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TWO NEW ALKALOIDS AND A NEW POLYPHENOLIC COMPOUND FROM *Cotula coronopifolia*

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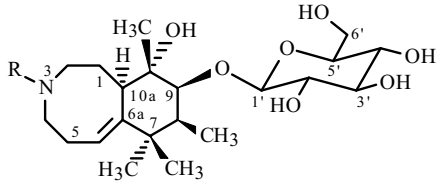
Two new alkaloid derivatives of the 3-benzazocine skeleton (cotuzine A and B) and a new polyphenolic substance (3-(1,3-dihydroxyprop-2-yl)-4-hydroxyanisol) (corimen) have been isolated from the aerial part (stems, leaves) of the plant Cotula coronopifolia *(L.). These three new structures were established by spectroscopic procedures (^{1}H, ^{13}C, one- and two-dimensional NMR). The stereochemistry of the new alkaloids was established based on different NOE effects revealed on the NOESY spectrum. Mass spectrometry and IR spectroscopy were used to confirm these structures.*

Keywords: *Cotula coronopifolia*, new alkaloids, cotuzine, corimen, NMR.

The interest in nature as a source of potential chemotherapeutic agents continues. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world. Higher plants contribute no less than 25% of the total [1]. As part of our chemical investigation into some medicinal plants growing in Tunisia [2–4] and in order to continue our search for potential biological natural compounds [5], we report here the isolation and structure elucidation of two new alkaloids **1** and **2** and a new polyphenolic compound **3** isolated from the aerial part of *Cotula coronopifolia* (L.) (Asteraceae). So far not much is known on the ethnopharmacology of this plant [6].

Structures of cotuzine A and B (**1**, **2**) and corimen (**3**) were established by spectroscopic means (1D and 2D NMR) and confirmed by comparison with those of closely related compounds [7, 8]. The relative stereochemistry of the alkaloid structure was obtained from the NOESY experiment.

Cotuzine A (**1**) was obtained as an amorphous solid (29 mg) from the butanolic extract of the aerial part (stems, leaves) of the plant *Cotula coronopifolia* (L.). The IR spectrum indicated the presence of hydroxyl groups (3400–3200 cm^{-1}), methyl groups (2963, 2882 cm^{-1}), secondary amines (3029, 827 cm^{-1}), and double bonds (3021, 1651 cm^{-1}). The ^{13}C NMR spectrum (Table 1) indicates the presence of 21 carbons, including an ethylenic group relative to C-6 and C-6a at 119.9 ppm and 144.6 ppm, respectively, an anomeric carbon at 103.7 ppm, and two (-CH_2-) groups at 40.5 and 42.2 ppm forming the (-CH_2-NH-CH_2-) skeleton. The ^{1}H NMR spectrum shows a signal at δ 5.22 ppm attributable to H-6 coupled with H-5α (1.91 ppm, m) and H-5β (1.83 ppm, m), a doublet relative to CH_3-8 (0.89 ppm, d, J = 6.8 Hz), and three signals relative to three methyl groups: CH_3-7α (0.91 ppm, s), CH_3-7β (1.04 ppm, s), and CH_3-10 (0.91 ppm, s). These results were supported by the correlating signals H-6/H-5α, H-6/H-5β, and CH_3-8/H-8 observed in the ^{1}H–^{1}H COSY NMR spectrum. The same spectrum shows correlations between H-4α/H-2, H-4β/H-5, H-10a/H-1β, and H-10a/H-2β.



1, 2

1: R = H
2: R = CH_3

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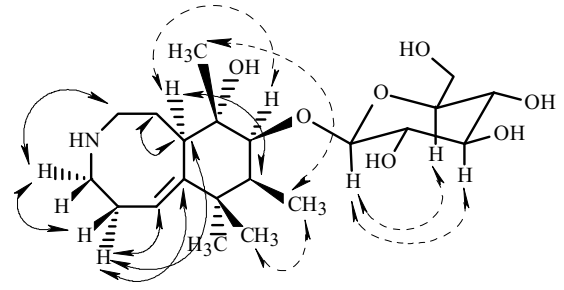
TABLE 1. ^{1}H (300 MHz; CD_3OD, δ, ppm, J/Hz) and ^{13}C NMR (75 MHz; CD_3OD, δ) Chemical Shifts for Cotuzine A (**1**)

C atom	δ_C	δ_H	HMBC
1	24.1	1.58, 1.83 (2H, m)	10a; 9
2	40.5	1.41, 1.69 (2H, m)	4
4	42.2	1.76, 1.91 (2H, m)	5
5	24.5	1.83, 2.04 (2H, m)	4; 6; 6a; 10a
6	119.9	5.22 (1H, d, $J_{6\text{-}5}$ = 4.88)	4; CH_3-7
6a	144.6	–	5; 4
7	40.1	–	CH_3-7
8	36.7	2.06 (1H, m)	10a
9	88.6	3.28 (1H, m)	8
10	73.3	–	–
10a	48.7	1.19 (1H, dd, J = 5.1, J = 6.9)	5; 8
CH_3-8	13.4	0.89 (3H, d, J = 6.8)	7; 8
CH_3-7α	22.1	0.91 (3H, s)	8
CH_3-7β	30.3	1.04 (3H, s)	7
CH_3-10	22.7	0.91 (3H, s)	10a; 10
1′	103.7	4.21 (1H, d, J = 7.5)	9; 2′; 5′; 6′
2′	73.4	3.14 (1H, m)	3′, 4′
3′	79.8	3.25 (1H, m)	5′; 6′
4′	79.6	3.15 (1H, m)	3′
5′	76.9	3.09 (1H, m)	6′
6′	63.3	3.57; 3.74 (2H, d, J = 5.4)	1′; 5′



1

Fig. 1. (⟵⟶) Dipolar and (⟵⟶) scalar correlations of the natural substance cotuzine A (**1**).

Information revealed from the COSY spectrum was in agreement with correlations observed on the HMBC spectrum. The heterocyclic ring structure was confirmed via the heteronuclear correlations H-5β/C-6, H-5β/C-6a, H-5β/C-10a, H-4β/C-5, H-1β/C-10a, H-10a/C-8, and H-6/(CH_3-7) (Fig. 1). The correlations H6′/C-5′, H4′/C-3′, H3′/C-5′, and H1′/C-2′ confirm the glycoside skeleton. The junction between the aglycon and the sugar moiety was established via the H1′/C-9 correlation.

Elucidation of the relative stereochemistry of compound **1** was established by the NOESY experiment. Dipolar correlations of the anomeric proton H-1′ with H-3′ and H-5′ prove the β-configuration of the glucopyranose (Fig. 1). Moreover, the coupling constant (J = 7.5 Hz) between H-1′ and H-2′ confirm the β-D-glucopyranose structure. Dipolar correlations revealed from the NOESY spectrum between H-9/H10a, (CH_3-10)/(CH_3-8), and (CH_3-8)/(CH_3-7) determine the relative stereochemistry of compound **1**.

Compound **3**, (2-(2′-hydroxy-5′-methoxyphenyl)propane-1,3-diol), (corimen), was obtained as a yellow oil. The aromatic moiety of compound **3** was established via the scalar correlations observed on the HMBC and COSY spectra.

The HMBC spectrum shows correlations between H-3′/C-5′, H-6′/C-5′, and H-6′/C-2′. The position of the methoxyl group at C-5′ was determined by the correlation OCH_3/C-5′, in the same way that the position at C-1′ of the bialcoholic moiety was established by the correlation H-2/C-1′. Dipolar correlations H-2/H-6′ and H-1/H-6′ observed on the NOESY spectrum confirm the position of the lateral bialcoholic chain.

Compound **1** showed strong activity [9–11] against *Citrobacter freundii* (inhibitory zone IZ = 9.5 mm; minimal inhibition concentration MIC = 500 μg/mL) and *Pseudomonas aeruginosa* (IZ = 8.5 mm; MIC = 500 μg/mL). Compound **1** also showed antifungal activity [12] toward *Trichophyton rubrum* (54%) and *Aspergillus fumigatus* (58%).

EXPERIMENTAL

General Experimental Procedures. ^{1}H, ^{13}C NMR, and two-dimensional NMR spectra were obtained with a Bruker WP 300 spectrometer at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. Measurements were made in CD_3OD at 27°C, and the resonances of residual solvent were used as internal references. Hxcoqf and inv4lplrndqf heteronuclear pulse programs were used respectively for CHCorr and HMBC experiments; 2D homonuclear correlation pulse programs Cosyqf45 and Cosyqfrl were used for COSY experiments. The NOESY experiment was performed based on the Noesyph pulse program. HR-MS spectrum was recorded using an ESI-TOF (Waters-Micromass) apparatus. FAB-MS spectrum was recorded using an HP 1100 MSD apparatus. FTIR spectrum was measured on a Perkin–Elmer 157G infrared spectrophotometer.

Plant Material. The aerial part of *Cotula coronopifolia* (stems, leaves) was collected in April 2008 in Monastir, Tunisia. A voucher specimen (C.C.R01-02) was deposited at the Herbarium in the Faculty of Sciences, University of Monastir, Tunisia, and identified by Dr F. Harzallah-Skhiri, Laboratoire de Biologie Vegetale, Ecole Superieure d′Horticulture et d′Elevage, Chott Meriem, Sousse, Tunisia.

Extraction and Isolation. Dried and finely powdered *Cotula coronopifolia* aerial part (2500 g) was immersed in methanol at room temperature for 24 hours and was extracted four times (4 × 3L) [13, 14]. The obtained extract was filtered and evaporated to dryness. The methanolic residue (620 g) was extracted with petroleum ether (1 L). A petroleum ether extract (57.2 g), symbolized E1, and a methanolic residue, symbolized R1, were obtained. Extraction of the residue R1 with chloroform yielded a chloroform extract (9.4 g), symbolized E2, and a second methanolic residue, symbolized R2. Extraction of residue R2 with ethyl acetate gave an ethyl acetate extract (6 g), symbolized E3, and a third methanolic residue, symbolized R3. Exhaustion of the residue R3 with butanol yielded a butanolic extract, symbolized E4 (15.5 g), and a polar methanolic residue R4. The butanolic extract E4 was fractionated on a chromatographic column (CC) over silica gel (600 g) eluted with a step-gradient of chloroform in acetone. Seventeen fractions were collected. Repeated CC separations of the eleventh fraction (285 mg) afforded compound **3** (18 mg) in pure form as an amorphous solid, while repeated CC separations of the fourteenth fraction (543 mg) yielded compounds **1** (29 mg) and **2** (11.6 mg) in pure form.

Cotuzine A (1). Amorphous solid. FAB-MS, *m/z* 416 $[M + H]^+$. ($C_{21}H_{37}NO_7 + H$); 398 $[M + H - H_2O]^+$ ($C_{21}H_{36}NO_6$); 236 $[M + H - C_6H_{12}O_6]^+$. ESI-HR-MS, ($C_{21}H_{37}NO_7 + H$): calcd 416.2643; found 416.2654. ^{1}H (300 MHz, CD_3OD 3.21 ppm) and ^{13}C NMR (75 MHz, CD_3OD 50.9 ppm), see Table 1.

Cotuzine B (2). Amorphous solid. IR (ν_{max}, cm^{-1}): 3400–3200 (hydroxyl groups); 2950, 2880 (methyl groups); 3010, 830 (secondary amine); 3020, 1650 (double bond). FAB-MS *m/z* 430 $[M + H]^+$. ($C_{22}H_{40}NO_7 + H$); 412 $[M + H - H_2O]^+$ ($C_{22}H_{38}NO_6$). ESI-HR-MS, ($C_{22}H_{40}NO_7 + H$): calcd 430.5171; found 430.5160. ^{1}H NMR (300 MHz, CD_3OD 3.21 ppm, δ, ppm, J/Hz): 5.22 (1H, d, J = 4.8, H-6), 3.33 (1H, m, H-9), 2.03 (1H, m, H-8), 1.82 and 2.02 (2H, m, H-5), 1.85 and 2.00 (2H, m, H-4), 1.55 and 1.77 (2H, m, H-1), 1.57 and 1.84 (2H, m, H-2), 1.27 (1H, dd, J = 5.1, J = 6.9, H-10a), 1.02 (3H, d, J= 6.8, CH_3-8), 1.07 (3H, s, CH_3-7α), 1.14 (3H, s, CH_3-7β), 1.06 (3H, s, CH_3-10), 4.21 (1H, m, H-1′), 3.24 (1H, m, H-3′), 3.17 (1H, m, H-4′), 3.13 (1H, m, H-2′), 3.12 (1H, m, H-5′), 3.51, 3.70 (2H, d, J = 5.4, H-6′). ^{13}C NMR (75 MHz, CD_3OD 50.9 ppm, δ): 145.8 (C-6a), 121.1 (C-6), 89.8 (C-9), 75.4 (C-10), 49.9 (C-10a), 43.3 (C-4), 41.7 (C-2), 41.5 (C-7), 37.9 (C-8), 32.1 (CH_3-7β), 26.2 (C-5), 24.7 (C-1), 23.5 (CH_3-10), 23.5 (CH_3-7α), 12.5 (CH_3-8), 104.9 (C-1′), 80.0 (C-3′), 79.9 (C-4′), 77.2 (C-5′), 74.5 (C-2′), 65.3 (C-6′).

Corimen (3) (2-(2′-Hydroxy-5′-methoxyphenyl)propane-1,3-diol). FAB-MS *m/z* 198 $[M + H]^+$. ($C_{10}H_{14}O_4 + H$); 181 $[M + H - H_2O]^+$ ($C_{10}H_{13}O_3$); 125 $[M + H - C_3H_6O_2]^+$ ($C_7H_9O_2$). ^{1}H NMR (300 MHz, CD_3OD 3.21 ppm, δ, J/Hz): 6.72 (1H, d, J = 2.5, H-6′), 6.65 (1H, d, J = 8.4, H-3′), 6.60 (1H, dd, J = 8.4, 2.5, H-4′), 3.71 (3H, s, O-CH_3), 3.69 (4H, d, J = 6.3, H-1 and H-3), 2.75 (1H, m, H-2). ^{13}C NMR (75 MHz, CD_3OD 50.9 ppm, δ): 150.7 (C-2′), 148.2 (C-5′), 135.5 (C-1′), 123.6 (C-3′), 118.0 (C-4′), 114.9 (C-6′), 67.1 (C-3), 67.0 (C-1), 58.2 (O-CH_3), 53.5 (C-2).

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