

FURTHER NORDITERPENOID ALKALOIDS FROM DELPHINIUM NUTTALLIANUM

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Abstract - A study of the minor bases of *D. nuttallianum* resulted in the isolation and identification of eight known and five new diterpenoid alkaloids.

In the course of an investigation of the toxic properties of *D. nuttallianum* Pritz., a cow-poisoning plant of the rangeland of Interior British Columbia, we identified sixteen known and four new norditerpenoid alkaloids (first designated A-D),¹ as well as one new diterpenoid base.² We now report the outcome of further fractionation of the minor bases of this plant, by vsec on basic alumina³ followed by repeated ptlc on silica gel 60, which resulted in the separation of another thirteen components. Individually these were obtained as amorphous solids in very small amounts (< 5 mg, 0.56% of the total plant alkaloids) but the application of modern spectrometric procedures, including in some cases proton-detected (inverse) heteronuclear proton-carbon nmr chemical shift correlations according to Bax and Subramanian,⁴ allowed us to deduce their structures. Eight were thus identified⁵ as alkaloids which had been described before: N-acetyldelectine, bicolorine, 2-dehydrohetisine, delectinine, hetisine,⁷ hetisine-11,13-di-O-acetate,⁷ lycoctonine and takosamine. The other five alkaloids (*E* - *I*) appear to be new. The evidence which led us to their structures may be summarised as follows.

Alkaloid *E* was suspected to have the composition $C_{24}H_{39}NO_7$ on the basis of its eims^{8a} (apparent molecular ion at m/z 453) and ^{13}C -nmr spectrum^{8b} (resonances corresponding to 24 carbon atoms). Its 1H nmr spectrum^{8c} contained signals which indicated the presence of an N-ethyl (δ 1.11, 3H, t, $J=7.2$ Hz for $CH_3CH_2N<$) and three methoxyl groups (δ 3.37, 3.41, and 3.51, each 3H, s). This suggested that *E* had a C_{19} -norditerpenoid skeleton of the aconitine or lycoctonine kind. A decision in favour of the latter followed from the assignment of an absorption (δ 4.40, 1H, s) to a 6α -H adjacent to a hydroxylated C-7. The presence of a 14α -hydroxyl group was inferred from another low-field carbonyl resonance (δ 4.04, t, $J=4.5$ Hz). Since there was no signal corresponding to a tertiary C-methyl group we knew that C18 was also oxygenated. The distribution of the three methoxyl groups at C8, 16 and 18 followed from an examination of the ^{13}C -nmr spectrum of *E*: there being resonances characteristic of the tertiary methoxyl (δ 52.0), β -methoxylated C16 (δ 82.0), and C18 (methoxy)methylene (δ 78.9). The structure (2) thus deduced for *E* corresponded to deacetylated 6-*epi*-pubescenine (our original alkaloid-A) (*I*) and the ^{13}C -nmr spectra of the two alkaloids were very

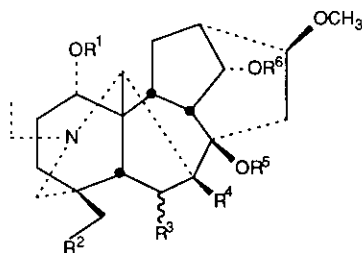
Table 1. ^{13}C -Nmr chemical shift data for the desacetyl-6-*epi*-pubescenine (2), 6-*epi*-neolinine-14-acetate (4), nuttalline (6), bicolorine-14-acetate (8), delectinine-14-acetate (10) and related models.

Carbon	1 ¹	2	3 ¹	4	5 ⁹	6	7	8	9	10
1	72.6	72.6	72.4	72.4	86.1	85.3	73.0	72.6	85.1	84.1
2	26.9	27.1	26.9	26.7	26.0	25.6	29.8 ^b	29.7 ^b	25.3	26.1
3	29.2	28.5	30.0	29.9	35.2	31.9	32.4	31.6	31.6	31.6
4	37.2	37.2	37.7	37.6	39.2	39.0	32.9	32.6	38.8	38.6
5	44.2	45.0	44.4	43.5	48.8	46.3	46.0	44.0	46.1	45.7
6	81.1	80.7	82.5	82.9	82.5	82.3	72.3	72.4	90.1	90.4
7	90.1	90.0	50.9	51.1	52.8	51.2	54.8	54.6	89.0	88.3
8	84.7	84.3	75.4	75.1	72.6	74.1	76.1	76.2	76.3	- ^c
9	41.5	43.0	46.2	44.7 ^b	50.3	49.6	49.8	50.4	45.1	42.6
10	49.3	49.3	45.2	44.0 ^b	45.7 ^a	46.1	44.4 ^a	43.5	49.5	49.3
11	49.6	49.1	48.5	48.5	50.4	48.4	48.4	48.7	48.2	48.8
12	27.2	27.3	29.6	29.6	28.6	28.1	29.1 ^b	29.4 ^b	27.5	28.2
13	36.5	39.6	39.8	37.9	38.4 ^a	37.2	39.7 ^a	36.7	36.5	38.1
14	75.4	74.7	76.1	76.7	75.5	75.6	76.2	77.3	75.3	76.0
15	29.4	29.1	40.7	40.2	39.2	37.2	42.9	42.7	33.1	33.7
16	82.2	82.0	81.8	81.7	82.2	82.1	82.0	82.2	81.8	82.3
17	66.5	66.8	65.3	65.0	64.2	64.2	65.2	65.0	65.4	64.8
18	78.9	78.9	67.4	67.3	80.8	68.1	27.4	27.5	67.6	67.8
19	57.7	57.6	57.7	57.6	54.0	53.7	61.8	61.9	52.8	52.5
NCH ₂	50.6	50.7	48.5	48.5	49.3	49.5	48.6	48.4	51.3	51.1
CH ₃	13.8	13.8	12.9	12.9	13.6	13.6	13.1	13.0	14.2	14.2
OCH ₃										
1'					56.3	56.4			56.0	55.8
6'			57.6	57.6	57.6	57.7			58.1	57.9
8'	51.5	52.0								
16'	56.3	56.6	56.3	56.2	55.9	56.3	56.3	56.1	56.5	56.2
18'	59.6	59.5			59.2					
CO ₂	170.2			171.6				170.7		171.9
CH ₃	21.3			21.5				21.4		21.5

a = reassigned [9].

b = may be interchanged.

c = hidden under the solvent signals.



1. Alkaloid A (6-*epi*-pubescenine)
 $\text{R}^1 = \text{H}, \text{R}^2 = \text{OCH}_3, \text{R}^3 = \beta\text{OH}, \text{R}^4 = \text{OH}, \text{R}^5 = \text{CH}_3, \text{R}^6 = \text{Ac}$
2. Alkaloid E (desacetyl-6-*epi*-pubescenine)
 $\text{R}^1 = \text{H}, \text{R}^2 = \text{OCH}_3, \text{R}^3 = \beta\text{OH}, \text{R}^4 = \text{OH}, \text{R}^5 = \text{CH}_3, \text{R}^6 = \text{H}$
3. Alkaloid D (6-*epi*-neolinine)
 $\text{R}^1 = \text{H}, \text{R}^2 = \text{OH}, \text{R}^3 = \beta\text{OCH}_3, \text{R}^4 = \text{R}^5 = \text{R}^6 = \text{H}$
4. Alkaloid F (6-*epi*-neolinine-14-acetate)
 $\text{R}^1 = \text{H}, \text{R}^2 = \text{OH}, \text{R}^3 = \beta\text{OCH}_3, \text{R}^4 = \text{R}^5 = \text{H}, \text{R}^6 = \text{Ac}$
5. Chasmanine
 $\text{R}^1 = \text{CH}_3, \text{R}^2 = \text{OCH}_3, \text{R}^3 = \alpha\text{OCH}_3, \text{R}^4 = \text{R}^5 = \text{R}^6 = \text{H}$
6. Alkaloid G (nuttalline)
 $\text{R}^1 = \text{CH}_3, \text{R}^2 = \text{OH}, \text{R}^3 = \beta\text{OCH}_3, \text{R}^4 = \text{R}^5 = \text{R}^6 = \text{H}$
7. Bicolorine
 $\text{R}^1 = \text{R}^2 = \text{H}, \text{R}^3 = \beta\text{OH}, \text{R}^4 = \text{R}^5 = \text{R}^6 = \text{H}$
8. Alkaloid H (bicolorine-14-acetate)
 $\text{R}^1 = \text{R}^2 = \text{H}, \text{R}^3 = \beta\text{OH}, \text{R}^4 = \text{R}^5 = \text{H}, \text{R}^6 = \text{Ac}$
9. Delectinine
 $\text{R}^1 = \text{CH}_3, \text{R}^2 = \text{OH}, \text{R}^3 = \beta\text{OCH}_3, \text{R}^4 = \text{OH}, \text{R}^5 = \text{R}^6 = \text{H}$
10. Alkaloid I (delectinine-14-acetate)
 $\text{R}^1 = \text{CH}_3, \text{R}^2 = \text{OH}, \text{R}^3 = \beta\text{OCH}_3, \text{R}^4 = \text{OH}, \text{R}^5 = \text{H}, \text{R}^6 = \text{Ac}$

similar apart from the absence in that of *E* of the acetate resonances (see Table 1). Confirmation of this relationship was provided by saponification of *I* with methanolic sodium methoxide which yielded material spectroscopically identical to *E*, which we have therefore called desacetyl-6-*epi*pubescenine (*2*).

Alkaloid *F* was similarly deduced to have the composition $C_{25}H_{39}NO_7$ (eims apparent molecular ion m/z 465; ^{13}C -nmr spectrum with 25 resonances). Its 1H nmr spectrum contained signals for the methyl of an *N*-ethyl group (δ 1.12, 3H, t, $J=7.2$ Hz), two methoxyls (δ 3.34 and 3.39, each 3H, s) and an acetoxyl substituent (δ 2.08, 3H, s). As before this information indicated that *F* was a norditerpenoid. The 14α - location of the acetoxyl followed from the appearance of the 14β -H resonance at low-field (δ 4.78, 1H, t, $J=4.5$ Hz), while a broad methine signal (δ 3.80, $w_{1/2} = \sim 9$ Hz) suggested 1α -hydroxylation. The absence of a 1H nmr absorption corresponding to a quaternary methyl, together with ^{13}C resonances characteristic of a methoxylated C18 (δ 67.3) and a β -methoxylated C16 (δ 81.8) led us to *4* as the structure of *F*. This corresponds to the 14-O-acetate of 6-*epi*-neoline (alkaloid *D* of our earlier investigation) (*3*), a conclusion which was supported by the similarities in the ^{13}C -nmr spectra of the two alkaloids (see Table 1). Saponification of *F* with methanolic sodium methoxide gave a base spectroscopically indistinguishable from *D*. Thus *F* is 6-*epi*-neoline 14-O-acetate (*4*).

Alkaloid *G* had composition $C_{24}H_{39}NO_6$ (hr-eims m/z 437.2772, calcd 437.2777) with prominent high-mass ions at m/z 422 and 406, corresponding to losses of OH and OCH_3 respectively. Once again, an inspection of the 1H nmr spectrum of *G* revealed signals corresponding to the methyl of an *N*-ethyl (δ 1.06, 3H, t, $J=7.2$ Hz), and three methoxyl groups (δ 3.28, 3.36 and 3.44, each 3H, s) and indicated a C_{19} -skeleton. In addition, the 1H nmr spectrum contained resonances characteristic of a 14α -hydroxylated methine (δ 4.0, 1H, t, $J=4.5$ Hz), and another carbinyl proton (δ 3.88, 1H, d, $J=7.4$ Hz) which was coupled to a higher-field proton (δ 2.36). An inverse 1H - ^{13}C correlation spectrum established that the high-field proton was attached to C-7 not C-5. Models reveal that in an aconitane system a 6α -H has a dihedral angle of $\sim 20^\circ$ with respect to H-7, and 90° to H-5, and the observed $J_{6,7}$ of 7.4 Hz is consistent with 6β -oxygenation. The ^{13}C -nmr spectrum of *G* (see Table 1) allowed us to arrive at a complete structure for the alkaloid. Thus a low field signal (δ 85.3) was typical of a 1α -methoxylated carbon (and the location of a methoxy group at C1 was also indicated by the prominent M-31 ion in the eims of *G*). Two other low field resonances (δ 82.3 and 82.1) were as expected for β -methoxylated C6 and C16, while others corresponded to α -hydroxylated C14 (δ 75.6), hydroxylated C8 (74.1) and C18 (68.1). We therefore deduced that *G* has the structure *6*. This compound, does not seem to have been described before, and we have named it nuttallianidine.

Alkaloid *H* had the composition $C_{24}H_{37}NO_6$ (hr-eims m/z 435.2623, calcd 435.2621). The ir spectrum^{8d} of *H* contained a strong carbonyl absorption (ν_{max}^{KBr} 1740 cm^{-1}), and an acetate functionality was also apparent from a signal in its 1H nmr (δ 2.07, 3H, s). The 1H nmr spectrum also exhibited resonances corresponding to the methyl of an *N*-ethyl group (δ 1.11, 3H, t, $J=7.2$ Hz), a quaternary methyl (δ 1.04, 3H, s), and a methoxy function (δ 3.29, 3H, s). As before, the sum of these requires the alkaloid to have a C_{19} skeleton. The ^{13}C -nmr spectrum of *H* (see Table 1) suggested 1α -, 6β -, and 8-hydroxylation, and 14α -acetoxylation which corresponded to *8*: this being the 14-O-acetate of bicolorine (*7*) (formerly Base B of *D. bicolor*^{6,10}). Indeed, saponification of *H* yielded bicolorine (*7*), and we have therefore named *H*

bicolorine 14-O-acetate.

Finally, alkaloid **1** appeared to have the composition $C_{26}H_{41}NO_8$ (eims m/z 495, with prominent fragment ions at m/z 480 and 464), while as before its 1H -nmr spectrum contained resonances which revealed the presence of N-ethyl (δ 1.05, 3H, t, $J = 7.2$ Hz), three methoxys (δ 3.44, 3.32 and 3.36, each 3H, s), and an acetate (δ 2.07, 3H, s) functionalities in accord with a norditerpenoid skeleton. A low-field resonance (δ 4.76, 1H, t, $J = 4.5$ Hz) indicated the 14 α -attachment of the acetoxyl, while another methine resonance (δ 3.84, 1H, s) was tentatively ascribed to a 6 α -H of a lycocotonine-type alkaloid. The ^{13}C -nmr of **1** (see Table 1) indicated that this skeleton was 1 α , 6 β and 16 β methoxylated, and that there was hydroxylation at C-18, as well as at 7 and 8. The structure (**10**) thus derived corresponded to the apparently previously undescribed 14-O-acetate of delectinine (**9**). Saponification of **1**, as before, yielded **9** thus confirming that **1** was delectinine 14-O-acetate.

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8. Spectroscopic measurements were made as follows:
 - (a) eims and hr-eims were measured using a 70 eV ionizing electron beam impacting on the sample placed on a heated probe using Kratos MS-80 and VG-7070 instruments;
 - (b) the ^{13}C -nmr spectra were measured with Bruker AM 400 and ACE 200 spectrometers, the samples being dissolved in $CDCl_3$ and the solvent signal (δ 77.0) used as an internal reference for which all chemical shifts (δ) are reported in ppm;
 - (c) the 1H nmr spectra were measured with the same spectrometers and solutions as for the ^{13}C -nmr spectra, the residual $CHCl_3$ signal (δ 7.27 ppm) was used as an internal reference for which all chemical shifts (δ) are reported in ppm;
 - (d) ir spectra were measured with a Nicolet system, the samples being dispersed in KBr.
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