absorption of a γ-lactone (1760 cm^{-1}). One of the lactones present was converted into a diacetate and was isolated by chromatography as a crystalline compound and identified as 9β,15-dihydroxygermacra-1(10)-4,dien-11β,13-dihydro-6α,12-olide.

RESULTS AND DISCUSSION

Stenophyllolide, isolated as the major sesquiterpene lactone from *C. aspera* subsp. *stenophylla*, was shown to be the 9β,15-dihydroxygermacra-1(10),4,11-trien-6α,12-olide (**1**) by spectroscopic and X-ray analysis [3]. Stenophyllolide was crystallized from ethanol, and the mother liquors from this crystallization still contained further sesquiterpene lactones which did not crystallize. These lactones were converted into their acetates and the mixture was chromatographed on silica gel, from which hexane–ether eluted a crystalline material identified as 9,15-diacetoxy-1(10)-4-dien-11β,13-dihydro-6α,12-olide (**2b**) on the basis of the following evidence. The molecular formula determined by low- and high-resolution mass spectrometry was $C_{19}H_{26}O_6$. Of the six oxygens, two formed part of a γ-lactone ring, as indicated by an IR absorption at 1760 cm^{-1} [4], and four were acetoxyl groups, as revealed by the IR spectrum (1735 cm^{-1}), the ^1H NMR spectrum which showed two singlets at δ2.05 and 2.00 for 6H (signal of two Me groups and the mass spectral peaks at *m/z* 350 $[M]^+$, 291 $[M-59]^+$ and 232 $[M-2\times59]^+$. Double bonds are placed at $\Delta^{1(10)}$ (since the signal for H-1 at δ5.24 was sharpened by irradiation of H-14) and $\Delta^{4(5)}$ (since the signal for H-5 at δ4.79, was modified by irradiation of H-6). Acetoxyl groups were placed at C-9 (H-9, δ5.12, modified by irradiation of H-8) and C-15 (H-15, δ4.58). There were two methyl groups, one at C-11 (H-13, δ1.27, *d*, for 3H). The unusual upfield position of the last C-10 vinylic methyl may be explained by the reduced anisotropic diamagnetic deshielding of the decadiene ring at the C-10 methyl group; a similar value



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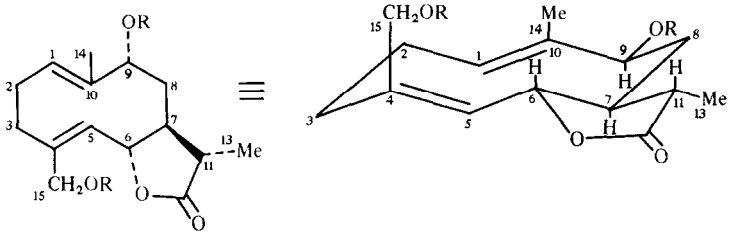

2a R = H
2b R = Ac

(1.39) has been observed for the same group of salonitolide [5]. Other points regarding the stereochemistry were determined by its partial synthesis from the stenophyllolide. The α-orientation of the C-11 methyl group was a consequence of the stereospecific nature of the sodium borohydride reduction, which introduces the hydride from the less-hindered axial position. Final identification was achieved as follows. The stenophyllolide, namely the 9β,15-dihydroxygermacra-1(10),4,11-trien-6α,12-olide (**1**), on treatment with sodium borohydride [6, 7] gave the 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide (**2a**), which with acetic anhydride and pyridine afforded 9β,15-diacetoxygermacra-1(10),4-dien-11β,13-dihydro-6α-12-olide (**2b**), identical to the compound isolated from acetylation of sesquiterpene lactones of the mother liquors from the crystallization of stenophyllolide.

EXPERIMENTAL

Isolation of 9β,15-diacetoxygermacra-1(10)-4-dien-11β,13-dihydro-6α,12-olide. From dried flowers of *C. aspera* subsp. *stenophylla* (6.6 kg) the stenophyllolide (574 mg) was isolated and crystallized from EtOH, following the method described in ref. [2]. The alcoholic mother liquor from this crystallization was removed *in vacuo* and the residue, treated with Ac_2O and pyridine, was chromatographed on silica gel, from which hexane–Et_2O (7:3) eluted a crystalline material (32 mg), mp 138–140° (hexane–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 2990–2870, 1760, (CO γ-lactone), 1735 (CO acetate), 1460, 1370, 1240 and 965 (C=C). MS 70 eV *m/z* (rel. int.): 350 $[M]^+$ (0.1), 291 $[M-59]^+$ (10.4), 232 $[M-2\times59]^+$ (2.7), 231 (13.1). Analysis of the peak *m/z* 291 $[M-59]^+$ found by HRMS: 291.1596 (required for $C_{17}H_{23}O_4$: 291.1596). Molecular formula: $MeCO_2+C_{17}H_{23}O_4 = C_{19}H_{26}O_6$. ^1H NMR (200 MHz, $CDCl_3$): δ5.24 (1H, *dd*, *J* = 10 and 5.0 Hz, H-1), 5.12 (1H, *dd*, *J* = 8 and 4 Hz, H-9), 4.79 (1H, *d*, *J* = 10 Hz, H-5), 4.58 (2H, *dd*, *J* = 10 and 18 Hz, H-15), 4.56 (1H, *t*, *J* = 10 Hz, H-6), 2.55 (2H, *m*, H-3), 2.24 (2H, *m*, H-1), 2.05 and 2.00 (6H, 2*s*, $2MeCO_2$), 1.90 (3H, *m*, H-8 and H-11), 1.80 (1H, *m*, H-7), 1.38 (3H, *s*, H-14), 1.27 (3H, *d*, *J* = 6 Hz, H-13).

Reduction of stenophyllolide by $NaBH_4$. Stenophyllolide (82 mg, 0.310 mM) dissolved in EtOH (3.0 ml) was treated at 0° with a soln of $NaBH_4$ (37 mg, 0.974 mM) in EtOH (4 ml) for 2 hr with magnetic stirring. The reaction was stopped by the addition of 30 ml of a saturated soln of NH_4Cl. The reaction product, extracted with EtOAc, washed with aq. NaCl, and dried over $MgSO_4$, was separated by prep. TLC into two bands. The band of low R_f was 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide (58 mg, 70%), mp 144–147°. Found by HRMS: 266.152; $C_{15}H_{22}O_4$ requires: 266.1518; IR ν_{max}^{KBr} cm^{-1}: 3300–3600 (OH), 1765 (CO γ-lactone), 1670 (C=C), 1210, 1025, 995, 875, 815 and 795; ^1H NMR (60 MHz, $CDCl_3$): δ5.16 (1H, *dd*, *J* = 10 and 1 Hz, H-1), 4.80 (1H, *d*, *J* = 10 Hz, H-5), 4.60 (1H, masked *t*, H-6), 4.25 (3H, *m*, H-15 and H-9), 2.70–1.80 (8H, *m*, H-2, H-3, H-7, H-8 and H-11), 1.40 (3H, *s*, H-14), 1.25 (3H, *d*, *J* = 6.5 Hz, H-13).

Acetylation of 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide. The 9β,15-dihydroxygermacra-1(10),4-dien-11β,13-dihydro-6α,12-olide (58 mg, 0.218 mM) was treated with an excess of Ac_2O (0.2 ml, 2.1 mM) and pyridine and the reaction product poured into H_2O. Recrystallization from hexane–Et_2O gave 9β,15-diacetoxygermacra-1(10)-4-dien-11β,13-dihydro-6α,12-olide (52 mg, 68%), mp 138–140°, identical to that obtained from the EtOH extract of the plant, as described above (mp, mmp, IR and ^1H NMR).

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GALEUTERONE AND PREGALEUTERONE, LABDANE DITERPENOIDS FROM *GALEOPSIS REUTERI*

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Key Word Index—*Galeopsis reuteri*; Labiatae; diterpenoids; furanic and prefuranic labdane derivatives; galeuterone; pregaleuterone.

Abstract—From the aerial part of *Galeopsis reuteri* a furanic labdane diterpenoid, galeuterone, and its prefuranic derivative, pregaleuterone, have been isolated. The structures of these substances have been established mainly by spectroscopic means.

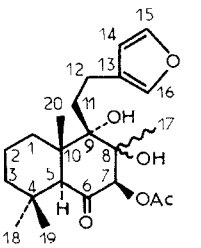
INTRODUCTION

In our search for new natural diterpenoids in the Labiatae plants belonging to the genus *Galeopsis* [1, 2], we have examined the aerial part of *G. reuteri* Reichenb. From this source two diterpenes, galeuterone (**1**) and pregaleuterone (**2**), have been isolated and their structures established mainly by spectroscopic means.

RESULTS AND DISCUSSION

Galeuterone (**1**) has a molecular formula $C_{22}H_{32}O_6$ and its IR spectrum showed hydroxyl (3570, 3510, 3460 cm^{-1}), furanic (3145, 1505, 875 cm^{-1}), acetate (1740, 1255 cm^{-1}) and ketone (1725 cm^{-1}) absorptions. The ^1H NMR spectrum of this diterpenoid showed signals in agreement with a structure such as **1**: δ7.35 (1H, *t*, $J_{15,14} = J_{15,16} = 1.8$ Hz, H-15), 7.30 (1H, *m*, $W_{1/2} = 3$ Hz, H-16), 6.30 (1H, *dd*, $J_{14,15} = 1.8$ Hz, $J_{14,16} = 0.6$ Hz, H-14), 5.31 (1H, *d*, $^4J_{7\alpha,5\alpha} = 0.8$ Hz, H-7α), 2.90 (1H, *d* (*br*), $^4J_{5\alpha,7\alpha} = 0.8$ Hz, H-5α), 2.18 (3H, *s*, OAc), 1.33 (3H, *s*, Me-17), 1.25 (3H, *s* (*br*), Me-20), 1.02 and 0.92 (3H each, *s*, Me-18 and Me-19). In addition, the ^1H NMR spectrum of **1** showed two one proton singlets at δ2.21 and 1.98 which disappeared after addition of D_2O and were assigned to the C-8 and C-9 tertiary hydroxyl groups. Double resonance experiments confirmed all the above assignments and established that the H-5α proton was coupled with the H-7 proton and the Me-20 group [3], because irradiation at δ2.90 transformed the H-7 doublet signal at δ5.31 into a singlet and caused a noticeable narrowness of the signal at δ1.25. The observed coupling between the H-5α and H-7 protons ($^4J = 0.8$ Hz) can only be explained if ring B of galeuterone adopts a boat conformation in which the Me-20–C-8β-substituent 1,3-diaxial interactions are minimized. In this conformation the pseudoequatorial C-7α proton is coupled through the C-6 carbonyl group with the C-5α pseudoaxial proton [4]. Thus establishing a C-7β configuration for the acetoxyl group of galeuterone (**1**).

A less probable alternative structure for galeuterone, with the acetoxyl group at the C-6β position and the



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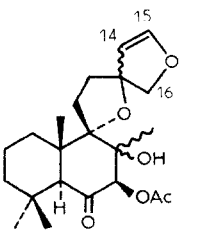

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