Pronuciferine *N*-Oxide, a Proaporphine *N*-Oxide Alkaloid from *Berberis coletioides*

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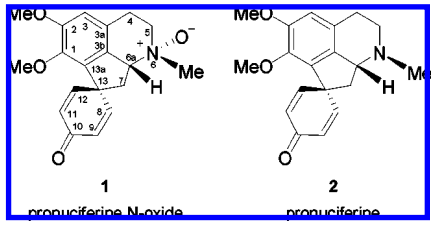
Pronuciferine *N*-oxide (**1**), a proaporphine *N*-methyl-*N*-oxide alkaloid, along with the parent alkaloid pronuciferine (**2**) were isolated from *Berberis coletioides*. The structure of the new compound was determined by spectroscopic evidence. Compound **1** is the first naturally occurring proaporphinoid alkaloid with an *N*-oxide functionality.

Proaporphines, dienone isoquinoline alkaloids, have been recognized as the biogenetic precursors of aporphine[1] and morphine alkaloids.[2] By 1989, 43 proaporphine alkaloids were known from natural sources,[3] and since then, 12 new members have been reported.[4−8] These 55 compounds have been isolated from 11 plant families (Annonaceae, Aristolochiaceae, Berberidaceae, Euphorbiaceae, Fumaraciae, Lauraceae, Menispermaceae, Nelumbonaceae, Papaveraceae, Ranunculaceae, and Siparunaceae).

On the basis of a survey of proaporphine alkaloids, they may be divided into five structural classes, A−E, according to the number and type of rings they possess, Figure S1, Supporting Information. All classes bear in common a completely or partially reduced dienone system. Class D members are proaporphine-tryptamine dimers and have been found only in the genera *Roemeria* (Papaveraceae) and *Phoebe* (Lauraceae), whereas class E members are proaporphine-benzylisoquinoline dimers so far limited in their distribution to the genus *Berberis*. It is noteworthy that whereas the proaporphine-benzylisoquinoline dimers of class E are exclusive of the genus *Berberis*, the proaporphinoid monomer congeners are very infrequent in this genus. In fact, only one proaporphinoid, coyhaiquine, isolated from a South American *Berberis empetrifolia*, has been reported.[9] Interestingly, both *B. empetrofolia* and *B. coletioides* have been collected in southern Chile.

Twelve Chilean species of genus *Berberis* have been chemically studied: *B. acthinacantha*, *B. buxifolia*, *B. chilensis*, *B. darwinii*, *B. empetrifolia*, *B. hakeoides*, *B. ilicifolia*, *B. linearifolia*, *B. microphylla*, *B. montana*, *B. serrato-dentada*, and *B. valdiviana*,[10] and our interest in the search of natural products from species of the Berberaceae family of Southern Chile prompted us to study the hitherto uninvestigated *Berberis coletioides* Lechler.



In this paper, we report on the structure of a new alkaloid (**1**) along with the known (*R*)-form of pronuciferine (**2**),[11,12] $[\alpha]^{20}_D$ +101 ($CHCl_3$). Compond **1** falls into class A of proaporphinoids and is the first example of a naturally occurring *N*-oxide belonging to this alkaloid class.

Compounds **1** and **2** were obtained from the crude extract of *B. coletioides* collected at the Rios Region (Valdivia, Chile), after flash chromatography followed by gel filtration on Sephadex LH-20 chromatography and HPLC. Plants from the *Berberis* genus have been used for years by the indigenous populations in traditional medicine,[13] and studies to date indicate that certain members of these alkaloids exhibit interesting biological activities.[13−16] Proaporphines have also been targets for total synthesis.[12]

Pronuciferine *N*-oxide (**1**) was obtained as an amorphous solid, $[\alpha]^{20}_D$ +20 (*c* 0.3, $CHCl_3$). It gave a molecular ion at *m*/*z* 327.1460 (HREIMS) consistent with the molecular formula $C_{19}H_{21}NO_4$. In its ^{1}H NMR spectrum an aromatic singlet at δ 6.72, cross-ring coupled olefinic signals at δ 6.83, 6.85, 6.39, and 6.28, and several aliphatic signals were observed (Table 1). Partial structures corresponding to a dienone system, $-CH_2-CH_2-$, and $-CH_2-CH-$ were deduced from COSY, ^{13}C NMR, and HMBC data. The NMR data of **1** resembled those of the known proaporphinoid pronuciferine (**2**), also isolated in this work, except for the chemical shifts corresponding to carbons and protons around the isoquinoline nitrogens (Table 1). The downfield chemical shift of a methyl singlet at δ 3.43, as well as the significant downfield signal at δ 5.03 for H-6a, indicated the presence of an electron-withdrawing group bonded to the isoquinolic nitrogen. These data are consistent with an *N*-methyl-*N*-oxide functionality, suggesting that compound **1** is the *N*-oxide derivative of **2**. This was further corroborated by the mass spectrum, which showed the loss of an oxygen atom, giving rise to a peak at *m*/*z* 311 (base peak) (Figure 2S, Supporting Information). Both compounds **1** and **2** gave the same ion at *m*/*z* 282, but HRMS suggested that in compound **1** this ion corresponds to fragment A.

NOESY experiments aided in establishing the stereochemistry of the substituents on the isoquinoline core of **1** (Figure 1). NOESY experiments of **1** revealed a *syn*- periplanar relationship between H-6a and the vicinal *N*-methyl group. Additional NOE interactions, particularly H-12 and H-8 with H-6a and H-7α, respectively, and H-6a with H-7β, were used to fix the spatial disposition of the two nonequivalent sides of the cyclohexadienone ring. In order to obtain an energy-minimized conformation of **1** to account for the observed NOE between H-12 and H-6a, molecular mechanics energy minimization was performed.[17] The minimized structure **1** showed a cyclohexadienone system orthogonal to the plane of the remaining core around the spiro carbon atom, which led to the calculation of a H-12−H-6a interatomic distance (2.442 Å) appropriate for the strong NOE observed (Figure 1). The absolute configuration at C-6a of **1** was assigned as *R* in comparison with **2**.[11]

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Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Pronuciferine *N*-Oxide (**1**) and Pronuciferine (**2**)

	pronuciferine *N*-oxide (**1**)					pronuciferine (**2**)		
position	δ_C, mult.		δ_H (*J* in Hz)	HMBC	COSY	δ_C, mult.		δ_H (*J* in Hz)
1	145.2	qC				144.5	qC	
2	154.6	qC				153.6	qC	
3	111.9	CH	6.72, s	1, 2, 3b, 4		111.8	CH	6.62, s
3a	125.3	qC				128.3	qC	
3b	127.0	qC				133.6	qC	
4	24.2	CH_2	β: 2.98, m α: 3.40, m		H-5	27.2	CH_2	β: 3.00,ddd (17.1, 11.4, 6.8) α: 2.80, dd (17.1, 5.4)
5	65.7	CH_2	3.85, m 4.03, m		H-4	54.8	CH_2	β: 2.54, td (12.0, 5.4) α: 3.15, dd (12.0, 6.8)
6								
6a	74.2	CH	5.03, m	13	H-7	65.6	CH	3.51, dd (11.0, 6.2)
7	39.4	CH_2	β: 2.31, m α: 2.94, m	13, 13a, 6a 13, 6a	H-6a	47.1	CH_2	β: 2.35, dd (11.7, 6.2) α: 2.28, dd (11.4, 11.0)
8	151.8	CH	6.83, dd (10.0, 1.7)	10, 12	H-9	153.3	CH	6.86, dd (9.9, 1.7)
9	128.8	CH	6.39, dd (10.0, 1.7)	13	H-8	128.3	CH	6.38, dd (9.9, 1.7)
10	185.6	qC				186.1	qC	
11	128.1	CH	6.28, dd (9.8, 2.6)	9, 13	H-12	127.5	CH	6.27, dd (9.9, 2.8)
12	148.7	CH	6.85, dd (9.8, 2.6)	8, 10, 13	H-11	149.7	CH	6.99, dd (9.9, 2.8)
13	50.3	qC				51.1	qC	
13a	133.6	qC				132.7	qC	
1-OMe	60.9	CH_3	3.61, s	1		60.9	CH_3	3.58, s
2-OMe	56.5	CH_3	3.81, s	2		56.3	CH_3	3.78, s
6-*N*-Me	56.4	CH_3	3.43, br s	5, 6a		43.2	CH_3	2.40, s

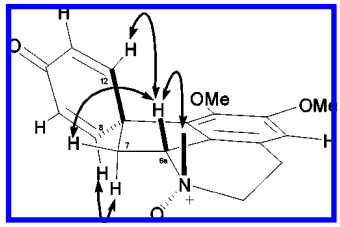

Figure 1. Selected NOEs of pronuciferine *N*-oxide (**1**).

^1H NMR and ^{13}C NMR data of pronuciferine (**2**) have been previously reported,[12] but they were unassigned. We have recorded ^1H and ^{13}C NMR spectroscopic data for **2** as reported in Table 1.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 20 °C. IR spectra were obtained with a Perkin-Elmer 1600/FTIR spectrometer. EIMS and HRMS were taken on a Vg-Micromass Zab 2F spectrometer. ^1H NMR and ^{13}C NMR, HSQC, HMBC, COSY, and NOESY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^1H NMR and at a 125.7 MHz for ^{13}C NMR, using $CHCl_3$ as internal standard. Two-dimensional spectra were obtained with the standard Bruker software. HPLC separations were performed with a Hewlett-Packard HP 1050 chormatograph (Jaigel-Sil semipreparative column, 10 μm, 20 × 250 mm) with hexane/EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane/MeOH/$CHCl_3$ (3:1:1) as eluant. Merck Si gel numbers 7734 and 7741 were used in column chromatography. The spray reagent for TLC was H_2SO_4/H_2O/AcOH (1:4:20).

Plant Material. *Berberis coletioides* was collected at Los Rios Region (Valdivia, Chile). A voucher specimen has been deposited at the Department of Marine Biology, Universidad de Magallanes, Punta Arenas, Chile (number: BB-145/06).

Extraction and Isolation. The dried plant (1 kg) was extracted with acetone at room temperature. The extract was concentrated to give a dark green residue (38 g) and partitioned with H_2O/CH_2Cl_2. The resulting CH_2Cl_2 fraction (7.5 g) was then submitted to a gel filtration column to give fraction A (822.9 mg), which, after flash chromatography on Si gel ($CHCl_3$/MeOH (30:1)) and HPLC (hexane/EtOAc (1:9), 2 mL/min), yielded compound **1** (7 mg, t_R = 35 min) and pronuciferine **2** (12 mg, t_R = 32 min).

Compound 1: amorphous solid; $[\alpha]^{20}_D$ +20 (*c* 0.3, $CHCl_3$); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 327 $[M]^+$, 311 $[M - O]^+$ (base peak), 282 $[M - O - CO - H]^+$, 268, 253; HREIMS *m/z* 327.1460 (calcd for $C_{19}H_{21}O_4N$, 327.1471), 311.1517 (calcd for $C_{19}H_{21}O_4N$, 311.1521), 282.1493 (calcd for $C_{18}H_{20}NO_2$)..

Compound 2: amorphous solid; $[\alpha]^{20}_D$ +101 (*c* 1.5, $CHCl_3$); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 311 $[M]^+$ (base peak), 282 $[M - CH_3N]^+$, 268, 253.

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Supporting Information Available: Figures and NMR spectra of compounds **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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