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ENT-2α-HYDROXY-13-EPI-MANOYL OXIDE FROM *SIDERITIS PERFOLIATA*

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Key Word Index—*Sideritis perfoliata*; Labiatae; diterpenoid; *ent*-2α-hydroxy-13-epi-manoyl oxide.

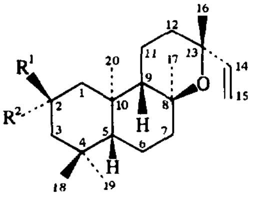
Abstract—A new *ent*-13-epi-manoyl oxide derivative was isolated from the aerial part of *Sideritis perfoliata*. Its structure, *ent*-2α-hydroxy-8,13β-epoxy-labd-14-ene, was established by chemical and spectroscopic means.

In continuation of our studies on the diterpenoids from *Sideritis* spp. [1–3], we have now investigated the aerial part of *S. perfoliata* L. From this source a new diterpenoid, *ent*-2α-hydroxy-13-epi-manoyl oxide (**1**), has been isolated and its structure established by chemical and spectroscopic means, including a chemical correlation with *ent*-13-epi-manoyl oxide.

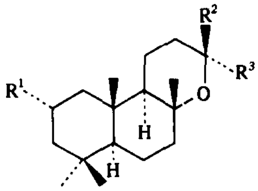
The new diterpenoid (**1**) had a molecular formula $C_{20}H_{34}O_2$ and its IR spectrum was consistent with the presence of a hydroxyl (3325 cm^{-1}) and a vinyl (3085, 1645, 963, 910 cm^{-1}) group. The ^1H NMR spectrum (300 MHz) of compound **1** showed signals of five tertiary methyl groups at δ1.22, 1.14, 0.93, 0.83, and 0.77, of a vinyl group attached to a fully substituted sp^3 carbon atom (an ABX system, δ_A 4.91, δ_B 4.98, δ_X 6.00; $J_{AB} = 0.8$ Hz, $J_{AX} = 10.8$ Hz, $J_{BX} = 17.8$ Hz), and of a proton geminal to a secondary hydroxyl group at δ3.92. This last signal appeared as a triplet of triplets with coupling values of $J_1 = 11.4$ Hz and $J_2 = 4.4$ Hz, thus establishing that the secondary hydroxyl group of the diterpenoid (**1**) is equatorial and placed between two methylene groups. In addition, the ^1H NMR spectrum of compound **1** showed two one-proton triplets at δ1.11 and 0.79 (both $J = 11.4$ Hz) and two one-proton double-doublet of doublets at δ2.04 and 1.78 (both $J_1 = 11.4$ Hz, $J_2 = 4.4$ Hz, $J_3 = 2.0$ Hz). Irradiation at δ3.92 transformed the triplets at δ1.11 and 0.79 into doublets (both $J = 11.4$ Hz) and the signals at δ2.04 and 1.78 (both double-doublet of doublets) into double doublets, with coupling values of 11.4 Hz and 2.0 Hz. This result established that the two methylene groupings vicinal to the secondary equatorial alcohol are attached to fully substituted carbon atoms and that their equatorial protons (signals at δ2.04 and 1.78) are reciprocally coupled through four bonds, because on irradiation of the signal at δ1.78, the signal at δ2.04 was transformed into a double doublet ($J_1 = 11.4$ Hz, $J_2 = 4.4$ Hz), with disappearance of the long-range coupling of 2.0 Hz.

The ^{13}C NMR spectrum of compound **1** (Table 1) showed signals arising from five methyl groups, six methylene carbons, two methine carbons, two quaternary carbon atoms, two olefinic carbons corresponding to the vinyl group, a methine carbon bonded to the oxygen atom of the secondary hydroxyl group, and two quaternary carbons bonded to another oxygen atom. The chemical shifts of the C-1 to C-7, C-10 and C-18 to C-20 carbon atoms of compound **1** (Table 1) are almost identical with those reported [4] for the corresponding carbon atoms of 2α-hydroxy-manoyl oxide (**2**), whereas the C-8, C-9 and C-11 to C-17 signals of the new diterpenoid (**1**) appeared at the same field as in 13-epi-manoyl oxide (**3**) [4].

All the above data are only compatible with a structure



1 $R^1 = OH, R^2 = H$
4 $R^1, R^2 = O$
5 $R^1 = R^2 = H$



	R^1	R^2	R^3
2	OH	Me	$CH=CH_2$
3	H	$CH=CH_2$	Me

Table 1. ^{13}C NMR chemical shifts of compound **1** (75.4 MHz, $CDCl_3$, TMS as int. standard)

Carbon No.	δ	Carbon No.	δ
1	48.5 *t**	11	16.1 *t*
2	65.3 *d*	12	34.7 *t*
3	51.3 *t*	13	73.4 *s*
4	34.8 *s*	14	147.5 *d*
5	55.9 *d*	15	109.6 *t*
6	19.5 *t*	16	32.6 *q*
7	42.9 *t*	17	24.0 *q*
8	75.9 *s*	18	33.4 *q*
9	58.4 *d*	19	22.1 *q*
10	38.5 *s*	20	16.9 *q*

*SFORD multiplicity.

such as **1** (or its enantiomer) for the new diterpenoid. Oxidation of compound **1** with chromium trioxide–pyridine yielded the keto-derivative **4**, the CD curve of which showed a negative Cotton effect ($\Delta\varepsilon_{293} = -1.52$). This is indicative of an *enantio* absolute configuration for compound **4**, because it is known [5] that positive Cotton effects are exhibited by 2-keto-derivatives of 5α-steroids.

Finally, Wolff–Kishner reduction of compound **4** yielded a substance identical in all respects (see the Experimental part) with *ent*-13-epi-manoyl oxide (**5**) [6, 7]. Thus, the new diterpenoid is *ent*-2α-hydroxy-8,13β-epoxy-labd-14-ene (**1**).

EXPERIMENTAL

Mps are uncorr. For general details on methods, see refs. [8–10]. Plant materials were collected in July 1983, at Antalya-Alanya, near Büklü village, Turkey, and voucher specimens (HUEF-1709) were deposited in the Herbarium of the Faculty of Pharmacy, University of Hacettepe, Turkey.

Extraction and isolation of the diterpenoid. Dried and finely powdered *S. perfoliata* L. aerial parts (300 g) were extracted with petrol in a Soxhlet apparatus for 120 hr. The petrol extract was treated as previously described [8–10] yielding a residue (12 g), which was subjected to column chromatography (silica gel, Merck No. 7733; eluted with *n*-hexane and *n*-hexane–Me_2CO, 9:1, 4:1 and 3:1 mixtures). Elution with *n*-hexane–Me_2CO (4:1) yielded the main diterpene constituent (**1**, 510 mg after several crystallizations from *n*-hexane): mp 134.5–135°; $[\alpha]^{17}_{D} -40.4°$ ($CHCl_3$; *c* 0.47); IR ν^{KBr}_{max} cm^{-1}: 3325 (hydroxyl), 3085, 1645, 963, 910 (vinyl group), 2960, 2860, 1457, 1380, 1095, 1080, 1035, 840; ^{1}H NMR (300 MHz, $CDCl_3$): see the text; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 306 $[M]^+$ (0.5), 291 (70), 288 (1.5), 279 (2.5), 273 (32), 261 (7), 255 (35), 190 (60), 175 (50), 147 (44), 135 (62), 121 (45), 95 (55), 81 (80), 69 (46), 67 (47), 55 (58), 43 (100). (Found: C, 78.60; H, 11.27. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%.)

Chromium trioxide–pyridine treatment of **1** *to yield compound* **4**. CrO_3–pyridine treatment of **1** (180 mg) in the usual manner gave **4** (165 mg after crystallization from Et_2O): mp 138–139°; $[\alpha]^{20}_{D} -71.0°$ ($CHCl_3$; *c* 0.55); CD nm ($\Delta\varepsilon$): 325 (0), 293 (−1.52), 247 (0) (MeOH; *c* 0.047); IR ν^{KBr}_{max} cm^{-1}: 1712 (ketone), 3090, 1645, 960, 925 (vinyl group), 2960, 2860, 1460, 1450, 1375, 1280, 1270, 1095, 1080, 1015, 835, 700; ^{1}H NMR (90 MHz, $CDCl_3$): vinyl group as an ABX system, δ_A 4.91, δ_B 4.95, δ_X 6.01 ($J_{AB} = 1$ Hz, $J_{AX} = 10.8$ Hz, $J_{BX} = 18$ Hz, H-14 and 2H-15), C–Me singlets at δ1.22, 1.14, 1.05, 0.86, and 0.75 (five tertiary methyl groups); EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 304 $[M]^+$ (2.5), 289 (100), 277 (5), 271 (58), 206 (28), 191 (15), 151 (18), 122 (18), 107 (15), 95 (15), 81 (23), 67 (17), 55 (17). (Found: C, 78.96; H, 10.49. $C_{20}H_{32}O_2$ requires: C, 78.89; H, 10.59%.)

ent-13-*Epi-manoyl oxide* (**5**) *from compound* **4**. Huang–Minlon reduction of compound **4** (110 mg) under N_2 atmosphere yielded *ent*-13-epi-manoyl oxide (**5**, 90 mg after crystallization from MeOH): mp 99–101°; $[\alpha]^{20}_{D} -37.0°$ ($CHCl_3$; *c* 0.23); IR ν^{KBr}_{max} cm^{-1}: 3080, 1640, 960, 930 (vinyl group), 2960, 2900, 2880, 1465, 1390, 1370, 1200, 1100, 1080, 1070, 1015, 985, 843, 700; ^{1}H NMR (90 MHz, $CDCl_3$): vinyl group as an ABX system, δ_A 4.89, δ_B 4.92, δ_X 6.01 ($J_{AB} = 1$ Hz, $J_{AX} = 10.8$ Hz, $J_{BX} = 18$ Hz, H-14 and 2H-15), C–Me singlets at δ1.21, 1.12, 0.85, 0.79, and 0.72 (five tertiary methyl groups); EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 290 $[M]^+$ (1.5), 275 (100), 263 (4), 257 (70), 245 (11), 192 (35), 191 (20), 177 (22), 137 (20), 123 (13), 109 (12), 95 (15), 81 (17), 69 (11), 55 (10). (Found: C, 82.73; H, 11.71. Calc. for $C_{20}H_{34}O$: C, 82.69; H, 11.80%.) Identical in all respects (mp, mmp, TLC, IR, $[\alpha]_D$, ^{1}H NMR, MS) with an authentic sample of *ent*-13-epi-manoyl oxide (**5**). Lit. [7]: mp 98–99.5°; $[\alpha]^{24}_{D} -37°$.

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