New Secondary Metabolites from *Croton sparsiflorus*

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Sparsifol (**1**), a new stereoisomer of inositol, and proaporphine alkaloid crotsparsidine (**2**), have been isolated from the EtOH extract of *Croton sparsiflorus*. Their structures were determined on the basis of ^{1}H and ^{13}C NMR spectra, DEPT, COSY, NOESY, HMBC, HMQC, EI-MS, and FAB-MS experiments.

Key words: Croton sparsiflorus, Euphorbiaceae, Inositol, Proaporphine Alkaloid

Introduction

The genus *Croton* belongs to the family Euphorbiaceae and comprises well over 1300 species growing as trees, shrubs, and herbs in tropical and subtropical regions of both hemispheres [1]. One of its species is *Croton sparsiflorus* (syn. *C. bonplandianus*) which is a shrub growing in sandy clay soil in Asia and South America [2]. It is used as a potent hypotensive agent [3] and for the treatment of a variety of ailments like fever, inflammation, and hypertension [4]. Different extracts of this plant show antibacterial activity [5]. Sifting of the literature revealed that alkaloids, diterpenes, and sterols have previously been reported from this species [6, 7]. The chemotaxonomic and ethnopharmacological importance of the genus *Croton* prompted us to carry out further phytochemical studies on *C. sparsiflorus.* As a result, we have isolated a new stereoisomer of *O*-methyl inositol named as sparsifol (**1**), and a new proaporphine alkaloid crotsparsidine (**2**).

Results and Discussion

The 80 % EtOH extract of the whole plant of *C. sparsiflorus* was suspended in water and successively extracted with *n*-hexane, CH_2Cl_2, AcOEt, *n*-BuOH, and H_2O. Column chromatography of the H_2O- and *n*-BuOH-soluble fractions as described in the Experimental Section resulted in the isolation of compounds **1** and **2**.

Sparsifol (**1**) was obtained as colorless crystals, m. p. 186 – 187 °C, $[\alpha]_D^{24} = -76.2$ (c = 0.18, H_2O).

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data and HMBC correlations of **1** recorded in D_2O.

C	δ_C	δ_H	HMBC (^{1}H-^{13}C)
1	81.1	3.23 (br. t, J = 3.0)	C-2, C-3, C-5, C-6, MeO
2	72.1	3.88 (br. dd, J = 3.6, 3.0 Hz)	C-1, C-3, C-4, C-6
3	73.4	3.39 – 3.46 (br. m)	C-1, C-2, C-4, C-5
4	70.5	3.57 (br. dd, J = 3.0, 2.4 Hz)	C-3, C-5, C-6
5	72.3	3.39 – 3.46 (br. m)	C-1, C-3, C-4, C-6
6	68.1	4.09 (br. dd, J = 3.6, 3.0 Hz)	C-1, C-2, C-4, C-5
MeO	57.1	3.27 (s)	C-1

The UV spectrum showed λ_{max} at 204 nm while the IR spectrum showed the presence of hydroxyl groups (3441 cm^{-1}) and an ether moiety (1280 cm^{-1}). The HR-FAB-MS (positive mode) exhibited an $[M+H]^+$ peak at *m/z* = 195.0861 which is consistent with the molecular formula $C_7H_{15}O_6$. The broad-band (BB) and DEPT ^{13}C NMR (Table 1) spectra of **1** revealed six methine and one methyl carbons. All these signals showed downfield shifts due to their attachment to oxygen atoms. The ^{1}H NMR spectrum (Table 1) showed the methoxyl protons as a singlet at δ = 3.27 (s, 3H) and six oxymethine protons in the range δ = 3.23 to 4.09. Since the molecular formula showed one double bond equivalent, compound **1** must be monocyclic.

Inositols have nine theoretically possible isomers among which *myo*-, D-*chiro*, L-*chiro*-, *neo*-, *muco*-, *scyllo*-, and *allo*-forms have previously been reported. Theoretically, 20 isomeric mono-*O*-methyl ethers of inositols are possible [8]. These isomers have distinct ^{1}H NMR spectra and characteristic coupling constants [8, 9]. Among the 20 possible isomers of mono-*O*-methylated inositols, *muco*-, *allo*- and *cis*-isomers have

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data and HMBC correlations of **2** recorded in CD_3OD.

C	δ_C	δ_H	COSY (^{1}H-^{1}H)	HMBC (^{1}H-^{13}C)
1	111.6	6.83 (s)	–	C-2, C-3, C-13, C-7a, C-14
2	144.2	–	–	–
3	151.3	–	–	–
4	24.1	3.07 (dd, J = 6.5, 7.0 Hz)	H_a-5, H_b-5	C-3, C-5, C-15, C-14
5	44.8	3.80 (dt, J = 6.5, 7.0 Hz)	H_b-5, H-4	C-4, C-6a, C-15
		3.51 (dt, J = 6.5, 6.5 Hz)	H_a-5, H-4	C-4, C-6a, C-15
6	NH	–	–	–
6a	57.9	4.85 (dd, J = 10.5, 11.0 Hz)	H_a-7, H_b-7	C-5, C-7, C-13, C-14, C-15
7	45.0	2.55 (dd, J = 10.5, 7.0 Hz)	H-6a, H_b-7	C-6a, C-7a, C-12, C-13, C-14
		2.45 (dd, J = 11.0, 7.0 Hz)	H-6a, H_a-7	C-6a, C-7a, C-8, C-13, C-14
7a	52.0	–	–	–
8	154.9	6.97 (dd, J = 7.5, 2.5 Hz)	H-9	C-7a, C-7, C-9, C-10, C-12
9	129.7	6.37 (dd, J = 7.5, 2.5 Hz)	H-8	C-7a, C-8, C-10, C-11
10	188.2	–	–	–
11	128.4	6.28 (dd, J = 8.0, 2.5 Hz)	H-12	C-7a, C-9, C-10, C-12
12	151.2	7.11 (dd, J = 8.0, 2.5 Hz)	H-11	C-7, C-7a, C-8, C-10, C-11
13	151.3	–	–	–
14	125.6	–	–	–
15	111.0	–	–	–
MeO	56.9	3.85 (s)	–	C-3

three axially and three equatorially oriented hydroxyl or methoxyl groups. However, in the *muco* form the three axially oriented substituents are adjacent to each other. On the other hand, in the *allo* form the two axially oriented substituents are adjacent to each other while the third axial substituent is next to the adjacent position. Lastly, the *cis* form has alternate axial and equatorial substituents. In the ^{1}H NMR spectrum of **1**, there were clearly three axially and three equatorially oriented protons and methoxyl functionalities. Therefore sparsifol has either a *cis*-, a *muco*- or an *allo*-configuration. The most upfield oxymethine protons at δ = 3.23 can be assigned to the axially oriented proton geminal to the methoxyl functionality. The signal was observed as a broad triplet ($J_{1,2}$ = $J_{1,6}$ = 3.0 Hz). The smaller coupling constant allowed us to assign an equatorial orientation to the oxymethine protons at C-2 and C-6. The signal of H-2 was observed at δ = 3.88 as a broad double doublet (J = 3.0, 3.6 Hz). It showed correlation in the ^{1}H-^{1}H-correlated experiment (COSY) with H-1 as well as with another multiplet of a proton at δ = 3.39 – 3.46, which could subsequently be assigned to H-3. The latter showed connectivity to H-2 as well as with another proton at δ = 3.57 (br. dd, J = 3.0, 2.4, 1H) assigned to H-4. Its connectivity with H-3 and with another proton at δ = 3.43 allowed us to assign the proton at δ = 3.43 to H-5. Both H-1 and H-5 showed connectivity to H-6 at δ = 4.09 (br. dd, J = 3.0, 3.6 Hz). From the relative coupling constants, it was evident that sparsifol has a *cis*-inositol configuration. This could further be confirmed by NOESY correlations between all the oxymethine protons. On the basis of these cumulative evidences, the structure of sparsifol could be assigned as 1-*O*-methyl-*cis*-inositol (Fig. 1).

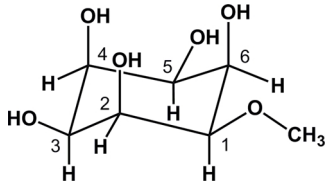

Fig. 1. Structure of sparsifol (**1**).

Crotsparsidine (**2**) was obtained as colorless crystals, m. p. 251 – 252 °C (decomp.), $[\alpha]_D^{25}$ = −26 (c = 0.012, MeOH). It gave a positive test with Dragendorff's reagent for an alkaloid. The UV spectrum showed characteristic bands of the dienone system of proaporphine bases at 286, 231, 201, and 195 nm [6]. The IR spectrum was also characteristic of proaporphine bases showing the presence of NH/OH (3383 cm^{-1}) groups, an α, β-unsaturated ketone (1624 cm^{-1}), an aromatic moiety (1600 – 1400 cm^{-1}), and an ether functionality (1286 cm^{-1}) [10]. The EI-MS showed an $[M]^+$ peak at *m/z* = 283 and an intense peak at *m/z* = 254, the latter peak being characteristic of proaporphine and aporphine bases [6, 11]. The high-resolution EI-MS showed the $[M]^+$ peak at *m/z* = 283.1239 which is consistent with the molecular formula $C_{17}H_{17}O_3N$. The ^{13}C NMR (BB and DEPT) (Table 2) spectra showed 17 well resolved signals comprising one methyl, three methylene, six methine, and seven quaternary carbons. The signals at δ = 188.2,

154.9, 151.2, 129.7, and 128.4 could be assigned to the dienone moiety. The methylene group adjacent to the nitrogen atom gave a signal at δ = 44.8 while the methine carbon adjacent to the nitrogen atom resonated at δ = 57.9. The signals of the aromatic ring at δ = 151.3, 144.2, 125.6, 111.0, and 111.6 revealed the penta-substitution.

The ^{1}H NMR (Table 2) spectrum showed typical signals for a dienone moiety at δ = 7.11 (dd, J = 8.0, 2.5, 1H), 6.97 (dd, J = 7.5, 2.5, 1H), 6.37 (dd, J = 7.5, 2.5, 1H), and 6.28 (dd, J = 8.0, 2.5, 1H). The aromatic proton was observed at δ = 6.83 (s). In addition the singlet at δ = 3.85 (s, 3H) could be assigned to the methoxyl group. The spectral data showed close agreement to those of crotsparine [6]. However, since these two compounds differ in melting point and optical rotation, compound **2** must be an isomer of crotsparine. The close comparison of the NMR data of the two compounds showed slight differences in the chemical shifts of the aromatic ring. The relative positions of hydroxyl and methoxyl groups could be ascertained through HMBC correlations: the H-1 (δ = 6.83) proton showed correlation with C-3 (δ = 151.3), C-2 (δ = 144.2), C-7a (δ = 52.0), and C-14 (δ = 125.6), whereas the methoxyl protons showed correlation with C-3 (δ = 151.3). All the dienone-containing proaporphine bases isolated so far from this species have the α-configuration of the proton at 6a. This could also be confirmed for compound **2** in which the coupling pattern of this proton showed complete agreement to those reported earlier for crotsparine and other proaporphine bases [12]. This could also be authenticated by the negative sign of optical rotation of compound **2** which is at par with other proaporphine bases previously isolated from this plant. Thus the structure of proapophine base **2** can be assigned as shown in Fig. 2.

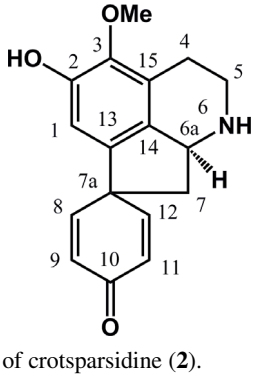

Fig. 2. Structure of crotsparsidine (**2**).

Experimental Section

General experimental procedures

Column chromatography (CC) was carried out using silica gel (230 – 400 mesh, E. Merck, Darmstadt, Germany) and Diaion HP-20 ion exchange resin (Nippon Rensui Co., Mitsubishi Chemical Corporation, Tokyo, Japan). Vacuum liquid chromatography (VLC) was carried out using silica gel (230 – 400 mesh, E. Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed with precoated silica gel F_{254} plates (E. Merck, Darmstadt, Germany), and detection was done at 254 and 366 nm, and by spraying with ceric sulfate in 10 % H_2SO_4 (heating). Melting points were determined on a Gallenkemp apparatus and are uncorrected. Optical rotations were recorded on a Jasco P-2000 polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrophotometer while the IR spectra were recorded from KBr pellets on a Jasco 302-A spectrometer. EI-MS and HR-EI-MS were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers, HR-FAB-MS were measured on a JEOL JMS-HX-110 mass spectrometer with glycerol as matrix, and ions are given in *m/z*. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 spectrometer in D_2O and CD_3OD solvents. The 2D (^{1}H-^{1}H COSY, HMQC, HMBC, NOESY) NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer. Chemical shifts (δ) are given in ppm, relative to tetramethylsilane as an internal standard, and scalar coupling constant (J) are reported in Hertz.

Plant material

The whole plant of *Croton sparsiflorus* Morong (Euphorbiacea) (18 kg) was collected from Thatta district, province Sindh, and identified by Prof. Dr. Surraiya Kahtoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited in the herbarium (voucher specimen No. 4309 KUH).

Extraction and isolation

The freshly collected whole plant material of *C. sparsiflorus* (18 kg) was shade-dried, cut into small pieces, and extracted with 80 % ethanol (3 × 20 L, 10 d for each) at r. t. The combined ethanolic extract was evaporated under reduced pressure at r. t. to yield a residue (300 g), which was suspended in water and successively extracted with *n*-hexane (50 g), CH_2Cl_2 (10 g), EtOAc (6 g), *n*-BuOH (14 g), and H_2O (220 g), respectively. The *n*-butanol-soluble fraction (14 g) was subjected to column chromatography over Diaion HP-20, eluting with H_2O, H_2O-CH_3OH and CH_3OH in decreasing order of polarity. The fraction obtained with H_2O-CH_3OH (2 : 1) (30 mg) was re-chromatographed over SiO_2 and eluted with $CHCl_3$-CH_3OH (9.0 : 1.0) to afford crotsparsidine (**2**) (20 mg).

The H_2O-soluble fraction (80 g) was subjected to VLC over SiO_2 and eluted with $CHCl_3$, $CHCl_3$-MeOH and MeOH in increasing order of polarity. The fraction obtained from $CHCl_3$-CH_3OH (7 : 3) was triturated with CH_3OH to afford sparsifol (**1**) (1.9 g).

*Sparsifol (**1**)*

Colorless crystals (MeOH). – M. p. = 186 – 187 °C. – $[\alpha]_D^{24}$ = −76.2 (*c* = 0.18, H_2O). – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 196 (0.8) nm. – IR (KBr): ν_{max} = 3441, 2935 cm^{-1}. – ^1H NMR and ^{13}C NMR: see Table 1. – MS (EI, 70 eV): *m/z* (%) = 158 (8), $[M–2H_2O]^+$, 144 (9), 129 (8), 116 (15), 102 (20), 87 (90), 73 (100), 60 (35), 55 (10). – HRMS ((+)-FAB): *m/z* = 195.0861 (calcd. 195.0868 for $C_7H_{15}O_6$, $[M+H]^+$).

*Crotsparsidine (**2**)*

Colorless crystals (MeOH). – M. p. = 251 – 252 °C (decomp.). – $[\alpha]_D^{25}$ = −26 (*c* = 0.012, MeOH). – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 286 (1.1), 231 (1.9), 201 (1.5), 195 (4.2) nm. – IR (KBr): ν_{max} = 3383, 2924, 1667, 1600 – 1400 cm^{-1}. – ^1H NMR and ^{13}C NMR: see Table 2. – MS ((+)-EI, 70 eV): *m/z* (%) = 283 (99), $[M]^+$, 266 (17), 254 (77), 239 (17), 222 (18), 211 (26), 183 (16), 128 (40), 115 (56), 91 (26), 77 (56), 65 (40), 55 (100). – HRMS ((+)-EI): *m/z* = 283.1258 (calcd. 283.1249 for $C_{17}H_{17}O_3N$, $[M]^+$).

[1] A. Salatino, L. Maria, F. Salatino, G. Negri, *J. Braz. Chem. Soc.* **2007**, *18*, 11.

[2] A. Radcliffe-Smith in *Flora of Pakistan*, Vol. 172 (Eds.: E. Nasir, I. Ali), Shamim Printing Press, Karachi, **1986**, p. 43.

[3] M. P. Dubey, R. C. Srimal, B. N. Dhawan, *Indian J. Pharmacol.* **1969**, *1*, 73.

[4] S. C. Mandal, S. Dewanjee, B. Parimaladeve, R. Boominathan, R. Mazumder, A. Mazumder, *Proceedings of the International Society of Ethnobiology – Ninth International Congress* (Ed.: E. Great), University of Kent, Canterbury, (U. K.) **2004**, p. 1.

[5] S. Bhuvaneswari, R. Aravind, V. Kaviyarasan, K. Kalaivanan, S. B. Hariram, *Int. J. Pharm. Bio Sci.* **2011**, *2*, 677.

[6] D. S. Bhakuni, S. Satish, M. M. Dhar, *Phytochemistry* **1970**, *9*, 2573.

[7] A. Chatterjee, P. L. Majumder, R. Mukherjee, S. K. Saha, S. K. Talapatra, *Tetrahedron Lett.* **1965**, *6*, 1539.

[8] K. M. Sureshan, T. Murakami, Y. Watanabe, *Tetrahedron* **2009**, *65*, 3998.

[9] S. J. Angyal, L. Odier, *Carbohydr. Res.* **1983**, *123*, 23.

[10] D. S. Bhakuni, M. M. Dhar, *Experientia* **1965**, *24*, 10.

[11] a) M. Tomita, A. Kato, T. Ibuka, H. Furukawa, M. Kozuka, *Tetrahedron Lett.* **1965**, 2825; b) M. Baldwin, A. G. London, A. Maccoll, L. J. Haynes, K. L. Stuart, *J. Chem. Soc.* **1967**, 154.

[12] C. Casagrande, L. Canonica, G. Severini-Ricca, *J. Chem. Soc., Perkin Trans. 1* **1975**, 1659.