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BEYERENE DERIVATIVES AND OTHER CONSTITUENTS FROM *PETUNIA PATAGONICA*

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Key Word Index—*Petunia patagonica*; Solanaceae; *ent*-beyerenes; *ent*-kauranes; flavonoids.

Abstract—Two new diterpenes of the beyerene type, *ent*-19-hydroxy-17-acetoxybeyer-15-ene and *ent*-beyer-15-en-17-oic acid, and two previously characterized kauranoids, *ent*-16β-hydroxy-17-acetoxykaurane and *ent*-16β,17-dihydroxykaurane, as well as two known flavonoids, luteolin-7,3′,4′-trimethyl ether and luteolin-7,3′-dimethyl ether, and a triterpenoid, oleanoic acid, were obtained from a chloroform extract of *Petunia patagonica*. The new structures were elucidated by spectral data and chemical transformations.

INTRODUCTION

Petunia patagonica (Speg) Millan is a species confined to Santa Cruz province (central district according to Soriano, 1956). This corresponds to the more arid zone of Patagonia with stony soils quite rich in mud and clay but poor in organic substances.

In this paper we report the isolation of four crystalline diterpenes (**1**, **4**, **7** and **9**), two of which appear to be novel, one triterpenoid and two flavonoids.

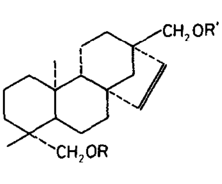
RESULTS AND DISCUSSION

Systematic fractionation of a chloroform extract of the aerial parts led to the isolation of four crystalline diterpenes (**1**, **4**, **7**, **9**) and two flavonoids by extensive silica gel and Sephadex chromatography.

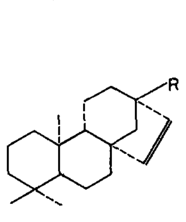
Diterpene **1** was recrystallized from methylethylketone as colourless crystals, mp 95–96°, $[\alpha]_D$ 51.0° ($CHCl_3$; *c* 1.1). Its molecular formula was determined as $C_{22}H_{34}O_3$ by mass spectrometry. A combination of IR and ^1H NMR spectral data indicated the presence of a primary alcohol [ν_{max}^{KBr}: 3400, 1025 cm^{-1}; AB δ3.45 and 3.80 (2H, *dd*, $J = 11$ Hz)]; a primary alcohol acetate [1740, 1245 cm^{-1}; δ3.95 (2H, *s*), 2.00 (3H, *s*)]; a *cis*-disubstituted double bond [3050, 745 cm^{-1}; δ5.71 and 5.53 (2H, *dd*, $J = 5.5$ Hz)]; and two methyl groups on saturated carbons [δ0.93 *s*, 0.70 *s* ($CDCl_3$); 1.16 *s*, 0.73 *s* (pyridine-d_5)]. On the basis of the above data, the diterpene nucleus must be tetracyclic and can be assigned to the beyerene series. The presence of only two methyl groups suggested that **1** had the remaining two methyl groups transformed into hydroxymethylene groups, only one of which was acetylated.

The mass spectrum exhibited a fragmentation at *m/z* 109 (ion b) which indicated that two methyl groups must be attached to C-4 and C-10, respectively, of the beyerene nucleus. On the other hand, the chemical shift values in $CDCl_3$ and pyridine-d_5 for the two methyl groups suggested that the alcohol is axially oriented at C-4 [1]. The acetoxymethylene group was placed on C-13. To characterize this diterpene further, a portion of the sample was saponified to afford a diol (**2**) which was identical in all respects (mp, $[\alpha]_D$, IR, ^1H NMR and MS) to erythroxydiol A [2]. Moreover, **2** was acetylated with acetic anhydride–pyridine to afford erythroxydiol A diacetate (**3**). Therefore, the chemical structure of **1** was indicated to be *ent*-19-hydroxy-17-acetoxybeyer-15-ene.

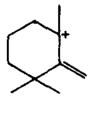
Compound **4** was recrystallized from acetone as colourless crystals, mp 218–220°. The IR spectrum showed absorptions for a carboxylic group (ν_{max}^{KBr}: 3200–2500 broad, 1695 cm^{-1}), a gem-dimethyl group (1385, 1370 cm^{-1}) and a *cis*-disubstituted double bond (745 cm^{-1}), confirmed by the presence in the ^1H NMR spectrum of an AB system at δ5.78 and 5.74 (2H, *dd*, $J = 5.5$ Hz) ($CDCl_3$); δ6.08 and 5.85 (pyridine-d_5). The ^1H NMR spectrum also significantly showed three tertiary methyl groups at δ0.83, 0.80 and 0.72, ($CDCl_3$); δ0.83, 0.78 and 0.72 (pyridine-d_5). These spectral properties characterized **4** as *ent*-beyer-15-ene. Treatment of **4** with diazomethane gave the methyl ester **5**, whose ^1H NMR spectrum exhibited the signal of a methyl group at δ3.66 confirming the presence of the carboxylic group. The mass spectral molecular ion peak occurred at *m/z* 316 (100%). The peaks at *m/z* 137 (ion a) and 123 indicated



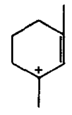
1 R=H ; R'=Ac
2 R=R'=H
3 R=R'=Ac



4 R= $-CO_2H$
5 R= $-CO_2Me$
6 R= $-CH_2OH$



m/z 137
(a)



m/z 109
(b)

that the three methyl groups were present in ring A. Reduction of **5** with lithium aluminium hydride afforded an alcohol identical to erythroxylol B (**6**) [2]. The identity was established by NMR spectroscopy and mp. Additionally, the ^{13}C NMR spectral data of **5** were obtained and they supported the proposed structure (see Table 1). A literature search revealed that no compound with such physical and spectral data has been described.

Petunia patagonica contained two known *ent*-kauranoids which are closely related: *ent*-16β, 17-dihydroxykaurane (**9**) and *ent*-16β-hydroxy-17-acetoxykaurane (**7**). The physical and spectroscopic properties of **9** and **7** were identical to those reported in the literature [3].

The last and most polar pigments were identified from their UV, ^{1}H NMR and mass spectral data as luteolin-7,3′,4′-trimethyl ether (**10**) and luteolin-7,3′-dimethyl ether (**11**). The triterpene was identified as oleanoic acid by direct comparison with an authentic sample.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR: 60 MHz, CDCl$_3$, TMS as internal standard; ^{13}C NMR: 20 MHz, CDCl$_3$, TMS as standard; MS: 70 eV, direct inlet. CC was carried out on silica gel and TLC on silica gel, UV-254 in the solvent systems C_6H_6–dioxane–HOAc (90:10:2) and (90:25:4).

Plant material. This was collected and identified by N. R. Figueroa Romero, Prov. of Santa Cruz. A voucher has been deposited at the Herbarium of Instituto Lillo (Tucumán, Argentina), No. 543 (LIL).

Extraction and isolation. Dried and finely powdered *P. patagonica* aerial part (250 g) was extracted with CHCl$_3$ and after solvent evapn the residue was chromatographed on a silica gel column, packed in C_6H_6 and eluted with C_6H_6–EtOAc mixtures of increasing polarity to yield the following compounds in order of elution: mixture of **1** and **10**; mixture of **4** and **11**; **7** (0.49 g); **9** (3.70 g) and **12** (3.85 g). These mixtures were subjected to further chromatography over Sephadex LH-20 columns, which were eluted with MeOH to yield: **1** (0.03 g); **10** (0.04 g); **4** (0.3 g) and **11** (0.12 g).

Table 1. ^{13}C NMR spectral data of compound **5**

Carbon	δ
1	38.9
2	18.4
3	41.9
4	33.5
5	55.7
6	19.9
7	36.4
8	48.3
9	52.3
10	37.1
11	19.2
12	28.2
13	53.7
14	56.6
15	136.6
16	129.9
17	176.3
18	33.5
19	21.8
20	14.9
21	51.5

Ent-19-*hydroxy*-17-*acetoxybeyer*-15-*ene* (**1**). Colourless crystals from methylethylketone, mp 95–96°; $[\alpha]_D$ 51.0° (CHCl$_3$; *c* 1.1); IR ν_{max}^{KBr} cm^{-1}: 3400, 1025 (–CH$_2$OH), 1740, 1245 (–OAc), 3050, 745 ($^{H}{>}C{=}C{<}^{H}$); ^{1}H NMR (CDCl$_3$): δ0.70 (3H, *s*, H-18), 0.93 (1.16 in pyridine-d_5) (3H, *s*, H-20), 2.00 (3H, *s*), 3.80 and 3.45 (2H, AB, J = 11 Hz, H-19), 3.95 (2H, *s*, H-17), 5.71 and 5.53 (2H, AB, J = 5.5 Hz, H-16 and H-15); MS *m/z* (rel. int.): 346 $[M]^+$ (68), 331 $[M-15]^+$ (3), 328 $[M-18]^+$ (4), 315 $[M-31]^+$ (38), 303 $[M-43]^+$ (4), 286 $[M-60]^+$ (27), 255 $[M-60-31]^+$ (77), 227 (50), 159 (100), 147 (25), 133 (45), 131 (40), 123 (95), 109 (33).

Ent-17,19-*dihydroxybeyer*-15-*ene* (**2**). Saponification of **1** with methanolic KOH (5%) at 20° overnight gave **2** as colourless crystals from Me$_2$CO, mp 180–181°; $[\alpha]_D$ 57.6° (CHCl$_3$; *c* 1.4). IR ν_{max}^{KBr} cm^{-1}: 3400, 1025 (–CH$_2$OH), 3050, 745 ($^{H}{>}C{=}C{<}^{H}$); ^{1}H NMR (CDCl$_3$): δ0.73 (3H, *s*, H-18), 0.95 (3H, *s*, H-20), 3.76 and 3.43 (2H, AB, J = 11 Hz, H-19), 3.50 (2H, *s*, H-17), 5.78 and 5.53 (2H, AB, J = 5.5 Hz, H-16 and H-15); MS *m/z* (rel. int.): 304 $[M]^+$ (100), 286 $[M-18]^+$ (11), 273 $[M-31]^+$ (93), 255 $[M-31-18]^+$ (41), 227 (10), 177 (31), 159 (33), 150 (32), 147 (23), 145 (18), 135 (46), 133 (30), 131 (15), 123 (78), 109 (43).

Ent-17,19-*diacetoxybeyer*-15-*ene* (**3**). Acetylation of **1** with Ac$_2$O–pyridine at 20° for 12 hr gave **3** as colourless crystals, mp 57–58°; IR ν_{max}^{KBr} cm^{-1}: 1740, 1245 (–OAc), 3050, 745 ($_{H}{>}C{=}{<}_{H}$); ^{1}H NMR (CDCl$_3$): δ0.73 3H, *s*, H-18), 0.93 (3H, *s*, H-20), 2.00 (6H, *s*), 4.20 and 3.80 (2H, AB, J = 11 Hz, H-19), 3.93 (2H, *s*, H-17), 5.74 and 5.53 (2H, AB, J = 5.5 Hz, H-16 and H-15).

Ent-*beyer*-15-*en*-17-*oic acid* (**4**). Colourless crystals from Me$_2$CO, mp 218–220°; IR ν_{max}^{KBr} cm^{-1}: 3500, 2300, 1695 (–CO$_2$H), 1385, 1370 (gem-dimethyl), 745 ($_{H}{>}C{=}C{<}_{H}$); ^{1}H NMR (CDCl$_3$): δ0.72, 0.80 and 0.83 (3H × 3, each s, H-18, H-19 and H-20), 5.78 and 5.74 (2H, AB, J = 5.5 Hz, H-16 and H-15); (pyridine-d_5): δ0.72, 0.78 and 0.83 (3H × 3, each *s*, H-18, H-19 and H-20), 5.87 and 6.08 (2H, AB, J = 5.5 Hz, H-16 and H-15); MS *m/z* (rel. int.): 302 $[M]^+$ (38), 287 $[M-15]^+$ (25), 284 $[M-18]^+$ (8), 256 $[M-46]^+$ (62), 246 (16), 227 (15), 217 (24), 213 (41), 199 (17), 191 (25), 185 (33), 171 (30), 157 (30), 138 (33), 137 (34), 129 (100), 123 (80), 109 (51).

Methyl ent-*beyer*-15-*en*-17-*oate* (**5**). Methylation of **4** with CH$_2$N$_2$ gave **5**, colourless crystals, mp 82–83°; $[\alpha]_D$ 53.88° (CHCl$_3$; *c* 1.1); IR ν_{max}^{KBr} cm^{-1}: 3025, 745 ($_{H}{>}C{=}C{<}_{H}$), 1735 (–COOMe), 1385, 1370 (gem-dimethyl); ^{1}H NMR (CDCl$_3$): δ0.72, 0.81 and 0.83 (3H × 3, each *s*, H-18, H-19 and H-20), 3.66 (3H, *s*, –OMe), 5.78 and 5.74 (2H, AB, J = 5.5 Hz, H-16 and H-15); MS *m/z* (rel. int.): 316 $[M]^+$ (100), 301 $[M-15]^+$ (24), 257 $[M-59]^+$ (20), 241 $[M-60-15]^+$ (10), 205 (13), 191 (10), 178 (22), 163 (10), 150 (13), 138 (33), 137 (19), 123 (29), 119 (17), 109 (12).

Ent-*beyer*-15-*en*-17-*ol* (**6**). Reduction of **4** with LiAlH$_4$ by standard procedures and recrystallization from *n*-hexane gave **6**, colourless crystals, mp 120–122°; IR ν_{max}^{KBr} cm^{-1}: 3400, 1025 (–CH$_2$OH), 3050, 745 ($^{H}{>}C{=}C{<}^{H}$); ^{1}H NMR (CDCl$_3$): δ0.83, 0.89 and 0.93 (2H × 3, each *s*, H-18, H-19 and H-20), 3.58 and 3.52 (2H, AB, J = 11 Hz, H-17), 5.86 and 5.61 (2H, AB, J = 5.5 Hz, H-16 and H-15); MS *m/z* (rel. int.): 288 $[M]^+$ (100), 273 $[M-15]^+$ (22), 270 $[M-18]^+$ (12), 257 $[M-31]^+$ (40), 217 (11), 203 (9), 201 (10), 177 (11), 175 (11), 173 (10), 163 (17), 161 (15), 159 (15), 151 (20), 150 (65), 139 (23), 138 (26), 137 (18), 123 (43), 109 (31).

Ent-16β-*hydroxy*-17-*acetoxykaurane* (**7**). Colourless crystals from methylethylketone, mp 150–152°; IR ν_{max}^{KBr} cm^{-1}: 3400 (–OH), 1740, 1245 (–OAc), 1385, 1370 (gem-dimethyl); ^{1}H NMR (CDCl$_3$): δ0.78, 0.82 and 1.00 (3H × 3, each *s*, H-18, H-19 and H-

20), 2.08 (3H, *s*), 4.20 (2H, *s*, H-17); MS *m/z* (rel. int.): 348 $[M]^+$ (1), 330 $[M-18]^+$ (8), 315 $[M-18-15]^+$ (5), 288 $[M-60]^+$ (6), 275 $[M-73]^+$ (100), 257 $[M-73-18]^+$ (27), 232 (26), 217 (18), 192 (25), 177 (4), 137 (40), 123 (70), 109 (42).

Ent-16β-17-*diacetoxykaurane* (**8**). Acetylation of **7** with Ac_2O–pyridine at 70° for 12 hr gave **8** as colourless crystals, mp 134–135°; IR ν_{max}^{KBr} cm^{-1}: 1740, 1250 (–OAc), 1385, 1370 (gem-dimethyl); ^{1}H NMR ($CDCl_3$): δ1.00, 0.86 and 0.81 (3H × 3, each *s*, H-18, H-19 and H-20), 2.00 and 2.08 (3H × 2, each *s*), 2.50 (1H, *m*, H-13), 4.90 and 4.43 (2H, AB, $J = 11$ Hz, H-17).

Ent-16β,17-*dihydroxykaurane* (**9**). Colourless crystals from Me_2CO, mp 187–188°; $[\alpha]_D$ −33.4° (dioxane; *c* 1.76); IR ν_{max}^{KBr} cm^{-1}: 3400, 1020 (–CH_2OH), 1385, 1370 (gem-dimethyl); ^{1}H NMR ($CDCl_3$): δ0.80, 0.85 and 1.00 (3H × 3, each *s*, H-18, H-19 and H-20), 2.16 (1H, *m*, H-13), 3.83 (2H, *s*, H-17), (pyridine-d_5): δ0.79, 0.84 and 1.00 (3H × 3, each *s*, H-18, H-19 and H-20), 2.45 (1H, *m*, H-13), 4.03 (2H, *s*, H-17); MS *m/z* (rel. int.): 306 $[M]^+$ (2), 288 $[M-18]^+$ (3), 275 $[M-31]^+$ (100), 273 $[M-15-18]^+$ (12), 257 $[M-31-18]^+$ (37), 232 (11), 217 (6), 203 (3), 177 (5), 175 (7), 137 (35), 123 (51), 109 (42).

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