A Methoxyabietane Diterpenoid from the Root of *Salvia phlomoides* and Structural Correction of Another Diterpene from *Cryptomeria japonica*

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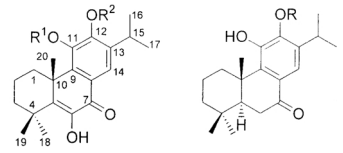
Dedicated to the memory of the late Prof. Dr. Antonio González (1917-2002), University of La Laguna, Tenerife, Spain

A reinvestigation of the acetone extract of the root of *Salvia phlomoides* (Labiatae) allowed the isolation of a new abietane diterpenoid whose structure (6,11-dihydroxy-12-methoxy-5,8,11,13-abietatetraen-7-one) was established by exhaustive NMR spectroscopic studies and by partial synthesis from 14-deoxycoleon U, another abietane constituent of the same extract. The physical and spectroscopic data of the methoxyabietane isolated from *S. phlomoides* were identical to those reported for another diterpenoid previously found in *Cryptomeria japonica* (Taxodiaceae), to which the regioisomeric structure 6,12-dihydroxy-11-methoxy-5,8,11,13-abietatetraen-7-one had been erroneously attributed by other authors.

Key words: Salvia phlomoides, *Cryptomeria japonica*, Methoxyabietanes

Introduction

Twenty years ago, the abietane diterpenoids of an acetone extract of the roots of *Salvia phlomoides* (Labiatae) were studied [1]. Now, an examination of another acetone extract of the roots of the same species, collected at the same place and in identical growing stage, allowed the isolation of a new abietane constituent (**1**) besides all the diterpenoids found in the first study [8,13-abietadiene, 8,11,13-abietatriene, royleanone, 7α-acetoxyroyleanone, taxodione, taxodone, sugiol, 14-deoxycoleon U (**2**), cryptojapanol (**3**), demethylcryptojaponol (**4**) and salviphlomone].



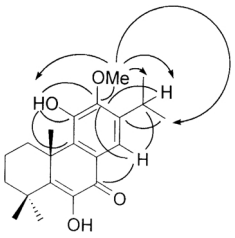
1 R^1 = H, R^2 = Me
2 R^1 = R^2 = H
5 R^1 = Me, R^2 = H

3 R = Me
4 R = H

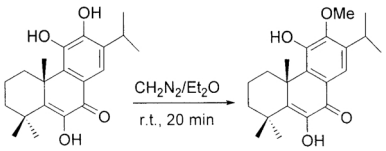
Results and Discussion

Combustion analysis and low-resolution mass spectrometry established a molecular formula $C_{21}H_{28}O_4$ for compound **1**, and its IR spectrum showed phenolic (3395 cm^{-1}) and additionally conjugated arylketone (3030, 1643, 1626, 1596, 1561 cm^{-1}) absorptions. The UV spectrum of **1** (see Experimental Section) was very similar to that of 14-deoxycoleon U (**2**, $C_{20}H_{26}O_4$) [1], thus suggesting that both compounds possessed the same chromophore. Moreover, the ^{1}H and ^{13}C NMR spectra of **1** and **2** were almost identical and the observed differences were compatible with the presence in **1** of a methoxyl group instead of one of the two C-11 and C-12 phenolic hydroxyl groups of **2** [**1**: δ_H 3.83 (s, 3H, PhOMe), 7.07 (s, 1H, diosphenol proton at C-6), 6.22 (s, 1H, phenolic proton at C-11 or C-12); δ_C 62.0 (q, *C*H_3OPh). **2**: no methoxyl signals, δ_H 7.48 (s, 1H, diosphenol proton at C-6), 8.00 (broad signal, 2H, C-11 and C-12 phenolic protons)] [1]. The location of the methoxyl group of **1** at the C-11 or C-12 position was strongly supported by its ^{13}C NMR spectrum as compared with that of **2** [1]. In fact, the ^{13}C NMR spectra of both compounds showed identical carbon atom resonances for the C-1 – C-7, C-10 and C-15 – C-20 carbons, whereas the aromatic C-8, C-9 and C-11 – C-14 carbons appeared at different fields in **1** and **2** (see Experimental Section and [1]).

From all the above data, it was evident that **1** was the C-11 or C-12 *O*-methyl derivative of **2**. The C-12



Scheme 1. Significant HMBC connectivities (⌒) and NOEs (⟶) observed for compound **1**.



Scheme 2. Transformation of 14-deoxycoleon U (**2**) into compound **1**.

position for the methoxyl group of **1**, the only possibly questionable point of its structure, needed a careful justification and this was achieved as follows. The HMBC spectrum of **1** showed connectivities between the methoxyl protons (δ 3.83, 3H, s) and a carbon resonating at δ 148.5 s, and this carbon atom was connected with the aromatic proton at δ 7.72 (s, 14-H) and with the 15-H proton belonging to the isopropyl group (δ_{15-H} 3.24, septet, $J = 6.9$ Hz, connected with a methine carbon at δ 26.7 d in the HSQC spectrum of **1**), as well as with the phenolic proton at δ 6.22 s. Moreover, the 16-Me and 17-Me protons [δ 1.28 (3H) and 1.26 (3H), both d, $J = 6.9$ Hz] were connected with an aromatic carbon at δ 139.9 s, which must be assigned to the C-13 carbon, whereas the aromatic 14-H proton (δ 7.72 s, which was correlated with the C-14 carbon at δ 116.2 d in the HSQC spectrum) showed HMBC cross-peaks with the C-7 carbonyl carbon (δ 179.9 s), the C-15 carbon (δ 26.7 d) and with four aromatic carbons at δ 139.9 s (C-13, see above), 124.4 s, 137.5 s (C-8 and C-9, respectively) and 148.5 s (C-12, the carbon that showed connectivity with the methoxyl protons, see above). In addition, the phenolic proton at δ 6.22 s was connected with three aromatic carbons at δ 137.5 s (C-9, see above), 145.8 s (C-11) and 148.5 s (C-12). All these correlations (see Scheme 1) firmly established that the methoxyl group of **1** was at the C-12 position. On the other hand, the differences in the chemical shifts for the aromatic carbons of **1** and **2** [$\Delta\delta = \delta$(**1**) – δ(**2**): +3.1 (C-8), −2.4 (C-9), +3.5 (C-11), −1.2 (C-12), +4.5 (C-13) and −0.2 (C-14) ppm, see Experimental Section and reference [1]] were similar to those observed between cryptojaponol (**3**) [2,3] and its 12-*O*-demethyl derivative (**4**) [3] [$\Delta\delta = \delta$(**3**) – δ(**4**): +4.0 (C-8), −0.5 (C-9), +4.1 (C-11), +1.8 (C-12), +6.5 (C-13) and +0.7 (C-14) ppm]. Furthermore, NOE experiments (Scheme 1) also supported the location of the methoxyl group of **1** at the C-12 position, because irradiation at δ 3.83 (methoxyl protons) caused NOE enhancement in the signals of the 15-H, 16-Me and 17-Me isopropyl protons (+3.1, +0.3 and +0.2% NOE enhancement, respectively) and in the signal of the C-11 phenolic proton (δ 6.22, +2.1%), thus confirming that the methoxyl group was placed between the C-11 hydroxyl and the C-13 isopropyl substituents. Finally, treatment of **2** with an ethereal solution of diazomethane (see Scheme 2) yielded a compound identical in all (m.p., $[\alpha]_D$ and IR, ^{1}H NMR and mass spectra) respects with **1**. This chemical correlation also supports the location of the methoxyl substituent of **1** at C-12, because it is known [4,5] that, in other 7-oxo-abietane derivatives possessing a C-6 diosphenol function and phenolic hydroxyls at the C-11 and C-12 positions, the reaction with diazomethane produces only monomethoxylation at C-12.

The physical (m. p., $[\alpha]_D$) and spectroscopic (IR, UV, ^{1}H and ^{13}C NMR and mass spectra) data of **1** were identical to those reported previously by Su *el al.* [6] for a methoxyabietane diterpenoid isolated from *Cryptomeria japonica* (Taxodiaceae) to which structure **5** was attributed. These authors located the methoxyl group of **5** at the C-11 position because irradiation of the signal of the C-20 methyl group (δ 1.66, 3H, s) caused NOE enhancement (+7%) in the signal of the methoxyl group (δ 3.83, 3H, s). In the case of **1**, irradiation at δ 3.83 (methoxyl protons) produced a weak NOE enhancement (+0.2%) in the signal of the Me-20 protons, whereas on irradiating at δ 1.66 (20-Me signal) a strong NOE enhancement (+3.8%) in the methoxyl signal was observed. This behaviour, and hence the mistake in structure **5** assigned to the diterpenoid found in *C. japonica* [6], must be rationalized considering that on irradiation at δ 1.66 (20-Me) the signal of the 1α-H proton (δ 1.67 m, see Experimental Section) was also irradiated, and this may produce an increment in the NOE of the C-12 methoxyl signal due to the spatial proximity of the 1α-H and 12-OMe protons, and this enhancement may be reinforced by a transference NOE through the C-11 hydroxyl proton.

From all the above results, it was evident that structure **5** attributed by Su *et al.* [6] to the diterpenoid of *C. japonica* must be amended to **1**. The complete and unambiguous assignments, based on HSQC and HMBC

experiments, of the ^{1}H and ^{13}C NMR spectra of **1** are reported in the Experimental Section, completing the ^{1}H NMR data and correcting previous ^{13}C NMR assignments for the pairs of carbons C-5/C-6, C-9/C-13 and C-11/C-12, which must be reversed [6].

Experimental Section

General

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. UV spectra were measured on a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian INOVA 400 apparatus at 400 and 100 MHz, respectively, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25) for protons and to the solvent signals (δ 77.0) for carbons. Mass spectra were registered in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analysis was made with a Carlo Erba EA 1108 apparatus. Merck silica gel 60 and petrol bp 50 – 70 °C were used for column chromatography.

Plant material

The roots of *Salvia phlomoides* Asso. were collected in April 2001, near Zaorejas (Guadalajara Province, Spain), and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Complutense University of Madrid.

Extraction and isolation of compounds

Dried and powdered *S. phlomoides* roots (600 g) were extracted with Me_2CO (3 l) in a Soxhlet apparatus for 24 h. After filtration, the solvent was evaporated yielding a red gum (30 g), which was subjected to silica gel column chromatography (600 g). The column was eluted with a solvent gradient from petrol 100% to EtOAc 100% to give 7 frs. (fr. 1 – 7). Fr. 1 (2.1 g) was rechromatographed [silica gel 50 g, petrol – EtOAc (99:1)] yielding the following compounds in order of elution: waxes (1 g), 8,13-abietadiene (13 mg, 0.0021% on dry plant material), 8,11,13-abietatriene (131 mg, 0.022%), royleanone (312 mg, 0.052%) and 7α-acetoxyroyleanone (410 mg, 0.068%). Fr. 3 (3.6 g) was rechromatographed [silica gel 100 g, petrol – EtOAc (19:1)] giving taxodione (2.23 g, 0.37%), whereas rechromatography of fr. 5 (230 mg) [silica gel 50 g, petrol – CH_2Cl_2(2:1)] successively yielded compound **1** (26 mg, 0.0043%), cryptojaponol (**3**, 105 mg, 0.018%) and taxodone (58 mg, 0.0096%). The residue of fr. 6 (3.7 g) was crystallized from EtOAc – pentane to give pure 14-deoxycoleon U (**2**, 1.36 g, 0.227%). Rechromatography of fr. 7 (670 mg) [silica gel 40 g, petrol – EtOAc (1:1)] successively eluted sugiol (7 mg, 0.0012%), demethylcryptojaponol (**4**, 135 mg, 0.022%) and salviphlomone (142 mg, 0.024%). The previously known diterpenoids were identified by their physical (m.p., $[\alpha]_D$) and spectroscopic (IR, UV, ^{1}H NMR and MS) data and by comparison (mmp, TLC) with authentic samples [1].

6,11-Dihydroxy-12-methoxy-5,8,11,13-abietatetraen -7-one (**1**)

Crystal, fine white needles. M.p. 188 – 190 °C (EtOAc – pentane); $[\alpha]_D^{24}$ +6.7° ($CHCl_3$, *c* 0.342); IR (KBr) ν_{max}: 3395 (PhOH and diosphenol), 3030, 1643, 1626, 1596, 1561 (conjugated aryl-ketone and diosphenol), 2961, 1322, 1136, 1011 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 333 (3.86), 280 (3.81), 246 (3.90), 237 (3.43); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (s, 1H, 14-H), 7.07 (s, 1H, 6-OH), 6.22 (s, 1H, 11-OH), 3.83 (s, 3H, 12-OMe), 3.24 (septet, $J = 6.9$ Hz, 1H, 15-H), 3.02 (ddd, $J = 14.0$, 7.4, 3.2 Hz, 1H, 1β-H), 2.04 (ddd, $J = 13.6$, 13.1, 5.8 Hz, 1H, 3α-H), 1.87 (ddddd, $J = 13.6$, 13.1, 9.4, 7.4, 3.5 Hz, 1H, 2β-H), 1.67 (m, 1H, 1α-H), 1.66 (m, 1H, 2α-H), 1.66 (s, 3H, 20-Me), 1.45 (s, 3H, 18-Me), 1.44 (s, 3H, 19-Me), 1.42 (ddd, $J = 13.6$, 6.4, 3.5 Hz, 1H, 3β-H), 1.28 and 1.26 (both d, $J = 6.9$ Hz, 3H each, 16-Me and 17-Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.9 (s, C-7), 148.5 (s, C-12), 145.8 (s, C-11), 144.5 (s, C-5), 143.0 (s, C-6), 139.9 (s, C-13), 137.5 (s, C-9), 124.4 (s, C-8), 116.2 (d, C-14), 62.0 (q, 12-O*C*H$_3$), 40.9 (s, C-10), 36.5 (s, C-4), 36.4 (t, C-3), 29.6 (t, C-1), 27.9 (q, C-18), 27.4 (q, C-20), 27.1 (q, C-19), 26.7 (d, C-15), 23.7 (q, C-16 or C-17), 23.5 (q, C-17 or C-16), 17.6 (t, C-2); EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 344 [M]$^+$ (86), 329 [M- Me]$^+$ (20), 301 [M-*i*Pr]$^+$ (21), 275 (100), 274 (91), 259 (71), 256 (31), 247 (26), 241 (20), 231 (17), 205 (10), 165 (6), 128 (9), 115 (9), 91 (6), 83 (6), 69 (4), 43 (10), 41 (9). $C_{21}H_{28}O_4$: calcd. C 73.2, H 8.2; found C 73.3, H 8.2.

*Selective methylation of 14-deoxycoleon U (**2**) to give compound* **1**

A solution of **2** (30 mg) in Et_2O (20 ml) was treated with an excess of an ethereal solution of CH_2N_2 at room temperature for 20 min. Evaporation of the solvent and crystallization of the residue from EtOAc – pentane gave 24 mg (yield 76.7%) of a substance [m.p. 187 – 190 °C, $[\alpha]_D^{24}$ +5.1° ($CHCl_3$, *c* 1.1)] identical in all (IR, ^{1}H NMR and MS) respects with the natural compound (**1**) isolated from the plant extract.

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