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

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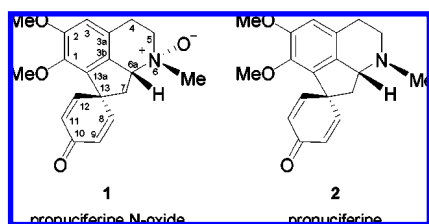
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Pronuciferine *N*-oxide (**1**), a proaporphine *N*-methyl-*N*-oxide alkaloid, along with the parent alkaloid pronuciferine (**2**) were isolated from *Berberis coletioides*. The structure of the new compound was determined by spectroscopic evidence. 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<sup>1</sup> and morphine alkaloids.<sup>2</sup> By 1989, 43 proaporphine alkaloids were known from natural sources,<sup>3</sup> and since then, 12 new members have been reported.<sup>4–8</sup> These 55 compounds have been isolated from 11 plant families (Annonaceae, Aristolochiaceae, Berberidaceae, Euphorbiaceae, Fumariaceae, 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, coyhaquine, isolated from a South American *Berberis empetrifolia*, has been reported.<sup>9</sup> Interestingly, both *B. empetrifolia* and *B. coletioides* have been collected in southern Chile.

Twelve Chilean species of genus *Berberis* have been chemically studied: *B. actinacantha*, *B. buxifolia*, *B. chilensis*, *B. darwinii*, *B. empetrifolia*, *B. hakeoides*, *B. ilicifolia*, *B. linearifolia*, *B. microphylla*, *B. montana*, *B. serrato-dentada*, and *B. valdiviana*,<sup>10</sup> 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**),<sup>11,12</sup> [ $\alpha$ ]<sub>D</sub><sup>20</sup>

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+101 (CHCl<sub>3</sub>). Compound **1** falls into class A of proaporphinoids and is the first example of a naturally occurring *N*-oxide belonging to this alkaloid class.

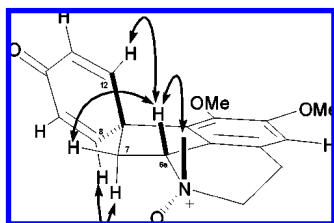
Compounds **1** and **2** were obtained from the crude extract of *B. coletioides* collected at the Rios Region (Valdivia, Chile), after flash chromatography followed by gel filtration on Sephadex LH-20 chromatography and HPLC. Plants from the *Berberis* genus have been used for years by the indigenous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

Pronuciferine *N*-oxide (**1**) was obtained as an amorphous solid, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +20 (c 0.3, CHCl<sub>3</sub>). It gave a molecular ion at *m/z* 327.1460 (HREIMS) consistent with the molecular formula C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>. In its <sup>1</sup>H NMR spectrum an aromatic singlet at  $\delta$  6.72, cross-ring coupled olefinic signals at  $\delta$  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<sub>2</sub>–CH<sub>2</sub>–, and –CH<sub>2</sub>–CH– were deduced from COSY, <sup>13</sup>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  $\delta$  3.43, as well as the significant downfield signal at  $\delta$  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-7 $\alpha$ , respectively, and H-6a with H-7 $\beta$ , 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.<sup>17</sup> 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**.<sup>11</sup>

**Table 1.** NMR Spectroscopic Data (500 MHz, CDCl<sub>3</sub>) for Pronuciferine *N*-Oxide (1) and Pronuciferine (2)

pronuciferine <i>N</i> -oxide (1)					pronuciferine (2)			
position	$\delta_C$ , mult.		$\delta_H$ ( <i>J</i> in Hz)	HMBC	COSY	$\delta_C$ , mult.		$\delta_H$ ( <i>J</i> 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 <sub>2</sub>	$\beta$ : 2.98, m $\alpha$ : 3.40, m		H-5	27.2	CH <sub>2</sub>	$\beta$ : 3.00,ddd (17.1, 11.4, 6.8) $\alpha$ : 2.80, dd (17.1, 5.4)
5	65.7	CH <sub>2</sub>	3.85, m 4.03, m		H-4	54.8	CH <sub>2</sub>	$\beta$ : 2.54, td (12.0, 5.4) $\alpha$ : 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 <sub>2</sub>	$\beta$ : 2.31, m $\alpha$ : 2.94, m	13, 13a, 6a 13, 6a	H-6a	47.1	CH <sub>2</sub>	$\beta$ : 2.35, dd (11.7, 6.2) $\alpha$ : 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 <sub>3</sub>	3.61, s	1		60.9	CH <sub>3</sub>	3.58, s
2-OMe	56.5	CH <sub>3</sub>	3.81, s	2		56.3	CH <sub>3</sub>	3.78, s
6- <i>N</i> -Me	56.4	CH <sub>3</sub>	3.43, br s	5, 6a		43.2	CH <sub>3</sub>	2.40, s

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

<sup>1</sup>H NMR and <sup>13</sup>C NMR data of pronuciferine (2) have been previously reported,<sup>12</sup> but they were unassigned. We have recorded <sup>1</sup>H and <sup>13</sup>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. <sup>1</sup>H NMR and <sup>13</sup>C NMR, HSQC, HMBC, COSY, and NOESY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for <sup>1</sup>H NMR and at a 125.7 MHz for <sup>13</sup>C NMR, using CHCl<sub>3</sub> as internal standard. Two-dimensional spectra were obtained with the standard Bruker software. HPLC separations were performed with a Hewlett-Packard HP 1050 chromatograph (Jaigel-Sil semipreparative column, 10 μm, 20 × 250 mm) with hexane/EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane/MeOH/CHCl<sub>3</sub> (3:1:1) as eluant. Merck Si gel numbers 7734 and 7741 were used in column chromatography. The spray reagent for TLC was H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O/AcOH (1:4:20).

**Plant Material.** *Berberis coletoides* 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<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>. The resulting CH<sub>2</sub>Cl<sub>2</sub> 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<sub>3</sub>/MeOH (30:1)) and HPLC (hexane/EtOAc (1:9), 2 mL/min), yielded compound 1 (7 mg, *t*<sub>R</sub> = 35 min) and pronuciferine 2 (12 mg, *t*<sub>R</sub> = 32 min).

**Compound 1:** amorphous solid; [α]<sub>D</sub><sup>20</sup> +20 (*c* 0.3, CHCl<sub>3</sub>); <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS *m/z* 327 [M]<sup>+</sup>, 311 [M - O]<sup>+</sup> (base

peak), 282 [M - O - CO - H]<sup>+</sup>, 268, 253; HREIMS *m/z* 327.1460 (calcd for C<sub>19</sub>H<sub>21</sub>O<sub>4</sub>N, 327.1471), 311.1517 (calcd for C<sub>19</sub>H<sub>21</sub>O<sub>4</sub>N, 311.1521), 282.1493 (calcd for C<sub>18</sub>H<sub>20</sub>NO<sub>2</sub>).

**Compound 2:** amorphous solid; [α]<sub>D</sub><sup>20</sup> +101 (*c* 1.5, CHCl<sub>3</sub>); <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS *m/z* 311 [M]<sup>+</sup> (base peak), 282 [M - CH<sub>3</sub>N]<sup>+</sup>, 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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