NEW C_{19}-DITERPENOID ALKALOIDS FROM DELPHINIUM NUTTALLIANUM PRITZ.

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Abstract - Sixteen known and four apparently novel C_{19}-diterpenoid alkaloids were isolated from D. nuttallianum and their structures were determined by eims, ^1H and ^{13}C nmr.

Previously[1] we have reported the isolation of a new C_{20}-diterpenoid alkaloid, hetisine 13-0-acetate, from Delphinium nutallianum Pritz. a poisonous herb of the interior ranges of British Columbia. We now present the results of our further investigations of this plant which resulted in the identification of some apparently hitherto undescribed C_{19}-diterpenoid alkaloids.

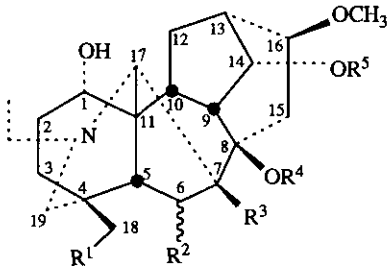
The mixture of weak bases obtained by $CHCl_3$-extraction at pH 5-7, was subjected to extensive chromatographic fractionation (conventional gravity-flow column chromatography over neutral Al_2O_3; vacuum short-column chromatography on basic alumina[2]; and ptlc on silica gel G) to yield twenty C_{19}-diterpenoid alkaloids. Of these sixteen were identified[3,4] as known compounds (anwheidelphine[5], browniine, browniine 14-O-acetate, condelphine, 14-dehydrobrowniine, delbonine, delcosine, delcosine 14-O-acetate, deltatsine, desacetylnudicauline[6], isotalatizidine, karacoline, karacoline 14-O-acetate, methyllycaconitine, nudicauline[7] and subcumine[8]) while four (**A-D**) appeared to be novel. The evidence for the structures of the latter alkaloids, all of which were very minor components (<0.01%) of the total mixture, may be summarised as follows.

Alkaloid **A**, was isolated as a colourless amorphous solid, homogeneous by tlc, with $[\alpha]_D^{23}$ + 23 ± 5° ($CHCl_3$), and ν_{max}(KBr) 3400(br) and 1737 cm^{-1}. Its eims (70 eV) contained as an apparent molecular ion the species m/z 495.2832 (11) ($C_{26}H_{41}NO_8$ requires 495.2832), with other ions at m/z 480 (7), 478 (12), and 464 (100) corresponding to losses of CH_3, OH, and OCH_3 respectively. Together with the ^1H nmr spectrum[9] of **A**, which contained inter alia signals attributable to the methyl of an N-ethyl group (δ1.10, 3H, t, J=7.2 Hz), an acetoxyl (δ2.04, 3H, s), and three methoxyl groups (δ3.33, 3.37 and 3.47, each 3H, s), this enabled us to deduce that **A** was a hexacyclic C_{19}-alkaloid of the

Table 1. ^{13}C Nmr chemical shift data[12] for the 6-*epi*-pubescenine, (**1**), nuttalianine (**4**), 8-O-methylkarasamine (**7**), 6-*epi*-neolinine (**9**) and related models.

Carbon	**1**	**2**[13]	**3**[10]	**4**	**5**[11]	**6**[14]	**7**	**8**[15]	**9**	**10**[8]
1	72.6	72.8	72.5	72.6	72.9	72.1	72.4	72.5	72.4	72.4
2	26.9	28.1	29.6	27.4	30.3	29.2	29.5	27.4	26.9	27.3
3	29.2	29.4	29.8	29.8	31.8	29.8	29.9	30.5	30.0	30.0[2]
4	37.2	37.5	38.5	36.9	32.6	37.9	32.7	37.6	37.7	37.0
5	44.2	45.1	44.1	43.7	43.5	48.2	38.2	41.5	44.4	44.2
6	81.1	79.1	70.8	72.1	72.5	72.6	24.4	24.9	82.5	82.4
7	90.1	nr	85.4	50.1	54.8	55.4	45.9	45.9	50.9	50.8
8	84.7	n.r	80.7	81.3	81.8	75.6	78.4	78.8	75.4	75.1
9	41.5	45.1	43.5	42.8	44.4[a]	44.2[a]	42.8	44.7	46.2	46.1
10	49.3	44.1	47.0	43.7	45.5[a]	45.6[a]	44.0	43.1	45.2	45.4
11	49.6	50.3	47.7	48.8	48.9	48.2	49.5	49.8	48.5	48.3
12	27.2	28.7	28.8	29.4	29.6	29.9	30.9	30.1	29.6	29.3
13	36.5	39.2	38.3	37.0	39.9[a]	40.6[a]	39.1	39.3	39.8	39.8
14	75.4	76.4	75.8	76.1	75.8	75.4	84.4	84.9	76.1	76.0
15	29.4	36.6	29.9	37.0	37.4	44.2	36.3	36.6	40.7	40.6
16	82.2	81.8	83.1	83.0	83.0	82.4	83.7	84.2	81.8	81.5
17	66.5	66.9	63.7	65.4	65.1	63.5	62.5	63.2	65.3	65.2
18	78.9	80.6	80.9	79.4	27.5	80.3	27.7	79.4	67.4	77.7
19	57.7	57.5	56.7	58.3	62.2	57.1	60.6	57.2	57.7	57.6
NCH_2	50.6	50.5	50.8	48.6	48.6	49.7	48.2	48.5	48.5	48.5
CH_3	13.8	13.9	13.8	13.0	13.1	12.9	13.0	13.1	12.9	12.9
OCH_3 6	-	-	-	-	-	-	-	-	57.6	57.1
8	51.5	-	52.9	48.2	48.5	-	48.2	48.2	-	-
14	-	-	-	-			57.6	57.3	-	-
16	56.3	56.2	56.6	56.3	n.r	56.3	56.4	56.0	56.3	56.3
18	59.6	59.6	59.3	59.5	-	59.2	-	59.1	-	59.1
O_2C	170.2	-	170.8	170.7	-	-	-	-	-	-
CH_3	21.3	-	21.2	21.3	-	-	-	-	-	-

n.r. = not reported.
a = reassigned.[8]



1 R^1=OCH_3, R^2=β-OH, R^3=OH, R^4=CH_3, R^5=Ac
2 R^1=OCH_3, R^2=β-OH, R^3=OH, R^4=R^5=H
3 R^1=OCH_3, R^2=α-OH, R^3=OH, R^4=CH_3, R^5=Ac
4 R^1=OCH_3, R^2=α-OH, R^3=H, R^4=CH_3, R^5=Ac
5 R^1=R^3=R^5=H, R^2=β-OH, R^4=CH_3
6 R^1=OCH_3, R^2=α-OH, R^3=R^4=R^5=H
7 R^1=R^2=R^3=H, R^4=R^5=CH_3
8 R^1=OCH_3, R^2=R^3=R^4=R^5=H
9 R^1=OH, R^2=β-OCH_3, R^3=R^4=R^5=H
10 R^1=OCH_3, R^2=β-OCH_3, R^3=R^4=R^5=H
11 R^1=OH, R^2=α-OCH_3, R^3=R^4=R^5=H

lycoctonitine/aconitine type[4], with the remaining oxygen atoms accounted for in three hydroxy groups.

Placement of the acetoxyl function at C(14) was indicated by the presence in the ^{1}H-nmr spectrum of a characteristic[4] triplet (δ 4.79, 1H, J=4.5 Hz), which also showed that C(9) and C(13) were unfunctionalised; while the presence of a 1α-hydroxyl group was similarly inferred from another low-field methine signal (δ 3.65, 1H, br, $w_{\frac{1}{2}} \sim 8$ Hz)[4,10,11].

The ^{13}C nmr spectrum[12] of **A** (see Table 1 where it is compared with those of delphinifoline (**2**) and pubescenine (**3**)) was consistent with these conclusions and allowed us to arrive at a complete structure. Thus, in addition to the low-field resonances due to an α-acetoxylated C(14) (δ 75.4) and α-hydroxylated C(1) (δ 72.6), there were others corresponding[4] to methoxylated C(18) (δ 78.9), β-methoxylated C(16) (δ 82.2), and β-hydroxylated C(7) (δ 90.1). Location of a methoxyl at C(8) was indicated by a low-field quaternary carbon signal (δ 84.7) and a characteristically high-field methoxyl resonance (δ 51.5).[11] (The alternative placement of the tertiary methoxyl at C-7 and hydroxyl at C-8 would be unprecedented, and also seemed unlikely in view of the facile loss of CH_3O in the eims fragmentation of **A**.) The remaining oxygenated carbon signal corresponded to a methine resonance (δ 81.1) in the position expected[4] for 6β-hydroxylation.

We had thus arrived at structure **1** for alkaloid **A**, which corresponds to the 6-epimer of the recently described pubescenine[10]. In full accord with this structure, the ^{1}H nmr spectrum of **A** contained a low field singlet (δ 4.40, 1H) corresponding to 6α-H with $J_{5\beta,6\alpha} \sim 0$ (models show the H(5)-H(6α) dihedral angle ~ 90°).[9,10] We have called **A** 6-*epi*-pubescenine.

Alkaloid **B** was obtained as colourless needles, mp 84-86°, $[\alpha]_D^{23} \sim 0°$ ($CHCl_3$) (plain negative dispersion curve to $[\alpha]^{23}_{365} \sim -15°$) and ν_{max}(KBr) 3400(br) and 1735 cm^{-1}. The eims revealed an apparent molecular ion at m/z 479.2888 (33) ($C_{26}H_{41}NO_7$ req. 479.2883) with high-mass ions at m/z 464 (51), 462 (97), and 448 (100) corresponding to losses of CH_3, OH, and CH_3O. As with **A**, an analysis of the ^{1}H nmr spectrum of **B** revealed the presence of N-ethyl (δ 1.13, 3H, t, J=7.2 Hz), acetoxyl (δ 2.05, 3H, s), and three methoxyl substituents (δ 3.33, 3.35, and 3.36, each 3H, s) and thus indicated a C_{19}-nucleus which, from the molecular composition and absence of olefinic groups, was hexacyclic. Since five oxygens were accounted for in the acetoxyl and methoxyl units, it seemed likely that the two remaining oxygens were present as hydroxyl groups.

The ^{1}H nmr spectrum of **B**, like **A**, contained three methine resonances at lower-field than the methoxyls. Of these one (δ 4.82, t, J=4.5 Hz) was consistent with 14α-acetoxylation, and another (δ 3.76, brs, $w_{\frac{1}{2}} \sim 8$ Hz) with 1α-hydroxylation, while the third (δ 4.47, d), (J=7.3 Hz) coupled to a single proton (δ 2.63, d, J=7.3 Hz) was tentatively located at C(6) of a lycoctonine/aconitine skeleton.

The ^{13}C nmr spectrum of **B** (see Table 1 where data for the similar alkaloids desacetylbicoloridine (**5**)[11] and senbusine A[14] (**6**) are presented for comparison) supported these conclusions and, as it also resulted in the location of the methoxyl groups at C(8) (δ 81.3, with the methoxyl resonance at δ 48.2), C(16)β (δ 83.0) and C(18) (δ 79.4), suggested that **B** corresponded to a 7-deoxy derivative of **A**, or pubescenine.

To settle the orientation of the C(6) hydroxyl substituent we resorted to an SFSD experiment[10,11]: irradiating the proton

resonance at δ 2.63 while observing the ^{13}C nmr spectrum. Enhancement of a resonance (δ 43.6) corresponding to C(5) established the identity of the proton signal as H(5) which to be coupled to H(6β) requires a 6β-orientation (the H(5)-H(6β) dihedral angle is then ca. 30°, while that for H(6β)-H(7) is ca. 90° i.e. $J_{6\beta,7} \sim 0$ Hz). We have therefore ascribed the structure **4** to **B**, and have named the alkaloid nuttalianine.

Alkaloid **C** was obtained as a colourless amorphous solid, $[\alpha]_D^{23} \sim -5°$ (plain negative dispersion curve to 405 nm) and its composition as $C_{24}H_{39}NO_4$ established as before by eims, ^1H and ^{13}C nmr. Thus its ms had M^+ at m/z 405.2872 (22) (calcd 405.2877) with fragment ions at m/z 390 (38) 388 (100) and 374 (29), and its ^1H nmr spectrum contained signals attributable to N-ethyl (δ 1.13, 3H, t, J=7.2 Hz) and three methoxyl groups (δ 3.38, 6H, s, 3.17, 3H, s), consistent with a hexacyclic C_{19}-diterpenoid. The remaining oxygen was presumed to be present as a hydroxyl group. The absence of oxygenation at C(18) was indicated by the presence of a quaternary C-methyl signal in the ^1H nmr (δ 0.90, 3H, s), and 1α-hydroxylation by a broad low-field methine resonance (δ 3.74, 1H, $w_{\frac{1}{2}}$ ca. 8 Hz). The ^{13}C nmr data (see Table I and the data for 8,14-di-O-methylisotalatizidine (**8**)[15]) were in agreement with these conclusions, and the placement of the methoxyl groups at C(8), C(14)α, and C(16)β: leading to the identification of **C** as karasamine 8-O-methyl ether (**7**).

The fourth alkaloid, **D**, a gum, $[\alpha]_D^{23} \sim +50°$ ($CHCl_3$) (plain positive dispersion curve to 365 nm) had composition $C_{23}H_{37}NO_6$ (m/z 423.2616, req. 423.2621; with high-mass fragment ions at m/z 408(100) and 406(44). Its ^1H nmr spectrum contained signals corresponding to an N-ethyl (δ 1.13, 3H, t, J=7.2 Hz), and two methoxyl groups (δ 3.37 and 3.41, each 3H, s) and three low-field methine resonances indicated the presence of 14α-, 1α; and 6-oxygenation (δ 4.12, t, J=4.5 Hz; 3.79, br s, $w_{\frac{1}{2}} \sim 8$ Hz; and 3.64, d, J=7.9 Hz respectively). The ^{13}C nmr (see Table, together with comparison data for subcusine (**10**)[8]) enabled us to elaborate our structure for **D** into **9** i.e. the C-6 epimer of neolinine (**11**)[16]; note in particular the methylene resonance at δ 67.4 in the range characteristic (**4**) of a hydroxylated C(18). The observed coupling of 6α-H only with 7-H is as reported for subcusine.[8] We have named **D** 6-*epi*-neolinine.

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