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.

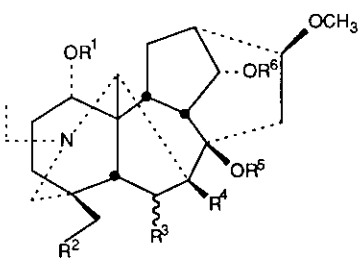
In the course of an investigation of the toxic properties of *D. nuttallianum* Pritz., a cow-poisoning plant of the rangeland of Interior British Columbia, we identified sixteen known and four new norditerpenoid alkaloids (first designated A-D),[1] as well as one new diterpenoid base.[2] We now report the outcome of further fractionation of the minor bases of this plant, by vscc on basic alumina[3] 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 identified[5] 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 ^{1}H nmr spectrum[8c] contained signals which indicated the presence of an N-ethyl (δ 1.11, 3H, t, J=7.2 Hz for $CH_3CH_2N\lesssim$) 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 carbinyl 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 | CH_3	50.6 / 13.8	50.7 / 13.8	48.5 / 12.9	48.5 / 12.9	49.3 / 13.6	49.5 / 13.6	48.6 / 13.1	48.4 / 13.0	51.3 / 14.2	51.1 / 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 | CH_3	170.2 / 21.3			171.6 / 21.5				170.7 / 21.4		171.9 / 21.5

a = reassigned [9].
b = may be interchanged.
c = hidden under the solvent signals.



1. Alkaloid ***A*** (6-epi-pubescenine)
   $R^1 = H$, $R^2 = OCH_3$, $R^3 = \beta OH$, $R^4 = OH$, $R^5 = CH_3$, $R^6 = Ac$
2. Alkaloid ***E*** (desacetyl-6-epi-pubescenine)
   $R^1 = H$, $R^2 = OCH_3$, $R^3 = \beta OH$, $R^4 = OH$, $R^5 = CH_3$, $R^6 = H$
3. Alkaloid ***D*** (6-epi-neolinine)
   $R^1 = H$, $R^2 = OH$, $R^3 = \beta OCH_3$, $R^4 = R^5 = R^6 = H$
4. Alkaloid ***F*** (6-epi-neolinine-14-acetate)
   $R^1 = H$, $R^2 = OH$, $R^3 = \beta OCH_3$, $R^4 = R^5 = H$, $R^6 = Ac$
5. Chasmanine
   $R^1 = CH_3$, $R^2 = OCH_3$, $R^3 = \alpha OCH_3$, $R^4 = R^5 = R^6 = H$
6. Alkaloid ***G*** (nuttalline)
   $R^1 = CH_3$, $R^2 = OH$, $R^3 = \beta OCH_3$, $R^4 = R^5 = R^6 = H$
7. Bicolorine
   $R^1 = R^2 = H$, $R^3 = \beta OH$, $R^4 = R^5 = R^6 = H$
8. Alkaloid ***H*** (bicolorine-14-acetate)
   $R^1 = R^2 = H$, $R^3 = \beta OH$, $R^4 = R^5 = H$, $R^6 = Ac$
9. Delectinine
   $R^1 = CH_3$, $R^2 = OH$, $R^3 = \beta OCH_3$, $R^4 = OH$, $R^5 = R^4 = H$
10. Alkaloid ***I*** (delectinine-14-acetate)
    $R^1 = CH_3$, $R^2 = OH$, $R^3 = \beta OCH_3$, $R^4 = OH$, $R^5 = H$, $R^4 = Ac$

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 ^{1}H 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, $w_{\frac{1}{2}}$ = ~9 Hz) suggested 1α-hydroxylation. The absence of a ^{1}H 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-neolinine (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*-neolinine 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 ^{1}H 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 ^{1}H 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 ^{1}H-^{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 ~20° 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 ^{1}H nmr (δ 2.07, 3H, s). The ^{1}H 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 ***I*** 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 ^{1}H-nmr spectrum contained resonances which revealed the presence of N-ethyl (δ 1.05, 3H, t, J = 7.2 Hz), three methoxyls (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 14α-attachment of the acetoxyl, while another methine resonance (δ 3.84, 1H, s) was tentatively ascribed to a 6α-H of a lycoctonine-type alkaloid. The ^{13}C-nmr of ***I*** (see Table 1) indicated that this skeleton was 1α, 6β and 16β 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 ***I***, as before, yielded ***9*** thus confirming that ***I*** was delectinine 14-O-acetate.

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   (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 ^{1}H 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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