Thalprzewalskiinone, a New Oxobenzylisoquinoline Alkaloid from *Thalictrum przewalskii*

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A novel oxobenzylisoquinoline alkaloid, thalprzewalskiinone (**1**), was isolated from the roots of *Thalictrum przewalskii*, and its structure was established via spectroscopic analysis. Five other alkaloids were isolated: the protoberberines pseudocoptisine and berberine, the aporphines (+)-*N*-methylnantenine and (+)-magnoflorine, and the simple isoquinolone *N*-methyl-6,7-dimethoxyisoquinolone. Isovanillin was also isolated from this extract.

The genus *Thalictrum* (Ranunculaceae) is an extremely rich source of benzylisoquinoline-derived alkaloids, with no fewer than 250 alkaloids having been isolated from some 60 different species.[1,2] In our continuing investigation of the alkaloids of this genus,[1,2] an extract of the Chinese medicinal herb *Thalictrum przewalskii* Maxim. was examined. The flowers and fruits of this plant have been used in the treatment of hepatitis and cirrhosis, while the roots have been employed in the treatment of influenza.[3,4] There has been only one literature report of the isolation of constituents from this species. Nine new alkaloids were recently obtained from an extract of the roots, including seven aporphine−benzylisoquinoline dimers (przewaline, przewalskine, przewalskinine, przewalstine, przewalstinine, przewalstidine, and przewalstidinine), one quaternary pavine (przewalidine chloride), and one protoberberine (8-hydroxypseudocoptisine chloride).[4]

A defatted ethanolic extract of the roots was partitioned according to accepted methods.[5] Extraction of the crude quaternary alkaloid iodide fraction with MeOH afforded a soluble and an insoluble portion. Repeated crystallization of the insoluble portion resulted in the isolation of (+)-magnoflorine iodide. Chromatography of the soluble portion over Si gel and elution with CH_2Cl_2 followed by CH_2Cl_2−MeOH mixtures supplied multiple alkaloid-rich fractions. Combination of these fractions via TLC similarity and repeated column chromatography or preparative-layer TLC gave the simple phenol isovanillin, the protoberberines pseudocoptisine iodide and berberine iodide, the aporphine (+)-*N*-methylnantenine iodide, the simple isoquinolone *N*-methyl-6,7-dimethoxyisoquinolone, and a novel oxobenzylisoquinoline assigned the trivial name of thalprzewalskiinone (**1**).

Thalprzewalskiinone (**1**) was isolated as an optically inactive yellow amorphous residue. The UV spectrum showed absorption maxima at 256 nm (log ϵ 4.54), 302 (4.05), and 326 (4.10), with a pronounced bathochromic shift in alkali of the 302-nm absorption band to 317 nm (log ϵ 4.11). This spectrum was characteristic of an aromatic compound with a phenolic hydroxy group and a carbonyl group in a para relationship.[6,7] The FTIR spectrum displayed strong peaks at 3354 and 1659 cm^{-1}, indicative of a hydroxy group and an extensively conjugated carbonyl group, respectively.[6] The HREIMS displayed the molecular ion at *m/z* 354 (22%) (obsd 354.1438, calcd 354.1431 for $C_{20}H_{20}O_5N$), with other significant or intense fragment ions at *m/z* 353 (100%), 338 (46), 310 (26), 204 (9) (**2**) (obsd 204.1024, calcd 204.1025 for $C_{12}H_{14}O_2N$), 189 (15), 165 (50), and 151 (38) (**3**) (obsd 151.1487, calcd 151.0395 for $C_8H_7O_3$). The ^{1}H NMR spectrum (DMSO-d_6) indicated the presence of three methoxy groups and one quaternary *N*-methyl function as four singlets at δ 3.74, 3.89, 4.08, and 4.14. The presence of seven aromatic protons was observed as follows: two one-proton doublets at δ 8.70 (H-3) (J = 6.8 Hz) and 8.45 (H-4) (J = 6.8 Hz), one one-proton doublet at δ 7.06 (H-6′), two one-proton singlets at δ 6.89 (H-8) and 7.88 (H-5), and two broad one-proton signals at δ 7.24 (H-5′) and 7.49 (H-2′). Repetition of the ^{1}H NMR spectrum at 90 °C aided in the resolution of the one-proton doublet and the two broad peaks. The broad proton signal at δ 7.24 appeared as a doublet at δ 7.27 (H-5′) at higher temperatures, and was ortho-coupled to the proton at δ 7.08 (H-6′). This latter proton appeared as a double doublet, indicating its meta-coupling to the proton at δ 7.45 (H-2′). In addition, there was a relatively lowfield broad signal at δ 9.6, characteristic of a phenolic proton. The ^{13}C NMR spectrum revealed the presence of 20 distinct carbon resonances, including one carbonyl carbon (δ 186.4), one quaternary *N*-methyl carbon (δ 45.4), and three aromatic methoxy carbons (δ 56.2, 56.3, 57.0). The aromatic region contained a total of 15 resonances: seven protonated carbons (δ 104.3, 106.4, 112.4, 115.0, 123.8, 127.0, and 135.9) and eight quaternary carbons (δ 121.5, 125.1, 136.5, 147.6, 149.6, 153.3, 155.6, and 158.0).

These spectral data suggested of a quaternary oxobenzylisoquinoline alkaloid, very similar in structure to gandharamine (**4**),[8] but bearing an additional methoxy group in the benzyl portion of the molecule. Furthermore, the data were also compatible with a structure quite similar to *N*-methylpapaveraldine (**5**) (prepared by the treatment of papaveraldine[9] with methyl iodide), where one of the two benzyl-ring methoxy groups had been replaced by a phenolic hydroxy group. The mass spectral fragment

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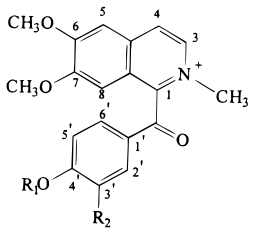
Table 1. Proton and Carbon Chemical Shift Assignments[a] and Long-range Connectivities Observed in the GHMBC Spectrum of Thalprzwalskiinone (1)

	NMR chemical shift (ppm)		
position	^{1}H (mult, J = Hz)	^{13}C	long-range couplings[b]
1		149.6	H-3, H-8, N-2 CH_3
2		45.4	
3	8.70 (d, 6.8)	135.9	
4	8.45 (d, 6.8)	123.8	H-5
4a		136.5	H-3, H-4 (2 bond), H-8
5	7.88 (s)	106.4	H-4
6		153.3	H-5 (2 bond), H-8, C-6 OCH_3
7		158.0	H-5, C-7 OCH_3, H-8 (2 bond)
8	6.89 (s)	104.3	
8a		121.5	H-4, H-5
α		186.4	
1′		125.1	
2′	7.49	115.0	H-6′
3′		155.6	H-5′
4′		147.6	H-6′
5′	7.24	127.0	
6′	7.06	112.4	
NCH_3	4.14	45.4	
$6OCH_3$	3.74	56.3	
$7OCH_3$	4.08	57.0	
$3'OCH_3$	3.89	56.2	

[a] The ^{1}H−^{13}C correlations were based on a GHSQC spectrum (DMSO-d_6). [b] Long-range couplings were based on a GHMBC spectrum (DMSO-d_6) and are specified from the proton indicated to the specified carbon.

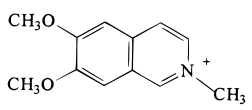
ion at *m*/*z* 204 (**2**) was consistent with the placement of two methoxy groups in the isoquinoline portion of the molecule, while the fragment ion at *m*/*z* 151 (**3**) was similarly consistent with the placement of one methoxy group plus one phenolic hydroxy group in the benzyl portion of the alkaloid.[8−10] The UV spectral data tentatively supported the placement of the phenolic hydroxy group at the C-4′ position because of the characteristic bathochromic shift in alkali that occurs when a phenolic hydroxy group is para to a carbonyl function.[7] Confirmation of this proposed structure and of the ^{1}H and ^{13}C NMR chemical shift assignments was accomplished via the GHSQC spectrum, in which one-bond proton−carbon chemical shift correlations were established, and via the GHMBC spectrum in which long-range heteronuclear correlations were ascertained (Table 1).



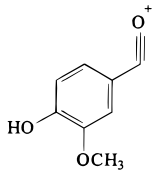
1 $R_1 = H$; $R_2 = OCH_3$

4 $R_1 = R_2 = H$

5 $R_1 = CH_3$; $R_2 = OCH_3$



2



3

Thalprzewalskiinone is the first example of a quaternary oxobenzylisoquinoline alkaloid to have been isolated from a *Thalictrum* species, although gandharamine (**4**) (4′-demethylthalprzewalskiinone) has been isolated from *Berberis baluchistanica* (Berberidaceae),[8] and *N*-methylpapaveraldine (**5**) (papaveraldinium, O-methylthalprzewalskiinone) has been found in *Stephania sasakii* (Menispermaceae).[11] Rugosinone (6,7-methylenedioxy-2′-hydroxy-3′,4′-dimethoxy-oxobenzylisoquinoline) and thalmicrinone (5,6,7,4′-tetramethoxy-oxobenzylisoquinoline) are the only nonquaternary oxobenzylisoquinoline alkaloids that have been isolated from *Thalictrum* species, the former being found as a metabolite of *T. rugosum*[9] and the latter being found in *T. minus* var. *microphyllum*,[10] respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher−Johns hot-stage apparatus and are uncorrected. The UV spectrum was recorded on a Hewlett−Packard HP-845 UV/vis spectrophotometer, while the FTIR spectrum was determined on a Nicolet, Impact 410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker model WH-300 (300 MHz) spectrometer, with DMSO-d_6 as a solvent. The GHSQC and GHMBC experiments were performed in DMSO-d_6 on a Bruker model AMX-400 spectrometer operating at a proton observation frequency of 399.952 MHz and equipped with a Z·SPEC MD-400-3 microdual or MID-400-3 micro inverse probe obtained from Nalorac Cryogenics Corp. (Martinez, CA). The EIMS were recorded on a Fison VG autospec spectrometer (high resolution) or a Fison VG analytical 70-G spectrometer. Column chromatography was carried out on Si gel 60 (70−230 mesh; Merck, Darmstadt, Germany), and column fractions were monitored via TLC over precoated sheets of Si gel 60 F_{254} (0.2 mm layer thickness) (E. Merck) under UV (254 and 365 nm) and by Dragendorff reagent. Preparative TLC was accomplished using 20 × 20 cm glass plates precoated with 1.0 mm Si gel GF_{254} (Analtech). Ion-exchange chromatography was performed using Amberlite IRA-400 (I) resin (Aldrich).

Plant Material. *T. przewalskii* Maxim. was collected in 1990, in various provinces of China and identified by faculty at Beijing Medical University. A voucher specimen is on deposit at the Department of Spectral Analysis, Beijing Medical University, Beijing, 100083, China.

Extraction and Isolation. The air-dried, ground roots (750 g) were extracted by percolation with petroleum ether, followed by air-drying of the plant material, and subsequent extraction with EtOH. The EtOH extract residue (34.9 g) was partitioned between 1% aqueous tartaric acid and Et_2O (fraction A), and the aqueous acidic phase was basified with NH_4OH to pH 8−9 and extracted with Et_2O (fraction B). The basic aqueous phase was acidified to pH 3−4 with HCl and treated with Reinecke salt (ammonium reineckate) solution (1%) until precipitation ceased. The resulting precipitate was filtered by suction, washed with H_2O, dissolved in MeOH (400 mL), and passed through a column of anion-exchange resin (200 g) (iodide form) to afford a dark-brown residue (1.53 g) (fraction C). Addition of MeOH to this residue afforded a soluble portion (fraction C-1) and an insoluble portion (fraction C-2), the former of which was repeatedly recrystallized from MeOH to yield (+)-magnofloroine iodide (600 mg), mp 249−250 °C; $[\alpha]^{23}_D$ +208° (*c* 0.25, MeOH). Fraction C-1 (640 mg) was dissolved in MeOH, adsorbed onto Si gel (1.9 g), and chromatographed over a column (column A) of Si gel (16 g) in CH_2Cl_2. Elution with CH_2Cl_2 afforded the nonalkaloid isovanillin as a white residue (1.5 mg). Elution with CH_2Cl_2−MeOH (99:1 and 98:2) gave a residue (180 mg), which, after rechromatography over Si gel (4 g) in $CHCl_3$−MeOH (99.8:0.2), afforded a residue from which *N*-methyl-6,7-dimethoxyisoquinolone (73 mg) crystallized from Me_2CO. Continued elution of column A with CH_2Cl_2−MeOH mixtures (98:2 and 95:5) gave a residue (54 mg), which, on rechromatography over Si gel (0.5 g), afforded a residue from which berberine iodide (5 mg) crystallized from MeOH. Continued elution of column A with CH_2Cl_2−MeOH mixtures

(95:5 and 98:2) afforded a residue (65 mg) that was adsorbed onto Si gel (60 mg) and rechromtographed over Si gel (1.8 g) in $CHCl_3$−MeOH (98:2) to yield a yellowish-brown residue (15 mg). Preparative TLC of this residue over Si gel plates using MeOH−NH_4OH−H_2O (8:1:1) yielded pseudocoptisine iodide as a yellow residue (2.5 mg). Continued elution of column A with CH_2Cl_2−MeOH mixtures (92:8; 90:10; 60:40) provided a residue (172 mg) that was rechromatographed over Si gel (6.8 g) in CH_2Cl_2−MeOH mixtures. Further rechromatography of two fractions from this column over Si gel columns afforded (+)-*N*-methylnantenine iodide (15 mg), $[\alpha]^{27}_D$ +31° (*c* 1.5, MeOH), as an amorphous brown residue and thalprzewalskiinone (**1**) (2.6 mg).

Thalprzewalskiinone (1): amorphous yellow residue; $[\alpha]^{26}_D$ 0° (c 0.025, MeOH); UV (MeOH) λ_{max} (log e) 256 (4.38), 302 (3.89), 326 (3.94) nm; (MeOH + 0.1N NaOH) 255 (4.45), 317 (3.95) nm; IR ν_{max} (film, NaCl) 3354, 3076, 2937, 2853, 1659, 1581, 1512, 1496, 1455, 1435, 1289, 1165, 1053, 1021, 758 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 354.1438 (calcd for $C_{20}H_{20}O_5N$, 354.1431).

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