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Alkaloids from *Delphinium fangshanense*

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Abstract

From the aerial parts of *Delphinium fangshanense* W.T. Wang, a new norditerpenoid alkaloid, 16-demethyldelsoline, and a new tetrahydrobenzylisoquinoline alkaloid, *O*-methylroefractine *N*-oxide, together with known alkaloids methylycaconitine, nudicauline, delavaine A, delavaine B and magnoflorine were obtained. Their structures were elucidated on the basis of 1-D and 2-D NMR spectroscopic techniques. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Delphinium fangshanense; Ranumculaceae; Diterpenoid alkaloids; 16-Demethyldelsoline; Tetrahydrobenzylisoquinoline alkaloid; *O*-methylroefractine *N*-oxide

1. Introduction

Delphinium fangshanense W.T. Wang is distributed in the northern part of China (Zhongguo & Bianji, 1979) and is used in Chinese medicinal herbs. *Delphinium* and *Aconitum* species (Ranunculaceae) generally contain C_{19}- or C_{20}-diterpenoid alkaloids (Pelletier, Mody, Joshi, & Schramon, 1984; Rahman, 1990); and only a few species have yielded isoquinoline alkaloids (Nambi Aiyar, Benn, Huang, Jacyno, & Jones, 1978; Slavík & Slavíkova, 1995). In continuation of our studies on *Delphinium* species in China, we report here the isolation and structural elucidation of a new norditerpenoid alkaloid, 16-demethyldelsoline (**1**), and a new tetrahydrobenzylisoquinoline alkaloid, *O*-methylroefractine *N*-oxide (**2**), from the aerial parts of *Delphinium fangshanense*. Known alkaloids also isolated from this plant were methyllycaconitine (**3**) (Pelletier, Dailey, & Mody, 1981), nudicauline (**4**) (Kulanthaivel & Benn, 1985), delavaine A (**5**) (Pelletier et al., 1986) and B (**6**) (Pelletier et al., 1986) and magnoflorine (**7**) (Stermitz, Castedo, & Dominguez, 1980).

2. Results and discussion

The aerial parts of *Delphinium fangshanense* were extracted with MeOH, as described in Section 3, to give five major fractions A–F. Chromatographic separation of fraction A on Al_2O_3 afforded 16-demethyldelsoline (**1**), methyllycaconitine (**3**) and nudicauline (**4**). Fraction B afforded delavaine A (**5**) and B (**6**). Fraction F was subjected to column chromatography and yielded the water-soluble alkaloid *O*-methylroefractine *N*-oxide (**2**) and magnoflorine (**7**).

16-Demethyldelsoline (**1**) was isolated as an amorphous powder. The HREIMS showed a molecular ion at *m/z* 453.2715 corresponding to the molecular formula $C_{24}H_{39}NO_7$, which required six degrees of unsaturation. The IR spectrum of **1** exhibited an OH absorption band at 3458 cm^{-1}; no carbonyl absorption band was observed. The ^1H NMR spectrum of **1** indicated the presence of NCH_2CH_3 ($\delta 1.10$, t, $J = 7.3$ Hz) and three methoxyl (δ 3.34 s, 3.39 s, 3.45 s). The DEPT spectrum revealed four quaternary carbon singlets at δ 37.6, 87.8, 78.2 49.1, nine doublets at δ 72.6, 44.0, 90.2, 45.1, 38.0, 42.2, 84.9, 72.9, 66.2, seven tri-

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plets at δ 27.2, 28.7, 29.0, 42.2, 77.3, 57.3, 50.6 and four quartets at δ 13.3, 57.5, 58.1, 59.1. The ^{1}H and ^{13}C NMR spectra resembled the reported spectrum of the norditerpenoid alkaloid delsoline (**8**) (Pelletier, Mody, Sawhney, & Bhattacharrya, 1977), The difference was that the methoxy at C-16 of **8** was replaced by a hydroxyl group. The lycoctonine-type norditerpenoid alkaloids are mostly oxygenated at C-1, C-7, C-8, C-14 and C-16, often at C-6 and C-18, and less commonly at other sites (Pelletier et al., 1984; Hanuman & Katz, 1994). In the HMBC spectrum of **1**, three methoxyl proton signals gave connectivities with C-6 (δ 90.2), C-14 (δ 84.9) and C-18 (δ 77.3), respectively, and long range correlations between H-14 (δ 3.70, t, $J = 4.6$ Hz) and the 14-OCH$_3$ carbon, H-6 (δ 4.03, s) and the 6-OCH$_3$ carbon and H-18 (δ 3.04 m, 4.42 m) and 18-OCH$_3$, indicating that the three methoxyl groups should be located at C-6, C-14 and C-18, respectively. Comparison of the ^{13}C NMR spectrum of **1** with that of **8** revealed an upfield shift of 10 ppm at C-16 and downfield shifts of 4.1 and 4.5 ppm at C-17 and C-13, respectively. The proton signal at δ 3.70 (1H, t, $J = 4.6$ Hz) for 14-βH revealed that the C-9 and C-13 positions were not oxygenated. Thus, the four hydroxyl groups should be located at C-1, C-7, C-8, and C-16, respectively. This conclusion is supported by 2-D NMR experiments, and thus the structure of **1** was assigned as 16-demethyldelsoline.

The alkaloid **2**, $C_{20}H_{25}NO_4$, was obtained as an amorphous solid, and the ^{13}C and ^{1}H NMR spectra Table 3 suggested a tetrahydrobenzylisoquinoline *N*-oxide alkaloid. The ^{1}H NMR spectrum of **2** was very close to that of roefractine **9** (Gözler, Kivcak, Gözler, & Shamma, 1990), except for the downfield shifts of ca. 1.2 and 0.8 ppm for H-1 and *N*-CH$_3$, respectively, which indicated that **2** was a *N*-oxide alkaloid (Debourges, Roblot, Hocquemiller, & Cavé, 1987). Its ^{13}C NMR and DEPT spectra indicated the presence of 20 carbon atoms, corresponding to six quaternary, seven methine, three methylene and four methyl carbon atoms in the molecule. The ^{1}H NMR spectrum of **2** displayed an AA′BB′ system at δ 6.90 (2H, d, $J = 8.5$ Hz) and 7.02 (2H, d, $J = 8.5$ Hz) ppm. Three-proton singlets at δ 3.55, 3.65 and 4.00 ppm represented three methoxyl groups and two one-proton singlets at δ 6.99 and 5.85 ppm were assigned to H-5 and H-8, respectively. The chemical shifts of C-1 (δ 72.4 d) and the *N*-CH$_3$ (δ 38.4 q) carbon atoms were observed at lower field in the ^{13}C NMR spectrum, which supported the above assumption. The UV spectrum of **2** displayed prominent maxima at 230 and 285 nm and was similar to that reported for the tetrahydrobenzylisoquinoline *N*-oxide alkaloids possessing the C-1 *S* configuration (Debourges et al., 1987; Gözler et al., 1990). Thus, the structure of **2** was assigned as (*S*)-*O*-methylroefractine *N*-oxide.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in CHCl$_3$ or H$_2$O with an Autopal-III automatic polarimeter. IR spectra were recorded as KBr discs on a Bio-Rad FTS-185 instrument. MS were recorded on a VQ Quattro mass spectrometer and HREIMS was recorded on Finnigan MAT mass spectrometer. ^{1}H, ^{13}C and 2-D NMR spectra were determined on a Bruker AM-400 spectrometer in CDCl$_3$, with TMS as an internal standard. UV spectra were recorded using a HP 8451A spectrophotometer. All solvents used were analytical grade. Al$_2$O$_3$ (neutral, 200–300 mesh) was used for column chromatography.

3.2. Plant material

The aerial parts of *D. fangshanense* W.T. Wang were

Table 1
^{13}C NMR chemical shift assignments of 16-demethyldelsoline (**1**) and delsoline (**8**)

Carbon	1	8	Carbon	1	8
1	72.6 d	72.6	14	84.9 d	84.5
2	27.2 t	27.2	15	38.0 t	33.9
3	28.7 t	29.3	16	72.9 d	82.9
4	37.6 s	37.4	17	66.2 d	66.0
5	45.1 d	43.9	18	77.3 t	77.3
6	90.2 d	90.4	19	57.3 t	57.2
7	87.8 s	87.8	*N*CH$_2$	50.6 t	50.3
8	78.2 s	78.5	CH$_3$	13.1 q	13.5
9	44.0 d	44.9	6-OCH$_3$	57.5 q	57.2
10	42.2 d	43.3	14-OCH$_3$	58.1 q	57.9
11	49.1 s	49.3	16-OCH$_3$		56.3
12	29.0 t	30.5	18-OCH$_3$	59.1 q	59.1
13	42.2 d	37.7			

Table 2
^{1}H NMR chemical shift assignments and HMBC of 16-demethyldelsoline (**1**)

H	δH	Mult. *J* (Hz)	^{1}H–^{13}C long range correlation
H-1	3.74	brs	C-3
H-2	1.52, 1.95	m, m	C-1, C-18, C-19
H-3	1.62, 1.90	m, m	C-1, C-5
H-5	1.85	s	C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-11, C-17, C-18, C-19
H-6	4.03	s	C-4, C-7, C-8, C-11, 6-OCH_3
H-9	1.87	m	C-10, C-12, C-15
H-10	2.35	m	C-1, C-9, C-13, C-14
H12	1.70, 2.15	m, m	C-9, C-10, C-11, C-14, C-16
H13	3.20	m	C-8, C-9, C-12, C-14, C-15
H-14	3.70	t, 4.6	C-8, C-13, C-16, 14-OCH_3
H-15	1.65, 2.80	dd, dd	C-7, C-8, C-9, C-13, C-16
H-16	3.72	m	C-14
H-17	2.99	brs	C-6, C-19
H-18	3.04, 3.42	m, m	C-2, C-4, C-19, 18-OCH_3
H-19	2.59	m	C-2, C-4, C-17, C-18
N-CH_2	2.90, 3.17	m, m	CH_3
CH_3	1.10	t, 7.3	*N*CH_2
6-OCH_3	3.39	s	C-6
14-OCH_3	3.45	s	C-14
18-OCH_3	3.34	s	C-18

collected from Fansi County, Shanxi Province, People's Republic of China, in August, 1995. It was identified by Professor J. Ma, Department of Biology, Lanzhou University, P.R. China, where a voucher specimen is deposited.

3.3. Extraction and isolation

The aerial parts of the plant (1.8 kg) were percolated with MeOH at room temp. The filtrates were combined and concentrated in vacuum to a dark green syrup. This material was then partitioned between $CHCl_3$ (500 ml) and aq. HCl (2%, 500 ml). The $CHCl_3$ phase was further extracted with aq. HCl (3×300 ml) until the last extractions showed a negative reaction toward Dragendorff's reagent. The acidic soln. was adjusted with NH_4OH to pH 11 and extracted thoroughly with $CHCl_3$ to give the crude alkaloid fraction (2 g). The aqueous phase was extracted with *n*-BuOH to give aqueous alkaloids (fraction F). The crude alkaloids were chromatographed over Al_2O_3 (neutral) and eluted with petrol–Et_2O–EtOAc (5:5:1 to 5:5:5) to afford eight fractions A–D. Fr. A was further sepd. on Al_2O_3 column chromatography (petrol–Et_2O–Me_2CO 7:3:0.5) to yield **1** (20 mg), **3** (50 mg) and **4** (10 mg). Fr. B was subjected to repeated chromatography on Al_2O_3 (neutral) to yield a mixture (140 mg) of **5** and **6**. Fr. F was further purified to give **2** (15 mg) and **7** (50 mg).

3.4. 16-Demethyldelsoline (**1**)

$[\alpha]_D$ +41.5° (*c* 0.4, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3458, 2939, 2879, 1464, 1396, 1298, 1202, 1116, 1085, 754. EIMS *m*/*z* (rel. int.): 453 $[M]^+$ (9), 438 $[M–CH_3]^+$ (100), 420 $[M–CH_3–H_2O]^+$ (90), 404 $[M–OCH_3–H_2O]^+$ (20), 250 (45), 209 (16), 194 (30), 182 (42). HREIMS *m*/*z* 453.2715 ($C_{24}H_{39}NO_7$, 11), 438.2529 ($C_{23}H_{36}NO_7$, 100). ^{1}H and ^{13}C NMR ($CDCl_3$): Table 1Table 2.

3.5. O-methylroefractine N-oxide (**2**)

$[\alpha]_D$ −2.26° (*c* 1.7, H_2O); UV λ_{max} nm: 230 (log ε, 4.40) and 285 (log ε, 3.61); EIMS *m*/*z* (rel. int.): 343 (0.5) $[M]^+$, 327 (2), 325 (1), 313 (5), 284 (2), 207 (50),

Table 3
^{1}H NMR chemical shift assignments of (**2**) and roefractine (**9**)

Atoms	δ_H Mult.*J* (Hz)	
	2	**9**
α–αH	3.82 dd (12.4, 3.4)	
α–βH	3.02 dd (12.4, 10.6)	
1-αH	4.81 dd (3.4, 10.6)	3.67 m
3-αH	4.09 m	
3-βH	3.50 m	
4-H	3.34 m	
5-H	6.99 s	6.62 s
8-H	5.85 s	5.95 s
2′, 6′	7.02 d (8.5)	7.02 d (8.4)
3′, 5′	6.90 d (8.5)	6.82 d (8.4)
N-CH_3	3.35 s	2.54 s
6-OCH_3	4.00 s	
8-OCH_3	3.55 s	3.56 s
4′-OCH_3	3.65 s	3.79 s

206 (100), 191 (35), 190 (40), 162 (12), 149 (5); CIMS: 343 $[M]^+$; ^1H NMR ($CDCl_3$): Table 3; ^{13}C NMR ($CDCl_3$) δ (ppm): 38.4 t (C-α), 74.2 d (C-1), 53.3 t (C-3), 24.6 t (C-4), 123.1 s (C-4α), 113.3 d (C-5), 150.8 s (C-6), 148.3 s (C-7), 112.5 d (C-8), 121.8 s (C-8α), 127.1 s (C-1′), 116.7 d (C-3′, 5′), 132.7 d (C-2′, 6′), 158.0 s (C-4′), 51.6 q (*N*-CH_3), 55.8 q (OCH_3), 56.2 q (OCH_3), 56.7 q (OCH_3).

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References

Rahman, A. U. (1990). *Handbook of natural products data, diterpenoid and steroidal alkaloids*. Amsterdam: Elsevier Science Publishers.

Debourges, D., Roblot, F., Hocquemiller, R., & Cavé, A. (1987). *Journal of Natural Products*, *50,* 852.

Gözler, B., Kivcak, B., Gözler, T., & Shamma, M. (1990). *Journal of Natural Products*, *53,* 666.

Hanuman, J. B., & Katz, A. (1994). *Journal of Natural Products*, *57,* 1473.

Kulanthaivel, P., & Benn, M. (1985). *Heterocycles*, *23,* 2515.

Nambi Aiyar, V., Benn, M., Huang, Y. Y., Jacyno, J. M., & Jones, A. J. (1978). *Phytochemistry*, *17,* 1453.

Pelletier, S. W., Dailey Jr., O. D., & Mody, N. V. (1981). *Journal of Organic Chemistry*, *46,* 3284.

Pelletier, S. W., Mody, N. V., Sawhney, R. S., & Bhattacharrya, J. (1977). *Heterocycles*, *7,* 327.

Pelletier, S. W., Mody, N. V., Joshi, B. S., & Schramon, L. C. (1984). Alkaloids: chemical and biological perspectives, vol. 2 (pp. 206). New York: Wiley.

Pelletier, S. W., Harraz, F. M., Badaw, M. M., Tantiraksachai, S., Wang, F. P., & Chen, S. Y. (1986). *Heterocycles*, *24,* 1853.

Slavík, J., & Slavíkova, L. (1995). *Collection of Czechoslovak Chemical Communications*, *60,* 1041.

Stermitz, F. R., Castedo, L., & Dominguez, D. (1980). *Journal of Natural Products*, *43,* 140.

Zhongguo, Z. Z., & Bianji, W. (1979). Zhongguo zhiwu zhi, vol. 27 (pp. 447). Beijing: Science Press.