NORDITERPENOID ALKALOIDS FROM *DELPHINIUM MENZIESII* DC

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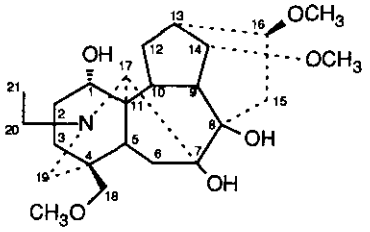
Abstract - An investigation of the alkaloids of *Delphinium menziesii* resulted in the isolation and identification of eleven norditerpenoid alkaloids, of which ten were known and one was new: the latter, named delmenzine, corresponded to 1-*O*-desmethyldelelatine.

As a continuation of our studies of the alkaloids of species of *Delphinium* native to Western Canada we have examined *D. menziesii* DC from Vancouver Island. Conventional processing[1] of air-dried epigeal plant material yielded a mixture of bases which was then subjected to fractionation by vacuum short-column chromatography on alkaline alumina,[2] followed by extensive ptlc on alumina or silica gel 60. By these means eleven compounds were isolated which were homogeneous, or nearly so, as judged by analytical tlc and their ms, ^{1}H and ^{13}C nmr spectra. All proved to be norditerpenoids of the kind normally associated with *Delphinium* and nine were recognized[3] as the known alkaloids anhweidelphinine,[4] browniine, delcosine, deltatsine, desacetyl 6-*epi*pubescenine,[5] methyllycaconitine, nudicauline, takaosamine and virescenine. A tenth was judged to have the structure (*I*) which corresponds to umbrosine,[6] but in this case we were unable to obtain a complete set of data from the original isolate to compare with those of our base (in particular the ^{13}C nmr data, see Table 1) in order to clinch the identification. The eleventh alkaloid, which we have called delmenzine, appeared to be previously unknown. The evidence which led us to its structure may be summarized as follows. Delmenzine, obtained amorphous in very small amount, gave a hreims with an apparent molecular ion m/z (rel. abundance) 437.2473(28) (calcd for $C_{23}H_{35}NO_6$ 437.2465) with other prominent high-mass ions at m/z 404 (94), 391 (100), 362 (14) and 194 (20) amu. The broadband ^{13}C nmr spectrum of delmenzine contained absorptions corresponding to twenty-three magnetically nonequivalent nuclei, of which seven appeared to be at

low-field as a result of substitution by oxygen, one being a methylenedioxy resonance (see Table 1). Thus the molecular composition was inferred to be $C_{23}H_{35}NO_6$, with an index of hydrogen deficiency of seven. The 400 MHz ^1H nmr spectrum of delmenzine was very well resolved and contained signals which could be attributed to an *N*-ethyl, a quaternary *C*-methyl, a methoxyl, and a methylenedioxy functions. Taken together with the absence in both the ^1H and ^{13}C nmr spectra of absorptions which corresponded to esters or olefinic units, this



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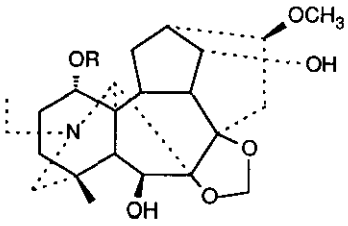

2 R=H
4 R=CH_3

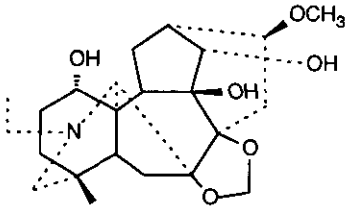

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Table 1 ^{13}C nmr data[a] for delmenzine (*2*) and the models (*3*,*4*); as well as the presumed umbrosine (*1*).

		2	*4*[7]	*3*[8]	*1*
	C1	72.2	83.9	72.2	72.3
	2	29.7	26.3[b]	29.2	29.4
	3	32.1	36.8	30.8	30.1
	4	32.9	34.2[c]	32.9	37.8
	5	52.9	55.9	43.1	41.6
	6	79.1	78.8	30.1	33.6
	7	92.4	94.0	89.8	85.5
	8	84.2	81.8	83.2	77.1
	9	42.3	42.6[d]	78.5	47.1
	10	45.6	47.8[d]	50.1	43.2
	11	50.5	49.7	52.2	49.9
	12	28.6	27.0[b]	26.3	26.8
	13	38.6	36.5	38.9	37.0
	14	74.6	73.9	80.4	84.8
	15	34.2	32.2[c]	36.7	36.2
	16	81.4	81.8	81.6	82.6
	17	65.0	63.9	62.7	64.7
	18	26.6	25.3	27.0	78.8
	19	61.4	57.5	60.3	55.8
	20	50.0	50.6	49.8	50.5
	21	13.7	14.0	13.5	13.7
OCH_3	1	---	56.4	---	---
	14	---	---	---	57.6
	16	56.3	56.4	56.4	56.2
	18	---	---	---	59.4
$-OCH_2O-$		93.3	93.2	93.9	---

[a] The chemical shifts (δ) are in ppm for samples dissolved in $CDCl_3$ (δ 77.0); [b,c] values with the same superscripts may be interchanged; [d] these values have been switched following findings[9] with similar methylenedioxy systems.

indicated that delmenzine was most probably a methylenedioxy derivative of a heptacyclic alkaloid of the lycoctonine type. The application of a variety of nmr measurements (including DEPT ^{13}C, INVERSE ^{1}H ^{13}C, ^{1}H ^{1}H COSY, and ^{1}H ^{1}H NOESY spectra[10]) resulted in the generation of the complete structure as ***2***. In particular, the appearance in the ^{1}H nmr of a triplet-like double doublet resonance at δ 4.21 (J=5.0 Hz) indicated 14α-hydroxylation, with flanking hydrogens at C-9 and C-13 i.e. ruled out hydroxylation at either of these adjacent positions and thus differentiated delmenzine from the isomeric tatsidine (***3***).[8] The methylenedioxy functionality was placed between C-7 and 8 on the basis of precedent and the ^{13}C chemical shifts attributed to these atoms.[8,9,11,12] Similarly, norditerpenoid alkaloids almost invariably have a 16β-methoxyl group and a ^{13}C resonance (δ 81.4 ppm) in the spectrum of delmenzine was in accord with this location of its methoxyl group.[11,12] The remaining two oxygen-functionalized carbon atoms corresponded to secondary alcohols (DEPT spectra) and their chemical shifts corresponded well with expectation[11,12] for the 1α- and 6β-hydroxylation shown in ***2***.

These assignments were supported by additional observations. Thus the proton attached to the supposed C-6 appeared in the ^{1}H nmr spectrum of delmenzine as a singlet as required by ***2*** ($\theta_{5\beta,6\alpha} \approx 90°$ i.e. $J_{5\beta,6\alpha} \approx 0$ Hz), and showed NOESY correlations to the quaternary methyl (H-18) and one of the AB pair of hydrogens attributed to H-19. Similarly H-16 was identified and shown to be coupled to the H-15 AB pair, and the assignment of C-16 was confirmed (INVERSE); as well H-1 was shown to be coupled to H-2, and to have an NOESY correlation to H-10 and/or H-12β, as anticipated for ***2*** (see Table 2). Finally, the structure deduced for delmenzine corresponds to the 1-*O*-desmethyl derivative of the recently described *D. elatum* alkaloid delelatine (***4***)[7], and the ^{13}C nmr spectra (Table 1) of the two alkaloids differ only as expected for this relationship.

D. menziesii has been reported to be toxic to livestock ,[13] and this is consistent with the methyllycaconitine content, since this is known to be a potent neurotoxin.[14]

EXPERIMENTAL

General. Melting points are uncorrected. The ^{1}H and ^{13}C nmr spectra were determined of solutions in $CDCl_3$ with residual $CHCl_3$ (δ 7.27) and $^{13}CDCl_3$ (δ 77.0) as internal references, using Bruker ACE-200 and AM-400 spectrometers. Chemical shifts (δ) are in ppm, coupling constants (J) in Hz. The ir spectra were recorded with a Mattson FT-IR model 4030 of samples dispersed in KBr discs. The eims data were obtained with a MS-80 mass spectrometer. All tlc separations were carried out on silica gel 60 F254 (Merck 5715) or aluminum oxide 60 Type E (Merck 5731) plates.

Extraction and preliminary separation of the alkaloids. Air-dried and powdered epigeal parts of *Delphinium menziesii* (88 g) were extracted with methanol (5 × 500 ml) in a Waring blendor. The extracts were concentrated to a residual gum (26 g) which was partitioned between 5% aq. tartaric acid (250 ml) and Et_2O (500 ml). The aqueous layer was then basified with saturated aq. Na_2CO_3 and extracted with $CHCl_3$ (5 × 200 ml). Evaporation of the combined, dried ($MgSO_4$) $CHCl_3$ extracts gave the crude alkaloids as a tan-coloured glass (697 mg, 0.79% wt. of dry plant). This was then subjected to vacuum short column chromatography on alumina (Merck type E basic), eluted sequentially with hexane (100 ml), $CHCl_3$-hexane (1:1, 150 ml; 3:2, 100 ml; 3:1, 50 ml; 9:1, 200 ml), $CHCl_3$ (150 ml), $CHCl_3$-MeOH (99:1, 100 ml; 50:1, 150 ml; 95:5, 150 ml, 1:1, 50 ml) and MeOH (100 ml). Fractions (50 ml) were collected, analysed by tlc, and then subjected to further fractionation as follows.

Isolation of umbrosine (1), methyllycaconitine, and anhweidelphinine. Fraction 7 (eluted with hexane-$CHCl_3$, 3:2) (83 mg) was separated by ptlc (silica gel, $CHCl_3$-Me_3OH, 9:1) into 3 components.

The first of these was crystallized from CH_2Cl_2-Me_2CO to afford umbrosine (26 mg), mp 150-152°C (lit.,[6] mp 150-151°C; hreims 437.2777 (calcd for $C_{24}H_{39}NO_6$ 437.2737); ^{1}H nmr δ 3.74 (1H, t, J=4.7 Hz, H-14), 3.66 (1H, m, H-1), 3.41, 3.36 and 3.34 (each 3H, s, 3 × OCH_3), 3.28 (1H, br t, J≈8 Hz, H-16), 3.23 and 3.04 (each 1H, d, J=8.8 Hz, H-18), *ca.* 2.95 (3H, m, H-15A and H-20), 2.74 (1H, d, J=11 Hz, H-19A), 2.66 (1H, br s, H-17), 2.43 (1H, m, H-13), 2.40 (1H, d, J=11 Hz, H-19B), 2.28 (1H, dd, J=7.6 and 14.8 Hz, H-6A), 2.22 (1H, br dd, J≈5 and 7 Hz, H-9), *ca.* 2.02 (1H, ddd, J=7.8, 11.2 and 14.2 Hz, H-12A), 1.91 (1H, dd, J=4.7 and 13.8 Hz, H-3A), 1.83 (1H, ddd, J=5, 7.1 and *ca.* 12 Hz, H-10), 1.77 (1H, br d, J=7.6 Hz, H-5), 1.67 (1H, ddd, J=2.5, 5 and 14.2 Hz, H-12B), *ca.* 1.59 (4H, m, H-2, 6B and 15B), *ca.* 1.53 (1H, m, H-3B) and 1.11 (3H, t, J=7.2 Hz, H-21); ^{13}C nmr see Table 1.

The second component was obtained as a glass (30 mg) which was identified as methyllycaconitine by comparison of its eims, ^{1}H and ^{13}C nmr spectra with those reported[11] for this alkaloid.

The third substance, obtained amorphous (4.8 mg), was identified as anhweidelphinine on the basis of its eims m/z 652 (M^+) with prominent high-mass fragment-ions at m/z 637, 621, 436, 420, 370 and 216 amu, ν_{max} 3465, 1714, 1602, 1465, 1390 and 1089 cm^{-1}; ^{1}H nmr δ 8.05 (1H, d, J=7.7 Hz, aryl H-6), 7.71 (1H, dt, J=1.5 and 7.7 Hz, aryl H-4), 7.55 (1H, dt, J=1.5 and 7.7 Hz, aryl H-3), 4.05 (1H, s, H-17), 3.78 (1H, s, H-6), 3.67 (1H, t, J=4.4 Hz, H-14), 3.43 (3H, s, 6-OCH_3), 3.29 and 1.78 (each 1H, m, H-15), 3.29 (1H, m, H-16), 3.21 (1H, m, H-1), 2.85 (1H, br s, H-9), 2.05 (1H, br s, H-5), 2.04 (1H, m, H-10), 1.55 (2H, m, H-12), 1.75 (1H, m, H-2′) and 1.45 (3H, d, J=7 Hz, H-5″). This, its ^{13}C nmr spectrum, and tlc behaviour were identical with those of a sample of

anhweidelphinine.[15]

Isolation of nudicauline. Fraction 5 (eluted with hexane-$CHCl_3$, 3:2) yielded a solid residue (11 mg) which was subjected to ptlc (silica gel, $CHCl_3$-MeOH, 9:1) to give an amorphous solid (4.5 mg) whose ^1H and ^{13}C nmr properties were as reported[12] for nudicauline.

Isolation of deltatsine. Fraction 6 (eluted with hexane-$CHCl_3$, 1:1) gave a solid (111 mg) which was subjected to ptlc (silica gel, $CHCl_3$-MeOH, 9:1) to afford, besides browniine,[11] methyllycaconitine[11] and umbrosine,[6] a substance (15 mg) whose ^1H and ^{13}C nmr were as reported[12] for deltatsine.

Isolation of delcosine, and *delmenzine (2).* Fractions 15-17 (eluted with $CHCl_3$) were combined to give a glass (160 mg) which was crystallised from Me_2CO to afford colourless needles of delcosine (82 mg), whose mp 212°C, ^1H and ^{13}C nmr were as reported.[11]

The material present in the mother liquors was subjected to ptlc (alumina, $CHCl_3$-MeOH, 40:1) to give, besides more delcosine,[11] amorphous delmenzine (2 mg), ^1H nmr δ 5.16 and 5.10 (each 1H, s, OCH_2O), 4.29 (1H, s, H-6), 4.21 (1H, t, J=5.0 Hz, H-14), 3.73 (1H, br m, H-1), 3.59 (1H, dd, J=6.7 and 5.0 Hz, H-9), 3.40 (1H, m, H-16), 3.38 (3H, s, OCH_3), 3.05 (1H, br s, H-17), 2.83 and 2.72 (each 1H, m, H-20), 2.61 (1H, dd, J=16 and 9.2 Hz, H-15A), 2.54 (1H, d, J=11.6 Hz, H-19A), 2.39 (1H, br t, J=5 Hz, H-13), 2.31 (1H, d, J=11.6 Hz, H-19B), *ca.* 2.14 (2H, m, H-10 and 12A), 1.88 (1H, dd, J=16 and 5.3 Hz, H-15B), *ca.* 1.74 (2H, m, H-3A, 12B), *ca.* 1.62 (2H, m, H-2), *ca.* 1.56 (1H, m, H-3B), 1.40 (1H, br s, H-5), 1.15 (3H, t, J=7.2 Hz, H-21), 1.08 (3H, s, H-18); ^{13}C nmr see Table 1.

Isolation of virescenine and takaosamine. Fractions 19-25 (eluted with $CHCl_3$-MeOH, 50:1) were combined and evaporated. The residual gum (70 mg) upon ptlc (silica gel, $CHCl_3$-MeOH 40:1) gave one substance (9 mg) whose ^1H and ^{13}C nmr were as reported[11] for virescenine, and another (11 mg) similarly identical with takaosamine.[11,12]

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