Notes

The Structure of Cossonidine: A Novel Diterpenoid Alkaloid

Matias Reina, Jose A. Gavín, Alberto Madinaveitia, Rafael D. Acosta, and Gabriel de la Fuente*

Instituto de Productos Naturales y Agrobiología, CPNA, CSIC-Universidad de La Laguna, Tenerife, Spain

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A new diterpenoid alkaloid cossonidine (**1**) has been isolated from *Delphinium cossonianum* and *Delphinium cadiopetalum*. The structure of cossonidine was determined by spectroscopic methods, including ^{1}H COSY, HMQC, HMBC, and ROESY NMR experiments, and chemical transformations. The NMR data of kobusine (**5**) and sanyonamine (**6**) are also presented.

In the course of our work on *Delphinium* species, which possess diverse biological activities,[1] we have isolated three norditerpenoid alkaloids, cardiopetalidine, dehydrocardiopetaline, and 8-cinnamoylgraciline, and a diterpenoid alkaloid, cossonine, from *Delphinium cossoniamum* Batt. (Ranunculaceae), an annual taxon endemic of Morocco.[2] From *D. cardiopetalum* DC., an annual taxon distributed in Spain and the South of France, we have isolated the norditerpenoid alkaloids karakoline, dihydrogadesine, 14-acetyldihidrogedesine, 14-benzoylgadesine, 14-benzoyldihydrogadesine, cardiopetaline, and cardiopetalidine,[3,4] as well as the diterpenoid alkaloids cardiopetamine, 15-acetylcardiopetamine, cardionine, cardionidine, 13-acetylhetisinone, hetisinone, and atisinium chloride.[5−8] In this paper we present the structure elucidation of cossonidine (**1**), a new diterpenoid alkaloid isolated from both of the above-mentioned plants.

Cossonidine (**1**), $C_{20}H_{27}NO_2$, is a slightly functionalized hetisine-type diterpenoid alkaloid as inferred from its ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2), which were closely related to those of nominine,[9,10] 9-hydroxynominine,[10] kobusine (**5**),[11] and sanyonamine (**6**).[12] The NMR spectra of cossonidine gave signals at δ_H 1.02 s and δ_C 28.5 q for the angular methyl group, and δ_H 4.94 and 4.97 (each s), δ_C 108.9 t and 156 s, for the exocyclic methylene. The one-proton signal at δ 4.19 br s and 4.00 s, which correlated with the carbon signals at δ 66.3 d and 71.6 d, respectively, in the HMQC experiment (Table 1),[13] suggested that cossonidine has two secondary hydroxyl groups. In fact, treatment of cossonidine with Ac_2O and pyridine gave a diacetate (**2**), $C_{24}H_{21}NO_4$, δ_H 2.09 and 2.11 (3H each s), where the one proton signals at δ 4.00 s and 4.19 br s in the ^{1}H NMR of **1** were shifted downfield to δ 5.43 s and 5.23 br s, respectively. The one-proton signals at δ 2.39 and 2.56 (each d, J = 12.5 Hz) (HMQC δ_C 63.0 t) were assigned to the non-equivalent C-19 methylene protons because of their three-bond connectivities with the C-18 resonance observed in the HMBC spectrum (Table 1).[14] Also in the HMBC spectrum, the H-18 signal gave conectivities with the carbon signals at δ 27.9 t, 37.2 q, and 56.6 d, and both H-19 signals with the carbon signals at δ 27.9 t, 37.2 s, 65.8 d, and 75.8 d, enabling us to attribute the said carbon signals at δ 27.9 t, 37.2 s, 56.6 d, 65.8 d, and 75.8 d to C-3, C-4, C-5, C-6, and C-20, respectively. In the ^{1}H COSY spectrum of cossonidine (Table 3, supporting information) the signal at δ 3.40 br s (HMQC δ_C 65.8 d) showed scalar coupling to the one-proton signals at δ 1.68 (dd, J = 13.2, 3.1 Hz) and 2.02 (dd, J = 13.2, 2.4 Hz), which correlated with the methylene carbon resonance at δ 32.6 in the HMQC spectrum, and 2.49 s (W coupling, HMQC δ_C 75.8 d); thus, the signals at δ_H 1.68 dd, 2.02 dd, 2.49 s, and 3.40 br s, and δ_C 32.6 t could be assigned to H-7α, H-7β, H-20, H-6, and C-7, respectively, corroborating the assignment of the methine carbon resonances at δ 65.8 and 75.8 to C-6 and C-20, respectively.

Table 1. ^{1}H, HMQC, and HMBC NMR Data of Cossonidine (**1**)[a]

		correlated carbon	
		δ_C	
proton	δ_H	HMQC	HMBC
H-1α	4.19 br s ($W_{1/2}$ = 6)	66.3 d	C-3, C-5
H-2α	1.79 m	27.2 t	
H-2β	1.77 m	27.2 t	
H-3α	1.25 m	27.9 t	
H-3β	1.74 m	27.9 t	C-1, C-2, C-4, C-5
H-5	1.89 s	56.6 d	C-9, C-10, C-18, C-19, C-20
H-6	3.40 br s ($W_{1/2}$ = 6)	65.8 d	C-8, C-10, C-20
H-7α	1.68 dd (13.2, 3.1)	32.6 t	C-5, C-6, C-8, C-9
H-7β	2.02 dd (13.2, 2.4)	32.6 t	C-8, C-14
H-9	2.01 d (11.5)	41.4 d	C-5, C-8, C-12, C-14, C-20
H-11α	1.92 dd (14.2, 4.2)	26.8 t	C-8, C-9, C-10, C-12
H-11β	176 m	26.8 t	
H-12	2.21 m ($W_{1/2}$ = 8)	33.7 d	
H-13α	1.07 dt (13.2, 2.7)	33.1 t	C-20
H-13β	1.80 m	33.1 t	C-20
H-14	1.90 m	43.6 d	C-9, C-10, C-20
H-15α	4.00 s	71.6 d	C-7, C-8, C-9, C-12, C-16, C-17
H-17e	4.94 s	108.9 t	C-12, C-15
H-17z	4.97 s	108.9 t	C-12, C-15, C-16
H-18	1.02 s	28.5 q	C-3, C-4, C-5, C-19
H-19α	2.39 d (12.5)	63.0 t	C-3, C-6, C-18, C-20
H-19β	2.56 d (12.5)	63.0 t	C-3, C-4, C-18, C-20
H-20	2.49 s	75.8 d	C-1, C-6, C-8, C-13, C-19

[a] Chemical shifts in ppm relative to TMS; coupling constants J (in parentheses) are in Hz. C-multiplicities were established by DEPT data.

Cornforth's oxidation of cossonidine gave two α,β-unsaturated keto compounds (**3**) $C_{20}H_{25}NO_2$, and (**4**) $C_{20}H_{23}NO_2$. Their NMR spectra showed signals at δ_H

* To whom correspondence should be addressed. Phone: +34-22-250720. Fax: +34-22-630099.

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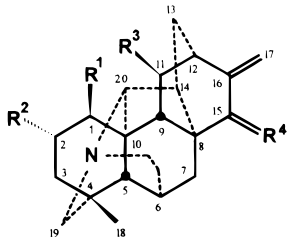
Table 2. ^{13}C-NMR Data of Compounds **1**−**6**[a]

	compound					
carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	66.3	69.7	66.1	214.9	26.9	34.2
2	27.2	23.9	27.4	38.1	19.5	66.6
3	27.9	28.2	27.8	38.6	33.8	40.2
4	37.2	37.4	37.5	38.4	37.9	35.8
5	56.6	57.3	56.6	68.5	61.0	60.0
6	65.8	65.4	65.2	66.6	65.2	65.2
7	32.6	32.3	28.3	28.0	32.4	32.2
8	45.8	44.1	52.7	51.7	45.8	41.8
9	41.4	42.6	47.6	45.5	54.8	44.6
10	55.1	53.3	55.7	66.1	49.2	48.0
11	26.8	26.3	27.6	30.4	67.7	26.8
12	33.7	33.4	33.7	33.8	41.4	33.6
13	33.1	33.1	32.4	32.9	30.1	32.8
14	43.6	43.3	45.6	45.9	41.7	43.2
15	71.6	72.4	201.8	201.3	71.7	71.5
16	156.4	150.9	147.6	147.3	150.6	155.0
17	108.9	110.9	115.2	115.6	114.6	108.8
18	28.5	28.3	28.4	27.3	28.8	29.3
19	63.0	62.5	62.6	64.4	62.5	62.0
20	75.8	75.5	76.0	79.6	75.0	73.8

[a] Chemical shifts in ppm relative to TMS. C-multiplicities were determined by DEPT experiments. Signals for OAc in **2**; 21.1 q, 170.6 s and 171.1 s.

5.08 (1H, d, J = 1.5 Hz), 5.89 (1H, d, J = 1.5 Hz), δ_C 147.3−147.6 s and 115.2−115.6 t for the exocyclic methylene, and δ_C 201.3−201.8 s for a carbonyl group, clearly indicating that one of the secondary hydroxyl groups in cossonidine is at C-15. The carbinyl proton signal at δ 4.00 s (HMQC δ_C 71.6 d) gave, among others, three-bond connectivities with the C-7 and C-17 carbon resonances in the HMBC experiment, and the signals at δ 1.68 dd (H-7α) and 2.02 (H-7β) gave spatial correlation to the one-proton signals at δ 4.00 s and 1.89 s for H-5 (HMQC δ_C 56.6 d), respectively, in the ROESY spectrum (Table 3, supporting information)[15]. These observations permitted the secondary hydroxyl group at C-15 to be placed in β configuration.



1 R^1 = βOH, R^2 = R^3 = H, R^4 = H, βOH

2 R^1 = βOAc, R^2 = R^3 = H, R^4 = H, βOAc

3 R^1 = βOH, R^2 = R^3 = H, R^4 = O

4 R^1 = R^4 = O, R^2 = R^3 = H

5 R^1 = R^2 = H, R^3 = OH, R^4 = H, βOH

6 R^1 = R^3 = H, R^2 = OH, R^4 = H, βOH

The other secondary hydroxyl group was situated at C-1, also in the β configuration, on the basis of the three-bond connectivities observed between the carbinyl proton signal at δ 4.19 br s (HMQC δ_C 66.3 d) and the C-3 and C-5 resonances in the HMQC spectrum and the spatial correlation between the one-proton signals at δ 4.10 brs and 2.49 s (H-20) revealed in the ROESY spectrum. The remaining NMR signals were in agreement with the proposed structure of cossonidine, and the assignments were made by comparison with the spectra of nominine, 9-hydroxynominine,[10] kobusine (**5**), sanyonamine (**6**) (Table 2 and Tables 4 and 6, supporting information) and the 2D NMR data (Table 1 and Table 3, supporting information).

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were obtained on a Reichert Thermovar apparatus. IR were obtained in $CHCl_3$ on a Shimadzu 408 spectrophotometer. Optical rotations were measured in EtOH, 1-dm cell, on a Perkin-Elmer 137 polarimeter. MS and exact measurements were recorded on a Hewlett-Packard 5995 and VG-Micromass ZAB-2F instruments at 70 eV, respectively. NMR spectra were obtained in $CDCl_3$ on a Bruker AMX-400 spectrometer with TMS and solvent as internal standard. The programs used for DEPT, ^{1}H COSY, HMQC, HMBC (J = 7 Hz) and ROESY (spin lock 700 ms) experiments were those furnished in the Bruker manual. Si gel Merck Art. 7734 and 5554 was used for CC and TLC, respectively. Neutral alumina Merck Art. 1097 and 5581 was also used for CC, and TLC and prep. TLC, respectively. Visualization was effected with Dragendorff's reagent. Cornforth's reagent was prepared by adding dropwise a solution of CrO_3 (100 mg) in H_2O (0.1 mL) to pyridine (1 mL) at 0 °C.

Plant Material. *Delphinium cossonianum* and *D. cardiopetalum* were collected as reported in previous works.[2,7] Voucher specimens BCF 36151 and 40491, respectively, are deposited at the Botany Department, Faculty of Pharmacy, University of Barcelona.

Extraction and Isolation. The fraction A_3 (5.12 g) from *D. cossonianum*[2] was chromatographed on Si gel with EtOAc−MeOH (9:1−3:2). The fractions eluted with EtOAc−MeOH (7:3) were rechromatographed over neutral alumina with EtOAc−MeOH (4:1) to give cossonidine (**1**) (650 mg). The product from the mother liquor of the crystallization of atisinium chloride from fraction F_2 (2.51 g) of *D. cardiopetalum*[7] was chromatographed on neutral alumina. The fractions eluted with EtOAc−MeOH (49:1) were rechromatographed on TLC plates of neutral alumina (20 cm × 20 cm, 0.25 mm) with EtOAc−MeOH (9:1) to afford cossonidine (45 mg).

Cossonidine (**1**) was obtained as colorless prisms (EtOAc): mp 243−245 °C dec; $[\alpha]^{25}_D$ + 34.7° (*c* 0.17, EtOH); IR ($CHCl_3$) ν max 3650, 2950, 2900, 1650, 1450, 1380, 1150, 1080, 1050, 1010, 990 cm^{-1}; HRMS *m/z* $[M]^+$ 313.2046 ($C_{20}H_{27}NO_2$ requires 313.2042); EIMS (70 eV) *m/z* $[M]^+$ 313 (100), 297 (12), 296 (45), 286 (12), 285 (55), 284 (27), 270 (15), 242 (8), 202 (4), 162 (26), 146 (53), 91 (27); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2, respectively.

Acetylation of Cossonidine. A mixture of cossonidine (**1**) (10 mg), pyridine (0.15 mL), and Ac_2O (0.2 mL) was kept at room temperature for 24 h. Then toluene was added and the solvent removed under vacuum. The residue was chromatographed over neutral alumina with hexane−EtOAc (1:1) to give 1,15-diacetylcossonidine (**2**) as a resin (9 mg, 71.4%); $[\alpha]^{25}_D$ + 5.2° (*c* 0.27, EtOH); IR ($CHCl_3$) ν max 3010, 2950, 2880, 1720, 1650, 1360, 1240, 1000, 950, 880 cm^{-1}; HMRS *m/z* $[M]^+$ 397.2257 ($C_{24}H_{31}NO_4$ requires 397.2253); EIMS (70 eV)-*m/z* $[M]^+$ 397 (5), 354 (3), 339 (30), 338 (100), 294 (2), 278 (4), 115 (5), 105 (5), 91 (8), 71 (8), 55 (10); ^{1}H NMR

δ 1.02 (3H, s, H-18), 1.10 (1H, dt, J = 13.5, 2.8 Hz, H-13α), 1.29 (1H, br d, J = 13.2 Hz, H-3α), 1.53 (1H, dd, J = 13.8, 9.2 Hz, H-11β), 1.86 (1H, s, H-5), 2.09 and 2.11 (3H each, s, 2×OAc), 2.17 (1H, *t*, J = 3.2 Hz, H-12), 2.38 (1H, d, J = 12.6 Hz, H-19α), 2.56 (1H, s, H-20), 2.57 (1H, d, J = 12.6 Hz, H-19β), 3.37 (1H, br s, $W_{1/2}$ = 7 Hz, H-6), 4.92 (1H, d, J = 1.4, H-17e), 4.95 (1H, t, J = 1.3 Hz, H-17z), 5.23 (1H, br s, $W_{1/2}$ = 6.4 Hz, H-1α), 5.43 (1H, s, H-15α); ^{13}C-NMR data, see Table 2.

Oxidation of Cossonidine. A mixture of cossonidine (**1**) (25 mg), pyridine (0.2 mL) and Cornforth's reagent (0.3 mL) was stirred at room temperature for 3 days. The excess of reagent was destroyed with EtOH, the solvent removed, and the residue chromatographed on a short neutral alumina column, eluting with EtOAc, to afford 15-dehydrocossonidine (**3**) (6 mg, 24.5%) and 1,15-didehydrocossonidine (**4**) (12.5 mg, 51.6%).

15-Dehydrocossonidine (**3**) was obtained as an amorphous compound: $[\alpha]^{25}_D$ +26.0° (*c* 0.058, EtOH); IR ($CHCl_3$) ν max 3650, 2950, 2900, 1705, 1630, 1400, 1325, 1285, 1050, 985, 950, 925, 895 cm^{-1}; HMRS *m/z* $[M]^+$ 311.1881 ($C_{20}H_{25}NO_2$ requires 311.1884); EIMS (70 eV) *m/z* $[M]^+$ 311 (3), 294 (4), 243 (4), 183 (5), 165 (5), 163 (7), 105 (10), 101 (15), 88 (22), 85 (57), 84 (25), 83 (100), 73 (22), 59 (16), 55 (13); ^{1}H NMR δ 1.03 (3H, s, H-18), 1.89 (1H, s, H-5), 2.14 (1H, dd, J = 14.2, 4.0 Hz, H-11α), 2.19 (1H, dt, J = 11.9, 1.6 Hz, H-14), 2.40 (1H, d, J = 12.5 Hz, H-19α), 2.54 (1H, d, J = 12.5 Hz, H-19β), 2.61 (1H, s, H-20), 2.64 (1H, m, H-12), 3.39 (1H, br s, $W_{1/2}$ = 7 Hz, H-6), 4.29 (1H, br s, $W_{1/2}$ = 6.4 Hz, H-1α), 5.08 (1H, d, J = 1.5 Hz, H-17e), 5.89 (1H, d, J = 1.5 Hz, H-17z); ^{13}C-NMR data, see Table 2.

1,15-Didehydrocossonidine (**4**) was obtained as an amorphous compound: $[\alpha]^{25}_D$ −13.0° (*c* 0.1, EtOH); IR ($CHCl_3$) ν max 3010, 2950, 2900, 1730, 1700, 1630, 1460, 1440, 1385, 1375, 1325, 1270, 1250, 1210, 1150, 1050, 1000, 840 cm^{-1}; HRMS *m/z* $[M]^+$ 309.1733 ($C_{20}H_{23}NO_2$ requires 309.1728); EIMS (70 eV) *m/z* $[M + 2]^+$ 311 (11), $[M + 1]^+$ 310 (26), $[M]^+$ 309 (100), 308 (13), 284 (7), 281 (10), 280 (10), 253 (18), 121 (14), 91 (11), 79 (10) and 77 (12), 55 (7); ^{1}H NMR δ 1.05 (3H, s, H-18), 1.34 (1H, dt, J = 13.6, 2.8 Hz, H-13α), 2.12 (1H, s, H-5), 2.47 (1H, dd, J = 14.2, 5.2 Hz, H-11α), 2.54 (1H, br *s*, $W_{1/2}$ = 10 Hz, H-12), 2.70 (1H, d, J = 12.7 Hz, H-19α), 2.77 (1H, d, J = 12.7 Hz, H-19β), 3.36 (1H, s, H-20), 3.48 (1H, br s, $W_{1/2}$ = 7 Hz, H-6), 5.08 (1H, d, J = 1.5 Hz, H-17e), 5.89 (1H, d, J = 1.5 Hz, H-17z); ^{13}C-NMR data, see Table 2.

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Supporting Information Available: Tables 3−7 (5 pages). Ordering information is given on any current masthead page.

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