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AJADININE: A NEW NORDITERPENOID ALKALOID FROM THE SEEDS OF *DELPHINIUM AJACIS*. THE COMPLETE NMR ASSIGNMENTS FOR SOME LYCOCTONINE-TYPE ALKALOIDS

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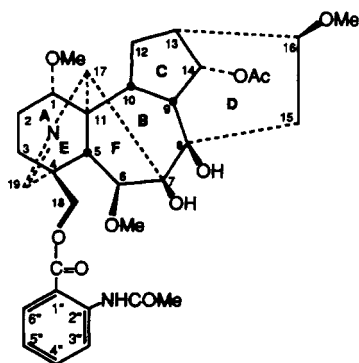
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ABSTRACT.—A new norditerpenoid alkaloid designated as ajadinine [**1**] has been isolated from the seeds of *Delphinium ajacis*. Its structure [**1**] was established from physical and spectroscopic data. The known alkaloids desoline, delcosine, and gigactonine have also been obtained from this same source. Complete and unambiguous nmr assignments for the lycoctonine-type norditerpenoid alkaloids 14-deacetyljadine [**2**], ajacine [**3**], delcosine [**4**], and ambiguine [**5**] are also presented in this work. Assignments for the aromatic protons of the C-18 *N*-acetyl anthranilic acid ester group were made by selective INEPT nmr experiments.

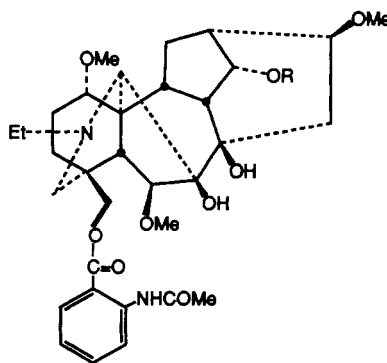
In continuation of our work on the isolation and identification of diterpenoid alkaloids from different parts of *Delphinium ajacis* L. (Ranunculaceae) (1), we have examined the seeds. We report here the isolation and characterization of a new norditerpenoid alkaloid, ajadinine [**1**]. Structure **1** was assigned to ajadinine on the basis of its spectroscopic data, including 2D studies. For complete ¹³C-

and ¹H-nmr chemical shifts assignments for **1** we compared accurate nmr assignments for the related lycoctonine-type norditerpenoid alkaloids, namely, 14-deacetyljadine [**2**], ajacine [**3**], delcosine [**4**], and ambiguine [**5**].

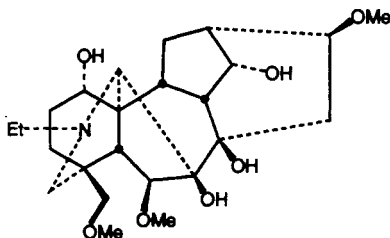
The ¹³C-nmr chemical shift assignments for alkaloids **2–5** that were reported earlier from this laboratory (2–4) were based on the determination of the



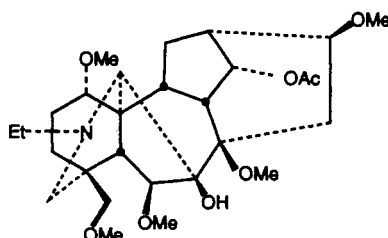
1



2 R=H
3 R=Me



4



5

noise-decoupled and single-frequency off-resonance decoupled (SFORD) spectra. The ^{13}C -nmr signals were assigned with the help of the single-frequency proton off-resonance decoupling technique, application of known chemical shift rules, steric effects and from comparison of the spectra of closely related compounds. Accurate and complete ^{13}C and ^1H -nmr assignments for alkaloids **2–5** are reported in the present work.

The molecular formula, $\text{C}_{33}\text{H}_{42}\text{N}_2\text{O}_{10}$, for ajadinine [**1**], an amorphous compound, $[\alpha]_{\text{D}} +83.7^\circ$, was derived from its eims (m/z 626 $[\text{M}]^+$) and ^{13}C -nmr spectral data. The ^1H -nmr spectrum revealed the presence of the several functional groups. The 3H singlets at δ 2.09 and 2.26 showed the presence of two acetyl groups, with the first being an *O*-acetyl and the latter an *N*-acetyl group. The presence of a one-proton broad singlet at δ 10.93 indicated that an $-\text{NHCOCH}_3$ group was present in a C-18 anthranoyl ester as in ajacine [**3**] (2). This conclusion was supported by the presence of four aromatic protons at δ 7.15 (ddd), 7.61 (ddd), 7.98 (dd), and 8.76 (dd). A broad 1H singlet at δ 7.55 and the absence of N-Et signals in the ^1H -nmr spectrum suggested the presence of a $-\text{N}=\text{C}_{19}\text{H}$ group as in acoseptridine (5) and barbeline (6). In addition, three methoxyl groups (δ 3.20, 3.33, 3.36, each 3H singlets), a H-14_B signal (δ 4.83, 1H, *t*, $J=4.5$ Hz), and an H-6_A resonance at δ 3.86 (1H, *s*) indicated the presence of a lycoctonine-type skeleton (for aconitine-type alkaloids H-14_B and -6_B appear 0.3 to 0.5 ppm downfield). The DEPT spectra indicated the presence of nine quaternary, fourteen methine, five methylene, and five methyl carbons, representing the thirty-three carbon atoms of the molecule. The pattern of the ^{13}C -nmr chemical shifts was similar to that reported for ajadine (3) except for some of the carbons in the A, B, E, and F rings of the molecule. The presence of the $-\text{N}=\text{C}_{19}\text{H}$ -C₄-grouping is supported by a methine car-

bon signal at 163.1 ppm and a quaternary carbon at 47.1 ppm, and these can be assigned to C-19 and C-4, respectively, as in acoseptridine (5). All the ^{13}C - and ^1H -nmr assignments (from ^1H - ^1H COSY, HMQC, and selective INEPT experiments, see Tables 1 and 2) obtained for ajadinine support structure **1**.

The ^{13}C - and ^1H -nmr chemical shift assignments for ajadinine [**1**], 14-deacetylajadine [**2**], ajacine [**3**], delcosine [**4**], and ambiguine [**5**] are reported in Tables 1 and 2, respectively. The ^1H -nmr assignments for compounds **2–5** are being reported for the first time (Table 2). DEPT, ^1H - ^1H COSY, HETCOR, and selective INEPT nmr spectral data were collected. This work revises the chemical shift assignments previously reported (2) for C-4, C-5, C-9, C-10, C-13, C-6-OMe, and C-16-OMe for ajacine [**3**]. In the case of delcosine [**4**], the assignments for C-2, C-3, C-5, C-10, and C-13 have been changed from the literature values (2). The chemical shifts of C-5, C-9, C-10, C-13, C-20, and C-8-OMe in ambiguine [**5**] differ from the published values (4). In the case of 14-deacetylajadine, the published ^{13}C -nmr shifts (7) are identical with those found in the present study, but the ^1H -nmr shifts for the four aromatic protons of the anthranoyl ester group (7) are now revised (Table 2).

The aromatic protons of the C-18 *N*-acetyl anthranilic acid ester group in lycoctonine-type compounds have now been assigned through a study of their selective INEPT nmr results. There are four aromatic protons in this group (H-3'', H-4'', H-5'', and H-6''). The pattern of these protons in ^1H -nmr spectra is similar for all the alkaloids bearing a C-18 anthranilic acid ester group. It consists of two triplets (ddd) and two doublets (dd) in the region δ 7.00 to 8.80. In the case of 14-deacetylajadine the reported (7) assignments for these protons are δ 7.10 (1H, *dt*, $J=1.4$ and 8.0 Hz, H-4''), 7.57 (1H, *ddd*, $J=8.0, 7.5$, and 1.8 Hz, H-5''), 7.98 (1H, *dd*, $J=8.0$ and 1.8 Hz, H-3'')

TABLE 1. ¹³C-Nmr Chemical Shifts and Assignments^a of Compounds 1-5.

Carbon	Compound				
	1	2	3	4	5
C-1	82.0 d	84.6 d	83.7 d	72.5 d	83.3 d
C-2	25.1 t	25.2 t	25.8 t	29.2 t ^b	25.4 t
C-3	29.6 t	32.1 t	31.8 t	27.3 t ^b	31.6 t
C-4	47.1 s	37.7 s	37.3 s ^b	37.5 s	38.2 s
C-5	45.6 d	50.2 d	50.0 d ^b	45.0 d ^b	53.9 d ^b
C-6	91.6 d	90.3 d	90.7 d	89.9 d	91.0 d
C-7	86.4 s	89.1 s	88.2 s	87.7 s	89.9 s
C-8	75.9 s	76.2 s	77.3 s	78.0 s	80.2 s
C-9	42.3 d	45.0 d	43.0 d ^b	45.1 d	40.9 d ^b
C-10	43.4 d	45.9 d	45.8 d ^b	43.8 d ^b	45.9 d ^b
C-11	50.1 s	48.2 s	48.9 s	48.7 s	46.9 s
C-12	25.1 t	27.4 t	28.5 t	29.2 t	27.2 t
C-13	38.6 d	36.2 d	37.8 d ^b	39.3 d ^b	35.6 d ^b
C-14	75.9 d	75.1 d	83.6 d	75.6 d	74.8 d
C-15	33.4 t	33.0 t	33.4 t	34.3 t	28.2 t
C-16	81.4 d	81.6 d	82.3 d	81.9 d	81.8 d
C-17	64.5 d	64.9 d	64.3 d	66.3 d	66.7 d
C-18	66.8 t	69.6 t	69.5 t	77.2 t	79.2 t
C-19	163.1 d	52.3 t	52.2 t	57.0 t	53.3 t
C-20	—	51.1 t	50.8 t	50.3 t	52.0 t ^b
C-21	—	14.1 q	13.8 q	13.6 q	15.0 q
C-1'	56.4 q	56.0 q	55.6 q	—	55.5 q
C-6'	58.6 q	58.2 q	57.9 q ^b	57.3 q	59.4 q
C-8'	—	—	—	—	52.5 q ^b
C-14'	—	—	57.6 q ^b	—	—
C-16'	56.4 q	56.4 q	56.1 q	56.3 q	56.2 q
C-18'	—	—	—	59.0 q	59.4 q
O=C (14)	171.7 s	—	—	—	171.2 s
CH ₃	21.5 q	—	—	—	21.4 q
NHC=O	169.1 s	169.0 s	168.9 s	—	—
CH ₃	25.5 q	25.4 q	25.3 q	—	—
Aromatic					
O=C	167.9 s	168.0 s	167.8 s	—	—
C-1''	114.0 s	114.4 s	114.3 s	—	—
C-2''	142.0 s	141.7 s	141.6 s	—	—
C-3''	120.7 d	120.4 d	120.3 d	—	—
C-4''	135.4 d	134.9 d	134.7 d	—	—
C-5''	122.7 d	122.5 d	122.3 d	—	—
C-6''	130.2 d	130.2 d	130.1 d	—	—

^aChemical shifts in ppm downfield from TMS. Multiplicities were determined from the DEPT experiments.

^bThese assignments are revised.

and 8.71 (1H, dd, $J=7.5$ and 1.3 Hz, H-6''). The unambiguous assignment for these four protons is described below.

Selectively pulsing the amide proton at δ 10.98 showed responses of the carbon signals at 169.0, 120.4, and 114.4 ppm. The signals at 169.0 and 114.4 ppm are

for the quaternary carbons which can be assigned to the amide carbonyl and to C-1'', which are two and three bonds away, respectively. The third signal at 120.4 ppm should be assigned to C-3'', also three bonds away. In the HETCOR plot the signal at 120.4 ppm showed a corre-

TABLE 2. $^1\text{H-Nmr}$ Chemical Shifts^a of Compounds 1-5.

Proton	Compound				
	1	2	3	4	5
H-1	3.32 m	3.05 m	3.01-2.97 m	3.63 br s	2.91 m
H-2	1.98-2.21 m	2.03-2.25 m	2.00-2.24 m	1.64-1.93 m	1.81-2.02 m
H-3	1.48-1.65 m	1.52-1.75 m	1.51-1.75 m	1.45-1.65 m	1.25-1.75 m
H-5	1.95 br s	1.83 br s	1.72 br s	1.84 br s	1.45 br s
H-6	3.80 br s	3.83 br s	3.86 s	3.99 s	3.51 s
H-9	3.08 m	3.10 m	3.03 m	2.95 m	3.33 m
H-10	2.11 m	1.85 m	2.07 m	1.93 m	1.94 m
H-12	1.65-2.55 m	1.82-2.83 m	1.75-2.85 m	1.90-2.03 m	1.90-2.13 m
H-13	2.50 m	2.95 m	2.29 m	2.30 m	2.51 m
H-14	4.83 t (4.5)	4.01 br s	3.56 t (4.6)	4.06 br s	4.68 t (4.8)
H-15	1.79-2.95 m	1.81-2.52 m	1.85-2.55 m	1.59-1.68 m	1.92-2.40 m
H-16	3.45 m	3.45 m	3.05 m	3.30 m	3.23 m
H-17	3.97 br s	3.25 m	2.91 br s	2.82 s	2.94 br s
H-18	4.40-4.57 d,d (9.7)	4.16 d (9.0)	4.13 br s	2.95-3.42 AB (9.3)	3.13 AB q (9.9)
H-19	7.55 br s	2.52-2.80 AB (10.5)	2.41-2.73 AB (11.9)	2.41 s	2.55 s
H-20	—	2.95 m	2.75-2.91 m	2.75-2.98 m	2.79-2.88 m
H-21	—	1.06 t (7.1)	1.03 t (7.2)	1.06 t (7.1)	1.02 t (7.1)
1-OMe	3.20 s	3.25 s	3.22 s	—	3.20 s
6-OMe	3.33 s	3.35 s	3.36 s	3.33 s	3.30 s
8-OMe	—	—	—	—	3.47 s
14-OMe	—	—	3.33 s	—	—
16-OMe	3.36 s	3.37 s	3.29 s	3.34 s	3.26 s
18-OMe	—	—	—	3.30 s	3.42 s
1-OH	—	—	—	7.30 br s	—
7-OH	2.62 br s	2.15 br s	2.05 br s	5.26 s	—
8-OH	3.55 s	3.87 s	4.13 s	3.95 s	—
14-OH	—	2.77 br s	—	3.40 s	—
14-OAc	2.09 s	—	—	—	2.01 s
NH-Ac	10.93 s	10.98 s	10.97 s	—	—
	2.26 s	2.22 s	2.18 s	—	—
Aromatic					
H-3"	8.76 dd (7.3, 1.5)	8.71 dd (7.2, 1.4)	8.65 dd (8.5, 1.3)	—	—
H-4"	7.61 ddd (7.7, 7.0, 1.4)	7.57 ddd (7.8, 7.1, 1.6)	7.52 ddd (7.6, 7.0, 1.5)	—	—
H-5"	7.15 ddd (8.0, 7.5, 1.3)	7.10 ddd (8.2, 7.6, 1.5)	7.09 ddd (8.0, 7.5, 1.5)	—	—
H-6"	7.98 dd (8.0, 1.5)	7.98 dd (8.1, 1.4)	7.92 dd (7.9, 1.5)	—	—

^aChemical shifts in ppm downfield from TMS, $J = \text{Hz}$ in parentheses. These shifts were assigned on the basis of $^1\text{H}-^1\text{H}$ COSY, HETCOR, and selective INEPT experiments. Assignment of -OH protons is from D_2O exchange studies.

lation to the proton doublet at 8.71 ppm and thus this proton signal can be assigned to H-3". When the signal at 8.71 ppm (H-3") was selectively pulsed the carbon signals at 114.4 and 122.5 ppm showed enhancements. The signal at 114.4 ppm is already assigned to C-1"

and the signal at 122.5 ppm is assigned to C-5", three bonds away from H-3". In the HETCOR plot the signal at 122.5 ppm showed a correlation to a proton triplet at δ 7.10 which should be that of H-5". When H-5" at δ 7.10 was selectively pulsed the carbon signals showing strong

responses were 120.4 (C-3'') and 114.4 ppm (C-1''), both being three bonds away.

A carbon signal at 134.9 ppm showed a correlation to a proton triplet at δ 7.57 in the HETCOR plot and when this triplet was selectively pulsed the carbon signals showing responses were at 141.7 and 130.2 ppm, and these can be assigned to C-2'' and C-6'', respectively, both being three bonds away from H-4''. Thus, the proton triplet at δ 7.57 is assigned to H-4''. The remaining proton doublet at δ 7.98, correlating to 130.2 ppm in the HETCOR plot is now assigned to H-6''. This was confirmed when the doublet at δ 7.98 was selectively pulsed to show the carbon signal responses of 168.0 ppm which should be the C-18 ester carbonyl, 141.7 ppm (C-2'') and 134.9 ppm (C-4''). The assignment of these protons was not possible from the results of the COSY and HETCOR plots. In the COSY plot these protons show correlations among themselves but not to any of the known protons in the molecule. Similar results were obtained in the case of the alkaloid ajacine [3]. The revised assignments of the aromatic protons of **2** are given in Table 2.

The known alkaloids desoline, delcosine, and gigactonine isolated from the seeds were identified by comparing their mp, tlc behavior, and spectroscopic data with those of authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. The optical rotation was measured on a Perkin-Elmer model 141 polarimeter. Ir spectra were determined on a Perkin-Elmer model 1420 spectrophotometer. Nmr spectra were recorded on Bruker AC 300 spectrometer operating at 300.13 MHz for ^1H and 75.47 MHz for ^{13}C . The pulse sequences employed in the 1D, DEPT, and 2D nmr experiments were those of the standard Bruker software. The pulse sequence for the selective INEPT experiments was obtained by modifying the Bruker standard INEPT sequence and the critical parameters used were as described in ref. (8). Chromatographic separations on a Chromatotron (9) were carried out on rotors coated with 1 mm thick layers of Al_2O_3 , 60 PF 254, 365 (EM 1104) or SiO_2 , 60HPF 254 (EM 7749); vacuum liquid chromatography (vlc)

(10) was carried out with Merck Al_2O_3 (EM 1085) and SiO_2 , 60H (EM 7736). All the known compounds isolated were identified by comparing their spectral data and tlc behavior with those of authentic samples.

SOURCE OF KNOWN ALKALOIDS.—Authentic samples of 14-deacetylajadine [2], ajacine [3], delcosine [4], and ambiguine [5] used for unambiguous nmr assignments, were of 30–40 mg size and were from our compound repository.

PLANT MATERIAL.—Seeds of *Delphinium ajacis* L. (Ranunculaceae) were purchased from Wild Flower International (lot no. D2418), Elk, California by the Grounds Department of the Physical Plant Division of the University of Georgia.

EXTRACTION OF ALKALOIDS.—The *D. ajacis* seeds (200 g) were ground and extracted with a mixture of solvents containing 95% EtOH (320 ml), hexane (100 ml), and H_2O (80 ml), by stirring vigorously at room temperature for 24 h and then refluxing for 24 h after which the contents were filtered. The extraction process was repeated twice and the combined extract was washed with hexane (400 ml). The hexane layer on evaporation gave a non-alkaloidal oily residue (6.5 g). The hexane washed extract was concentrated to a small volume *in vacuo* and CHCl_3 (100 ml) was added to it. The mixture was then extracted with 0.75 M H_2SO_4 (5×200 ml). The combined acidic layer was washed with CHCl_3 (2×200 ml) and then cooled in an ice- H_2O bath. The cold acidic layer was then basified to pH 10 with Na_2CO_3 and extracted with CHCl_3 (12×300 ml). The combined CHCl_3 extract was dried (Na_2SO_4) and evaporated *in vacuo* to give a crude alkaloid mixture (1.44 g).

CHROMATOGRAPHIC SEPARATIONS.—The crude alkaloid mixture was fractionated by vlc on an Al_2O_3 (70 g) column and elution was carried out with a gradient of hexane, CHCl_3 , and EtOH. Twelve fractions, each 150 ml, were collected. The vlc fraction 5 [0.24 g, eluted with hexane- CHCl_3 (1:1)] on further separation on an Al_2O_3 rotor with hexane- CHCl_3 (45:55) furnished fractions 4 and 5 (as a band visible at 365 nm) from which desoline (0.016 g), mp 212–213°, was isolated. Further elution with CHCl_3 /1% MeOH gave a band visible at 254 nm in fraction 14. The amorphous compound from fraction 14 was characterized as a new alkaloid, ajadinine [1] (0.006 g); $[\alpha]_{\text{D}} + 83.7^\circ$ ($c=0.166$, CHCl_3); ir ν max 3460, 3385, 1725, 1685, 1250, 1180, 905, and 725 cm^{-1} ; eims m/z (%) 626 $[\text{M}]^+$ (0.23), 611 $[\text{M}-\text{Me}]^+$ (0.94), 162 (10), 148 (0.65), 120 (15), 43 (100); for ^{13}C - and ^1H -nmr data, see Tables 1 and 2, respectively.

Vlc fraction 9 (0.18 g, eluted with CHCl_3 /1% EtOH) was further fractionated on an Al_2O_3 rotor using a gradient of hexane, CHCl_3 , and MeOH, and fractions were collected from the uv-

(365 nm) visible bands. Fraction 6 eluted with $\text{CHCl}_3/1\%$ MeOH gave gigactonine (0.012 g), mp 166–168°, and fraction 8 eluted with $\text{CHCl}_3/1\%$ MeOH gave delcosine [4] (0.0068 g), mp 202–204°.

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