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AN ABIETANE DITERPENOID FROM *SALVIA SAPINAE*

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Key Word Index—*Salvia sapinae*; Labiatae; abietane-type diterpenoid; 7β,15-dihydroxy abietatriene.

Abstract—A new abietane-type diterpene, 7β,15-dihydroxyabietatriene, along with three flavonoids, namely apigenin 7,4'-dimethyl ether, genkwanin and cirsimaritin, three triterpenes, including ursolic, oleanolic and 2α,3α-dihydroxyolean-12-en-28-oic acids and sitosterol were isolated from the aerial parts of *Salvia sapinae*. The structures were elucidated by spectroscopic methods.

INTRODUCTION

In the course of our studies on the terpenoid constituents from Labiatae [1–3], we previously reported several triterpenoids from *Salvia* [2]. As part of a continuing chemical investigation of this genus, which also elaborates diterpenes [4–6] and flavonoids [7, 8], we wish to describe the structure determination of the terpenoids of *Salvia sapinae* Epl.

This investigation has resulted in the isolation of three flavones: 4-hydroxy-7,4'-dimethoxyflavone (**1**), 5,4'-dihydroxy-7-methoxyflavone (genkwanin, **2**) and 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin, **3**), as well as sitosterol. In addition, four terpenoids including ursolic, oleanolic and 2α,3α-dihydroxyolean-12-en-28 oic acids along with the diterpenoid **4**, which was identified as 7β,15-dihydroxyabietatriene and represented a new natural product, were also isolated from this species.

RESULTS AND DISCUSSION

Exhaustive chromatography of the acetone extract of the air-dried aerial parts of *Salvia sapinae* allowed the isolation of sitosterol, oleanolic, ursolic and 2α,3α-dihydroxyolean-12-en-28 oic acids, three flavonoids **1–3** and a small amount of the new diterpenoid **4**.

Compound **4** analysed for $C_{20}H_{30}O_2$ (elemental analysis and mass spectrometry). The IR spectrum showed absorption for hydroxyl (3593 cm^{-1}) and a 1,2,4-trisubstituted aromatic ring (900, 831 cm^{-1}) [9]. The mass spectral analysis exhibited important ions associated with an abietane type skeleton [10] at *m/z* 287 (base peak) $[M-Me]^+$, 284 $[M-H_2O]^+$, 269 $[M-H_2O-Me]^+$ and 69. The ^1H NMR spectrum of **4** (see Experimental) showed signals for five methyl groups; three aromatic proton signals at δ7.13 (1H, *d*, *J* = 9 Hz), 7.28 (1H, *dd*, *J* = 2, 9 Hz) and 7.60 (1 H, *d*, *J* = 2 Hz); finally, a triplet signal at 4.78 was indicative of a secondary hydroxyl substitutent.

The second oxygen atom must be present as a tertiary hydroxyl group, since no other signal which could be attributed to geminal protons of an oxygen bearing function appeared in the ^1H NMR spectrum. The location of this substituent on C-15 was established by the downfield shift observed (δ1.56) for the C-16 and C-17 singlet signal (6H) [11]. The low chemical shift displayed for the triplet at δ4.78 (1H, $J_{ac} \approx J_{aa} = 8.5$ Hz), suggests the presence of a β-oriented hydroxyl group at C-7 [12]. Further support for the C-7 stereochemistry was obtained from the ^{13}C NMR data analysis of **4** (see Experimental). The estimated α and β deshielding effects caused by the presence of an equatorial hydroxyl group explained the values assigned to C-7 (δ70.98, Δδ = 39.9), C-6 (30.90, Δδ = 11.8) and C-8 (139.63, Δδ = 4.83) [13]. The strong attenuation of the γ-heteroatom effect exhibited by C-5 (δ49.82, Δδ = −0.58) confirmed the antiperiplanar arrangement of this nucleus and the C-7 hydroxyl substituent [14]. In addition, the observed downfield shift for C-10 (δ38.62, Δδ = 1.12) was consistent with the expected δ-equatorial effect [15]. The remaining carbons exhibited similar chemical shifts to those published for related compounds [16].

Chemical evidence for the benzylic position of the secondary hydroxyl group was achieved by oxidation of **4** with MnO_2 to yield the oxo derivative **5**. All the spectral data for compound **5** were in agreement with those values reported for similar structural environments [17].

Finally, further evidence supporting the proposed stereochemistry was provided by the rotatory dispersion curve of **5**, which was found to be very similar to those reported for related aryl ketones [18–21].

The results obtained by this investigation are in agreement with the chemical profile outlined for this genus. Further chemotaxonomic investigation on other species of *Salvia* should be useful, taking into account their possible pharmacological value as evidenced by the current use of some Labiatae species in traditional medicine [22].

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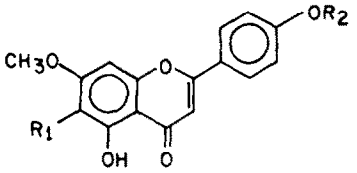

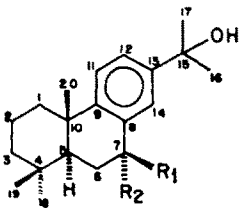

1 $R_1 = H$, $R_2 = CH_3$

2 $R_1 = H$, $R_2 = H$

3 $R_1 = OCH_3$, $R_2 = H$

4 $R_1 = OH$, $R_2 = H$

5 $R_1, R_2 := O$

EXPERIMENTAL

Plant material. Salvia sapinae was collected in February 1984, in Sierra Madre del Sur (Guerrero, México). Reference specimens are deposited in the National Herbarium, Instituto de Biología, UNAM, voucher No. 6166M.

Extraction. Dried and finely powdered aerial parts of the plant (6.0 kg) were exhaustively extracted with Me_2CO at room temp. After filtration, the solvent was evaporated yielding a gum (186 g).

Isolation procedure. The crude extract (186 g) was chromatographed on a colum of silica gel (3 kg deactivated with 10% H_2O) using *n*-hexane–EtOAc gradient elution system. Fractions of 1500 ml were collected.

The low polarity fractions 1–17 containing waxes and fats were discarded. Sitosterol (3.5 g), identified by standard sample comparison, was the main component in fractions 20–45.

Fractions 55–73 (46.76 g), eluted with *n*-hexane–EtOAc (3:1), were rechromatographed over silica gel (1.5 kg). Elution with $CHCl_3$–Me_2CO (9:1) left a residue which crystallized when triturated with Et_2O–Me_2CO to give oleanolic acid (11 mg) [23]. Subsequent fractions, eluted with $CHCl_3$–Me_2CO (4:1), were combined to afford 10 g of a residue which on treatment with CH_2N_2–Et_2O and further CC allowed the separation of oleanolic (382.5 mg, 0.0063% of the dry wt) and ursolic (428 mg) acids, as their methyl ester derivatives [23, 24], which were identified by comparison with authentic samples.

The medium polarity fractions 74–83 (3.63 g), eluted from the original column with *n*-hexane–EtOAc (7:3), were rechromatographed over silica gel (120 g) eluting with $CHCl_3$–Me_2CO (9:1). Fractions 35–80 were purified with activated charcoal and treated with CH_2N_2–Et_2O affording an additional 21 mg of methyl ursolate to give a total of 449 mg (0.0074% of the dry wt). Treatment of fractions 40–46 (45 mg) with activated charcoal and further alkylation with CH_2N_2–Et_2O yielded upon trituration with Et_2O (13 mg, 0.0002% of the dry wt) of 2α,3α-dihydroxyolean-12-en-28 oic acid, as its methyl ester derivative, mp 296° [25], which was identical in all respects with an authentic sample.

Fractions 84–88 (4.25 g), eluted with *n*-hexane–EtOAc (7:3), were resolved by CC over silica gel (160 g). Elution was started with $CHCl_3$, followed by increasing amounts of Me_2CO. Fractions 20–24, eluted with $CHCl_3$–Me_2CO (95:5), crystallized spontaneously to yield 25 mg (0.0004% of the dry wt) of apigenin 7,4'-dimethyl ether (1), mp 174° [26], which was identified by standard procedures. From fractions 49–52, eluted with $CHCl_3$–Me_2CO (9:1), were obtained 10 mg of genkwanin (2), mp 285–287° [27], which was characterized by standard methods. Finally fractions 74–78, eluted with $CHCl_3$–Me_2CO (4:1), were treated with activated charcoal. A crystalline powder was separated and recrystallized from Et_2O–$CHCl_3$ (1:1) to yield 17 mg of 4, as colourless needles, mp 136°. $[\alpha]_D^{25}$ + 80.8° (*c* 0.22; $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 203 (4.37), 266 (4.56), 274 (4.48); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3593, 3001, 2981, 2849, 1496, 1461, 1371, 1324, 1172, 1109, 1015, 951, 900, 831; ^1H NMR (80 MHz, $CDCl_3$): δ7.60 (1H, *d*, *J* = 2 Hz, H-14), 7.28 (1H, *dd*, *J* = 9, 2 Hz, H-12), 7.13 (1H, *d*, *J* = 9 Hz, H-11), 4.78 (1H, *dd*, *J* = 8.5 Hz, H-7) and C-Me singlets at 1.56 (6H), 1.26 (3H), 0.95 (6H); ^{13}C NMR (20.0 MHz, C_5D_5N): δ151.10 (*s*, C-13), 147.85 (*s*, C-9), 139.63 (*s*, C-8), 124.68 (*d*, C-14), 124.04 (*d*, C-12), 123.49 (*d*, C-11), 71.49 (*s*, C-15), 70.98 (*d*, C-7), 49.82 (*d*, C-5), 41.77 (*t*, C-3), 39.23 (*t*, C-1), 38.62 (*s*, C-10), 33.25 (*s*, C-4), 33.25 (*q*, C-18), 32.56 (*q*, C-16), 32.56 (*q*, C-17), 30.90 (*t*, C-6), 25.47 (*q*, C-20), 21.75 (*q*, C-19), 19.52 (*t*, C-2); EIMS 75 eV, *m/z* (rel. int.): 302 $[M]^+$ (12.9), 287 (100), 285 (8), 284 (18), 270 (4), 269 (17), 259 (1), 252 (2), 251 (4), 213 (4), 212 (2), 211 (6), 203 (5), 202 (2), 201 (6), 200 (3), 199 (13), 179 (4), 178 (12), 163 (14), 156 (5), 155 (2), 154 (7), 143 (10), 142 (4), 141 (18), 131 (7), 130 (2), 129 (10), 128 (12), 127 (4), 126 (1), 91 (8), 69 (13), 59 (19.5), 43 (52.2). (Found: C, 79.46; H, 9.96. $C_{20}H_{30}O_2$ requires C, 79.47; H, 9.93%.)

The polar fractions 89–94 (3.31 g) eluted with *n*-hexane–EtOAc (7:3) were rechromatographed on silica gel (140 g). The elution was accomplished with *n*-hexane–EtOAc (4:1). An additional crop of compound 2 (6.7 mg) was obtained from fractions 20–26, which had been shown to be present by TLC (bright yellow colour). The total yield of 2 was 16.7 mg (0.0002% of the dry wt). Fractions 29–45 crystallized spontaneously to afford an additional 65.7 mg of 4 to give a total of 82.7 mg (0.0013% of the dry wt).

Subsequent fractions 95–120 (7 g), from the original column, eluted with *n*-hexane–EtOAc (3:2) were rechromatographed on silica gel (300 g), starting elution with $CHCl_3$ and then with increasing amounts of Me_2CO. Fraction 58 eluted with $CHCl_3$–Me_2CO (9:1) afforded a residue that was washed with Et_2O to give cirsimaritin (3, 6.2 mg), mp 264–266° [28], identified by direct comparison with an authentic sample. The mother liquors obtained after isolation of 3 were combined with fractions 50–86 (450 mg) and resolved by CC, using $CHCl_3$–Me_2CO (9:1) as the eluent mixture. Fractions 11–14 crystallized to give an additional 43 mg of 3 to have a total yield of 49.2 mg (0.0008% of the dry wt).

Oxidation of 4. A soln of 50 mg of 4 in $CHCl_3$ (6 ml) was

oxidized with 625 mg MnO_2 at room temp. The mixture was left overnight. After removal of the oxidation reagent, the excess of solvent was evaporated and the residue was purified by a short CC of silica gel in $CHCl_3$–Me_2CO (9:1) yielding the keto derivative **5** (39.8 mg) oil. $[\alpha]_D^{25}$ + 21.70° ($CHCl_3$); ORD (*c* 0.00129; $CHCl_3$) $[\phi]_{578}$ + 58.1°, $[\phi]_{546}$ + 90.6°, $[\phi]_{436}$ + 360.4°, $[\phi]_{365}$ + 3255.8°; UV λ_{max}^{MeOH} nm (log ε): 205 (4.69), 249 (4.27), 295 (3.54); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3585, 2912, 2843, 1672, 1602, 1450, 1372, 1364, 1238, 1100, 970, 946, 900, 830; ^1H NMR (80.0 MHz, Me_2CO-d_6): δ 8.05 (1H, *d*, *J* = 3 Hz, H-14), 7.73 (1H, *dd*, *J* = 9, 3 Hz, H-12), 7.38 (1H, *d*, *J* = 9 Hz, H-11) and C–Me singlets at 1.53 (6H), 1.27 (3H), 1.05) (3H), 0.98 (3H); EIMS 75 eV, *m/z* (rel. int.): 300 $[M]^+$ (7.6), 286 (23.2), 285 (100), 69.1 (8.1), 43.1 (34.6).

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