THE DITERPENOID ALKALOIDS OF *DELPHINIUM DELAVAYI* FRANCH VAR. *POGONANTHUM* (H.-M.) WANG

S. William Pelletier*, Fathalla M. Harraz†, Mohamed M. Badawi, Sirirat Tantiraksachai, Feng-peng Wang††, and Szu-ying Chen†††
Institute for Natural Products Research and the School of Chemical Sciences,
The University of Georgia, Athens, Georgia 30602, U.S.A.

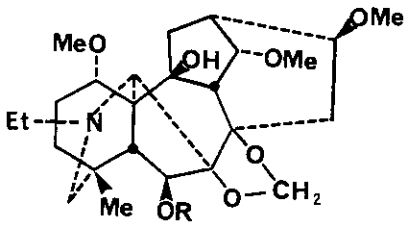
Abstract- Investigation of the alkaloids of *Delphinium delavayi* Franch var. *pogonanthum* (H-M) Wang has led to the isolation of nine known alkaloids and a new pair of regioisomeric alkaloids *delavaine A* (**10A**) and *delavaine B* (**10B**) whose structures have been determined based on spectroscopic evidence and two syntheses from methyllycaconitine (**3**). The known alkaloids that were isolated are the C_{19}-diterpenoid alkaloids: deltaline (**1**), deltamine (**2**), methyllycaconitine (**3**), anthranoyllycaconitine (**4**), lycoctonine (**5**), delsemine (**6**), and the C_{20}-diterpenoid alkaloids: hetisinone (**7**), hetisine (**8**), and ajaconine (**9**). Delsemine (**6**) has been synthesized from methyllycaconitine and chromatographically separated into its component regioisomers **6A** and **6B**.

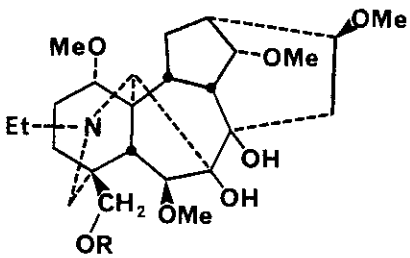
In continuation of our earlier phytochemical studies on the diterpenoid alkaloids of Chinese Ranunculaceae plants,[1,2] we report here the chemical investigation of the roots of *Delphinium delavayi* Franch var. *pogonanthum* (H.-M.) Wang. Nine known alkaloids have been isolated: deltaline (**1**), deltamine (**2**), methyllycaconitine (**3**), anthranoyllycoctonine (**4**), lycoctonine (**5**), delsemine (**6**), hetisinone (**7**), hetisine (**8**) and ajaconine (**9**), in addition to a pair of new regioisomeric alkaloids named *delavaine A* (**10A**) and *delavaine B* (**10B**). A fourth unknown crystalline alkaloid has been isolated in low yield.

The roots were extracted with 90% ethanol. Two approaches were followed to isolate the total crude alkaloids. The first approach involved gradient pH extraction when two fractions were obtained at pH 8 (E_1) and pH 12 (E_2). From the E_1 fraction six C_{19}-diterpenoid alkaloids were isolated by extensive chromatographic separation involving vlc[3], preparative tlc., column chromatography and a Chromatotron[4]: deltaline (**1**), deltamine (**2**), methyllycaconitine (**3**), anthranoyllycoctonine (**4**), lycoctonine (**5**), and delavaine (**10**). Fraction E_2 afforded after extensive chromatographic separation, involving dccc, vlc and a Chromatotron, three C_{20}-diterpenoid alkaloids: hetisinone (**7**), hetisine (**8**) and ajaconine (**9**). The second approach (see experimental) involved the isolation of the total crude alkaloids on a column of Dowex 50W-X8 cation exchange resin. Further chromatographic separation of the total crude alkaloids led to the isolation of the six C_{19}-diterpenoid alkaloids isolated from fraction E_1, and delsemine (**6**) (as a mixture of regioisomers).

On leave from: †College of Pharmacy, Alexandria University, Alexandria, Egypt; ††School of Pharmacy, West China University of Medical Sciences, Chengdu, China; †††Kunming Institute of Botany, Academy of Sciences, Kunming, China.

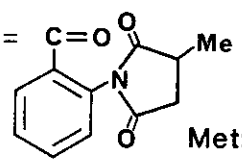
Delavaine (**10**) was obtained in an amorphous form, $[\alpha]_D^{24}$ +36.2° (c, 1.7 $CHCl_3$); its molecular formula $C_{38}H_{54}N_2O_{11}$ was derived from elemental analysis and mass spectrum. The mass spectrum exhibited a base peak at m/z 683 resulting from a loss of a methoxyl group from the molecular ion. Elemental analysis for nitrogen indicated the presence of only two nitrogen atoms in the molecule. The proton nmr spectrum exhibited the following signals: δ 1.07 (3H, t, J = 7.3 Hz, -N-CH_2-CH_3), two overlapping doublets centered at 1.28 and 1.31 (each 3H, J = 6.5 Hz, -CH(CH_3)-), 3.27, 3.35, 3.40 and 3.42 (each 3H, s, -OCH_3), 3.68 and 3.71 (each 3H, s, -COOCH_3), four aromatic protons at 7.10 (1H, t, J = 7.3 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz) and 8.72 (1H, d, J = 8.8 Hz) and 11.05 and 11.17 (each 1H, broad s, -NH-CO-).



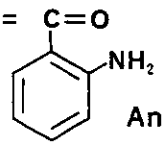


1: R = Ac **Deltaline**

2: R = H **Deltamine**

3: R = 

Methyllycaconitine

4: R = 

Anthranoyllycoctonine

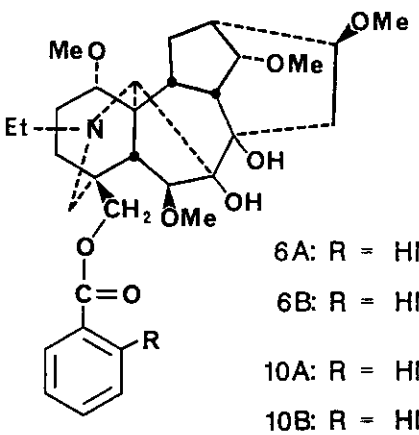
5: R = H **Lycoctonine**

The proton nmr spectrum showed a very close relation to that of septentriodine (**12**)[5], except for the presence of twin signals at δ 11.05 and 11.07 instead of one signal at δ 11.10, representing the secondary amide proton of the anthranoyl side chain. It also differs in the presence of twin singlets at δ 3.68 and 3.71 instead of the presence of only one singlet at δ 3.71 in septentriodine, which is attributed to the methyl group of the carboxymethyl ester function. In addition, two overlapping doublets centered at δ 1.28 and 1.31 (J = 6.5 Hz) appear in the proton nmr spectrum of delavaine. These two signals do not exist in the proton nmr spectrum of septentriodine (**12**).

The carbon-13 nmr spectrum of delavaine (Table 1) showed 44 peaks of which 32 peaks correspond with those of the basic skeleton of septentriodine. The twin peaks at 141.9 and 141.7, 114.8 and 114.7, 51.9 and 51.7, 41.4 and 39.0, and 17.9 and 17.1 ppm along with four downfield signals for four carbonyl carbons indicate that delavaine is actually a mixture of two closely related bases which

differ only in the side chain of the anthranilic acid moiety. The twin peaks at 51.9 and 51.7 ppm in the ^{13}C nmr spectrum, together with the twin singlets at δ 3.68 and 3.71 in the proton nmr spectrum indicate the presence of two methyl ester groups. The presence of two overlapping doublets centered at δ 1.31 and 1.28 (J = 6.5 Hz), attributed to two secondary methyl groups, indicate the presence of a mixture of two $-CH(CH_3)-$ groupings in the side chains. This conclusion was supported by the presence of two sets of peaks at 41.4 and 39.0 and 17.9 and 17.1 ppm for the methine and methyl carbons, respectively. These findings, combined with the mass spectral and elemental analysis, lead to structure **10** (A + B) for delavaine, a mixture of two isomers resulting from opening at either one of the two carbonyl sites of the methylsuccinimide ring of methyllycaconitine (**3**). The nmr and mass spectral data, as well as the tlc behavior, indicate that delavaine is identical with alkaloid B[6] from *Delphinium tricorne*.[7]

In order to confirm that delavaine contains a terminal methyl ester, its synthesis from methyllycaconitine (**3**) was undertaken in the absence of ammonia. When methyllycaconitine (**3**) was treated with sodium bicarbonate in methanol, it afforded after separation of the products on a Chromatotron, two products. The major component showed proton and carbon-13 (Table 1) nmr spectra, tlc behavior and specific rotations identical with those of delavaine (**10**). The second component isolated from the reaction was found to be the dimethyl ester (**13**). Its proton nmr spectrum showed signals at δ 11.0 and 11.22 (both for the -NH proton of the internal amide function of the anthranilic acid moiety) and four aromatic protons at δ 7.06, 7.53, 8.02 and 8.69. The methyl group of the aromatic methyl ester gave a singlet at δ 3.93. It also showed two singlets at δ 3.68

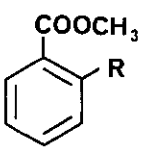


6A: R = HN–COCH(CH$_3$)–CH$_2$–CONH$_2$
6B: R = HN–COCH$_2$–CH(CH$_3$)–CONH$_2$ } **Delsemine**

10A: R = HN–COCH(CH$_3$)–CH$_2$–COOCH$_3$
10B: R = HN–COCH$_2$–CH(CH$_3$)–COOCH$_3$ } **Delavaine**

11A: R = HN–COCH$_2$–CH(CH$_3$)–COOC$_2$H$_5$ / HN–COCH(CH$_3$)–CH$_2$–COOC$_2$H$_5$

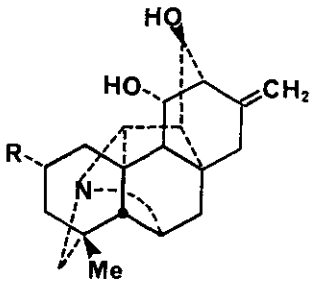
12: R = HN–COCH$_2$CH$_2$–COOCH$_3$ **Septentriodine**



13: R = HN–COCH$_2$–CH(CH$_3$)–COOCH$_3$ / HN–COCH(CH$_3$)–CH$_2$–COOCH$_3$

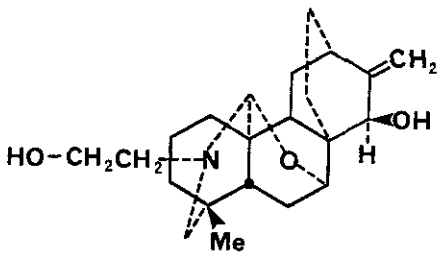
and 3.71 (both for the -COOC$\underline{H}_3$ of the side chain methyl ester of the two isomers). The mass spectrum showed the molecular ion at m/z 279. These data, as well as the carbon-13 nmr data (see experimental), confirmed the proposed structure (**13**). When methyllycaconitine was refluxed with methanol for four days, it afforded delavaine (**10**) as the major component, as well as dimethyl ester **13**.

When methyllycaconitine (**3**) was treated with sodium acetate in ethanol, it afforded a mixture of the ethyl esters (**11**). The proton nmr spectrum exhibited the the following signals: δ 1.02 (3H, $\underline{t}$, J = 7.3 Hz, -N-CH$_2$-C$\underline{H}_3$), two overlapping doublets centered at 1.31 and 1.28 (each 3H, J = 6.5 Hz, -CH(C$\underline{H}_3$)-), 3.29, 3.38, 3.41 and 3.45 (each 3H, $\underline{s}$, -OC$\underline{H}_3$), four aromatic protons at 7.14 (1H, $\underline{t}$, J = 7.8 Hz), 7.69 (1H, $\underline{t}$, J = 7.3 Hz), 8.02 (1H, $\underline{d}$, J = 7.3 Hz) and 8.75 (1H, $\underline{d}$, J = 7.8 Hz) and signals at δ 11.1 and 11.2 (each 1H, broad $\underline{s}$, -N$\underline{H}$-CO-). The proton nmr spectrum of **11** is very similar to that of **10** except for the absence of the twin singlets at δ 3.68 and 3.71 which are attributed to the methyl groups of the carboxymethyl ester function of delavaine (**10**).



7 : R = =O Hetisinone

8 : R = α-OH Hetisine



9 Ajaconine

These findings indicate without any doubt that delavaine has structure **10A** + **10B**. Because of its facile synthesis from methyllycaconitine (**3**) and methanol under mild basic conditions, it may be an artifact, formed from methyllycaconitine (**3**) and the methanol used in the separation procedures. However, under neutral conditions, conversion to substantial amounts of delavaine required refluxing in methanol for several days.

Extensive efforts to resolve the two components of delavaine (**10**) on tlc plates of aluminum oxide or silica gel using a variety of solvent systems were unsuccessful. However, when delavaine was chromatographed on an aluminum oxide rotor (Chromatotron) with hexene-chloroform (2:1) as an eluent, the earlier fractions consisted of homogenous delavaine A (**10A**), amorphous, $[\alpha]_D^{29}$ +39.4° ($CHCl_3$). The proton nmr spectrum showed a single signal for a methyl ester at δ 3.68 ppm and also a signal at δ 11.07 ppm for the secondary amide proton of the anthronyl side chain. In contrast to the twin signals at δ 17.1, 17.9 and 51.7, 51.9 ppm in the ^{13}C nmr spectrum of the delavaine mixture, the ^{13}C nmr spectrum of delavaine A, exhibited only one signal each at 17.9 and

51.7 ppm for the methyl groups of the *N*-ethyl and carbomethoxyl functions, respectively. In addition to the downfield signal at δ 168.1 ppm for benzoyl carbonyl in delavaine A, its ^{13}C nmr spectrum showed only two downfield signals at δ 172.5 and 174.1 ppm, for the two amide carbonyls of one anthronyl side chain (A).

The second compound eluted was delavaine B (**10B**), amorphous, $[\alpha]_D^{29}$ 31.7° (c, 0.8, $CHCl_3$). The proton nmr spectrum showed only one signal at δ 3.71 for the methyl ester group and one signal at δ 11.05 for the secondary amide proton of the second anthronyl side chain (B). The ^{13}C nmr spectrum of delavaine B exhibited signals at 17.1 and 51.9 ppm for the methyl groups of the *N*-ethyl and the carbomethoxyl functions, respectively. In addition to the benzoyl carbonyl signal at 168.1 ppm, there were two downfield signals at 170.0 and 175.9 ppm for the two amide carbonyls of the anthronyl side chain (B).

The alkaloid delsemine (**6**) was obtained in an amorphous form $[\alpha]_D^{31}$ +33.9 (c, 0.75 EtOH), with the following signals in the proton nmr spectrum: δ 1.07 (3H, t, J = 7.8 Hz, -N-CH_2-CH_3), two overlapping doublets (J = 6.5 Hz) centered at 1.28 and 1.31 (each 3H, -CH(CH_3)-), 3.26, 3.35, 3.38 and 3.41 (each 3H, s, -OCH_3), 5.44 and 5.85 (each 2H, broad m, -CO-NH_2), four aromatic protons of the anthranilic acid moiety at 7.10 (1H, t, J = 7.8 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz) and 8.68 (1H, d, J = 8.3 Hz), 11.06 and 11.17 (each 1H, broad s, -NH-CO-).

The carbon-13 nmr spectrum of delsemine (**6**) (Table 1) showed 42 peaks of which 32 peaks correspond with the basic skeleton of delavaine and septentriodine. The twin peaks at 141.8 and 141.6, 115.1 and 114.9, 42.0 and 39.4, and 18.1 and 17.8 ppm, along with four downfield signals for four carbonyl carbons, indicate that delsemine, as with delavaine, is a mixture of two closely related bases which differ only in the side chain of the anthranilic acid moiety. The proton nmr spectrum is very similar to that of delavaine (**10**) except for the absence of the twin signals at δ 3.68 and 3.71 which are attributed to the methyl groups of the carboxymethyl ester functions of the anthranoyl side chains. Instead, the proton nmr spectrum of delsemine showed two broad signals at δ 5.44 and 5.85 that are exchangeable with D_2O and are attributed to the protons of two primary amide groups in the anthranoyl side chains. As with delavaine, the presence of the -CH(CH_3)- grouping in the anthranoyl side chains of delsemine was indicated by the presence of two overlapping doublets (J = 6.5 Hz) centered at δ 1.31 and 1.28 attributed to two secondary methyl groups. It is also indicated by the presence of two sets of peaks at 42.0 and 39.4 and 18.1 and 17.8 ppm for the methine and methyl carbons, respectively, in the ^{13}C nmr spectrum of delsemine. The ^{13}C nmr spectrum of delsemine (**6**) did not show the twin peaks at 51.9 and 51.7 ppm (for the methyl groups of the carboxymethyl functions in the anthranoyl side chains) which appear in the ^{13}C nmr spectrum of delavaine. These findings support structure **6** for the isolated material. Confirmation of identity was provided by synthesis from methyllycaconitine (**3**) in ether with aqueous ammonium hydroxide following the procedure developed by Kuzovkov.[10] Isolation of the product on a Chromatotron afforded a product that showed proton and carbon-13 nmr spectra, tlc behavior and specific rotation identical with those of delsemine isolated naturally. Delsemine (**6**) was also obtained when methyllycaconitine (**3**) was subjected to the vapor of ammonium hydroxide for a prolonged time at room temperature.

As with delavaine (**10**), efforts to resolve the two components of delsemine (**6**) on tlc plates of alumina or silica gel using a variety of solvent systems were unsuccessful. The del-

semine mixture was preparatively separated on an alumina rotor (Chromatotron), using 1% MeOH in hexane-chloroform (1:1) as an eluent. The first eluted compound was delsemine B (**6B**),[11] amorphous, $[\alpha]_D^{30}$ +28.2 ($CHCl_3$). In addition to the downfield signal at 168.0 ppm, attributed to the benzoyl carbonyl carbon, the ^{13}C nmr spectrum of delsemine B exhibited two downfield signals at 170.6 and 177.8 ppm due to two amide carbonyl carbons of one anthronyl side chain.

The second eluted compound was delsemine A (**6A**), amorphous, $[\alpha]_D^{30}$ +36.8 ($CHCl_3$). The carbon-13 nmr spectrum showed two downfield signals at 173.6 and 174.7 ppm for the two amide carbonyl carbons of one anthronyl side chain.

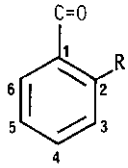
The terminal amide and carbomethoxyl carbonyl carbons are assigned to the signals at 174.7 and 174.1 ppm, respectively, in delsemine A (**6A**) and delavaine A (**10A**), paralleling the assignment of a signal at 173.3 ppm to the terminal carbonyl carbon in septentriodine and septentrionine (5). Delsemine A, delavaine A, septentriodine, and septentrionine all possess the terminal moiety $-CH_2CO-$. It follows that in delsemine B (**6B**) and delavaine B (**10B**) the terminal carbonyl carbons must be assigned to the signals at 177.8 and 175.9 ppm, respectively.

The assignment of the upperfield signals at 35.9 and 36.4 ppm to the methine carbons of the anthronyl side chain in the carbon-13 nmr spectra of delavaine B and delsemine B, respectively, was deduced from a study of a dept (distortionless enhancement by polarization transfer) carbon-13 nmr experiment, determined for a sample of delsemine B (**6B**) where the methine carbon signal appeared at 36.7 ppm. In this sample the methylene carbon of the anthronyl side chain appeared at 41.9 ppm. Consequently the lower field signals at 41.5 and 42.0 ppm in delavaine B and delsemine B, respectively, were assigned to the methylene carbons of the anthronyl side chain. In the carbon-13 nmr spectrum of delavaine A, the methine and methylene carbons of the anthronyl side chain resonated at the same position: 39.1 ppm. In delsemine A, there were two signals at 39.2 and 39.5 for the methine and methylene carbons of the anthronyl side chain.

Table 1. Carbon-13 nmr data of Delsemine (**6, 6A, 6B**), Delavaine (**10, 10A, 10B**) and Septentriodine (**12**)

Carbon	6	6A	6B	10	10A	10B	12
C-1	83.9	83.9	83.9	83.9	83.9	83.9	84.0
C-2	26.1	26.1	26.1	26.1	26.2	26.2	26.1
C-3	32.2	32.2	32.2	32.3	32.2	32.2	31.6
C-4	37.6	37.6	37.6	37.7	37.7	37.7	37.6
C-5	43.3	43.3	43.3	43.4	43.4	43.4	43.3
C-6	91.0	91.0	91.0	91.1	91.1	91.1	91.1
C-7	88.6	88.5	88.6	88.6	88.6	88.6	88.7
C-8	77.6	77.6	77.6	77.5	77.6	77.6	77.6
C-9	50.7	50.7	50.5	50.7	50.6	50.7	50.4
C-10	38.1	38.1	38.1	38.2	38.3	38.2	38.1
C-11	49.1	49.2	49.1	49.1	49.2	49.1	49.1
C-12	28.8	28.7	28.7	28.8	28.8	28.8	28.7
C-13	46.2	46.1	46.1	46.2	46.2	46.2	46.1

Table 1 continued

Carbon	6	6A	6B	10	10A	10B	12
C-14	83.9	83.9	83.9	84.0	84.0	84.0	84.0
C-15	33.9	33.7	33.7	33.9	33.9	33.9	33.7
C-16	82.6	82.6	82.6	82.6	82.6	82.6	82.7
C-17	64.5	64.5	64.5	64.55	64.5	64.5	64.6
C-18	69.9	69.8	69.8	69.9	69.8	69.8	69.9
C-19	52.5	52.4	52.4	52.6	52.6	52.6	52.4
N-CH_2	50.9	51.0	51.0	50.9	51.0	51.0	51.0
CH_3	14.0	14.1	14.1	14.0	14.0	14.0	14.1
C-1'	55.7	55.9	55.8	55.7	55.8	55.7	55.9
C-6'	57.8	57.9	57.9	57.8	57.9	57.8	57.9
C-14'	58.2	58.2	58.2	58.1	58.1	58.1	58.1
C-16'	56.3	56.4	56.4	56.3	56.3	56.3	54.4
C=O	168.0	168.0	168.0	168.1	168.1	168.1	168.3
 1	114.9, 115.1	114.8	114.9	114.7, 114.8	114.8	114.7	114.7
2	141.5, 141.8	141.7	141.5	141.7, 141.9	142.0	141.7	141.9
3	120.7	120.7	120.7	120.8	120.8	120.8	120.8
4	134.8	134.9	134.8	134.9	134.9	134.9	135.2
5	122.7	122.8	122.8	122.6	122.6	122.6	122.8
6	130.3	130.4	130.4	130.3	130.3	130.3	130.5
HN-C=O	173.7	173.6		172.4	172.5		
CH-CH_3	39.4, 18.1	39.5, 18.2		39.1, 17.9	39.1, 17.9		
CH_2	39.2	39.2		39.1	39.1		
C=O	174.7	174.7		174.1	174.1		
Y	Y = NH_2			51.7	Y=OCH_3 51.7		
HN-C=O	170.6		170.6	169.9		170.0	
CH_2	42.0		42.0	41.5		41.5	
CH-CH_3	36.5, 17.8		36.4, 17.8	35.9, 17.1		35.9, 17.1	
C=O	177.9		177.8	175.9		175.9	
Y	Y = NH_2			51.9	Y = OCH_3	51.9	
HN-C=O							170.6
CH_2							28.9
CH_2							32.7
C=O							173.3
OCH_3							51.9

Chemical shifts in ppm downfield from TMS, determined in $CDCl_3$.

Values given for primed carbons refer to chemical shifts for methoxyls.

EXPERIMENTAL

Melting points are corrected and were determined on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter.

Infrared spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer. ^{1}H nmr spectra were recorded on Varian EM-390, and JEOL model FX-90Q spectrometers. ^{13}C nmr spectra were recorded on JEOL FT models FX-60 and FX-90Q spectrometers. Mass spectra were recorded on a Finnegan Quadrupole 4023 mass spectrometer. Droplet countercurrent chromatography was performed in an assending mode on a Buchi-67 DCC chromagograph. For chromatographic separations on a Chromatotron,[4] rotors were coated with 1 mm thick layer of either alumina (Al_2O_3 60 GF254 neutral, type E, EM reagents, Art. No. 1092) or silica gel (silica gel 60 PF254 for preparative-layer chromatography containing gypsum, Art. No. 7149).

Extraction and separation — Powdered roots of *D. delavayi* (14.65 kg) were extracted with 90% ethanol. The solvent was evaporated *in vacuo* to give 1.36 kg of extract. Part of the extract (700.5 g) was dissolved in 1.4 l of $CHCl_3$ and then extracted with 2% aqueous sulfuric acid (5 x 400 ml). The acidic layer was basified to pH 8 with solid sodium bicarbonate and extracted with $CHCl_3$ (9 x 300 ml) to afford a crude alkaloid fraction (E_1, 55.66 g). The aqueous layer was made alkaline to pH 12 with 20% aqueous sodium hydroxide solution, and then extracted with $CHCl_3$ (5 x 300 ml) to give a crude alkaloid fraction (E_2, 2.89 g). Another part of the extract (25 g) was chromatographed on a column of Dowex 50W-X8 cation exchange resin to give 575 mg of a total crude alkaloidal residue. It was dissolved in 3 ml $CHCl_3$ and filtered to give a $CHCl_3$-soluble fraction (E_3, 250 mg) and a $CHCl_3$-insoluble fraction (E_4, 325 mg).

Isolation of hetisinone (7), hetisine (8), and ajaconine (9) — Fraction E_2 (2.27 g) was chromatographed on the Droplet Countercurrent Chromatograph (DCCC) in an ascending mode using a mixture of toluene-$CHCl_3$-MeOH-water (2:5:5:2). The lower organic layer was used as the mobile phase and the upper aqueous layer was used as the stationary phase. Fractions of 12 ml each were collected at a flow rate of 12 ml/h. Fractions 26-35 (226 mg) in 1.5 ml $CHCl_3$ was treated with excess ether when a heavy precipitate was formed. The latter was collected (40 mg) and the filtrate gave after evaporating the solvent 186 mg of yellow, oily residue that was subjected to preparative tlc, using 3, 20 x 20 cm 1 mm thick basic alumina plates. The main band was extracted with 10% methanol in chloroform to afford 40 mg of a yellowish white solid residue. Crystallization from acetone gave 28 mg of colorless needles of hetisinone (7), mp 268-270°C; $[\alpha]_D^{25}$ +40.0° (c, 0.9, $CHCl_3$); MS: m/z (327, M^+, 45%); mmp was undepressed, and the ir, ^{1}H and ^{13}C nmr spectra were identical with those of an authentic sample.[12,13] The methanol wash from the DCCC experiment was chromatographed by vacuum liquid charomatography (vlc)[3] using a column of basic alumina (40 g). Elution was started with chloroform, then with increasing concentrations of methanol (1-50%). Fractions 1-5 (310 mg) were rechromatographed on a Chromatotron using a 1 mm thick alumina rotor. Elution was carried out as mentioned earlier. Fractions 4-5 (108 mg) were rechromatographed on a Chromatotron using a 1 mm thick alumina rotor. Elution was started with chloroform, then with increasing concentrations of methanol (2-60%). Fractions 3-6 (75 mg) were rechromatographed on a Chromatotron using the same alumina rotor used above and elution was started with chloroform, then with increasing concentrations of methanol (4-30%). Fractions 3-5 (58 mg) crystallized from acetone to give 40 mg of ajaconine (9); mp 170-172°C; $[\alpha]_D^{27}$ -135.0° (c, 0.8, EtOH); MS: m/z 359 (M^+, 10%); mmp was undepressed; ir, ^{1}H and ^{13}C nmr spectra were identical with those of an authentic sample.[14,15] Fractions 8-10 (64 mg) crystallized from acetone to give 47 mg of hetisine (8) as fine needles; mp 254-256°C; $[\alpha]_D^{27}$ +10.0° (c, 0.4, $CHCl_3$); MS: m/z 329 (M^+, 50%), mmp was undepressed. Tlc behavior and ir, ^{1}H and ^{13}C nmr spectra were identical with those of an authentic sample.[12]

Isolation of deltaline (1), anthranoyllycaconitine (4), delavaine (10), deltamine (2), methyllycaconitine (3), lycoctonine (5) and delsemine (6) — Fraction E_3 (250 mg) was chromatographed on a Chromatotron[4] using a 1 mm thick alumina rotor. Elution was carried out as in the following sequence: ether-hexane (1:1), 50 ml; ether, 100 ml; ether-$CHCl_3$ (4:1), 50 ml; ether-$CHCl_3$ (3:2), 50 ml; ether-$CHCl_3$ (1:1), 50 ml; then with increasing concentrations of methanol (1-30%) in ether-$CHCl_3$ (1:1), 30% MeOH in $CHCl_3$, 50 ml, and finally with 50% MeOH in chloroform, 50 ml.

Isolation of deltaline (1) — Fraction 3 (30 mg) crystallized from ether to give 24 mg of deltaline (1), mp 189-191°C; $[\alpha]_D^{29}$ +31.4° (c, 1.1, MeOH); mmp undepressed; tlc, ir, ^1H and ^{13}C nmr spectra were identical with those of an authentic sample.[16,17] Another preparation of this isolate showed MS: m/z 507 (M^+, 1%).

Isolation of anthranoyllycoctonine (4) — Fraction 7 (7 mg) occurred as an amorphous product, homogeneous by tlc and ^1H nmr; $[\alpha]_D^{23}$ +48.8° (c, 0.9, EtOH); ^1H and ^{13}C nmr were identical with those of an authentic sample.[8,18] Another preparation of this isolate showed MS: m/z 586 (M^+, 7%).

Isolation of delavaine (10) — Fraction 4-6 (50 mg) was rechromatographed on a Chromatotron. A 1 mm thick alumina rotor was used and elution was performed as in the following sequence: ether, 50 ml; ether-$CHCl_3$ (4:1), 50 ml; ether-$CHCl_3$ (1:1), 50 ml; ether-$CHCl_3$-MeOH (1:1:1%), 50 ml and ether-$CHCl_3$-MeOH (1:1:2%), 50 ml. Fraction 2 gave 31 mg of delavaine as an amorphous product; $[\alpha]_D^{29}$ +36.2° (c, 1.7, $CHCl_3$); MS: m/z 714 (M^+, EI) and 715 [$(M+H)^+$, CI], EI: 683(100%), 682(12%), 651(28%), 248(10%) 216(98%), 188(54.5%), 146(45%), 129(87%), 120(33%), 115(27%), 101(37%), 85(12%), 75(22.5%), 71(30%), 69(72.8%) and 59(70%); ir (nujol) 3460, 3310, 1735, 1690, 1605, 1590 and 1257 cm^{-1}; ^{13}C nmr (Table 1).

Isolation of deltamine (2) — Fraction 8-9 (15 mg) was crystallized from ether to give 13 mg of deltamine (2), mp 232-234°C; mmp was undepressed; tlc, ir ^1H and ^{13}C nmr spectra were identical with those of an authentic sample.[17,19] Another preparation of this isolate showed $[\alpha]_D^{29}$ -20.7° (c, 0.8, EtOH); MS: m/z 465.

Isolation of methyllycaconitine (3) — Fractions 11-12 (9 mg) gave methyllycaconitine as a homogeneous amorphous product; $[\alpha]_D^{23}$ +41.4° (c, 0.9, EtOH); MS: m/z 667 (M^+, $-CH_3$, 1%); tlc, ^1H and ^{13}C nmr spectra were identical with those of an authentic sample.[8,16]

Isolation of lycoctonine (5) — Fractions 15-17 (33 mg) were crystallized from acetone to afford 29 mg of lycoctonine (5); mp 96-98°C; $[\alpha]_D^{29}$ +51.2° (c, 0.8, EtOH); MS: m/z 467 (M^+, 3%). TlC, ir, ^1H and ^{13}C nmr spectra were identical with those of an authentic sample.[8,20,21]

Isolation of delsemine (6) — Fractions 19-20 (35 mg) were combined and chromatographed on a Chromatotron using a 1 mm thick silica gel rotor. Elution was started with chloroform, then increasing concentrations of methanol (2-20%) were used. Fraction 5 (22 mg) gave delsemine as an amorphous product. Fraction E_4 (325 mg) was chromatographed on a Chromatotron using a 1 mm thick alumina rotor, and elution was performed as in the following sequence: ehter, ether-$CHCl_3$ (1:1), $CHCl_3$, then increasing concentrations of methanol in chloroform. Fractions 8-10 (44 mg) were re-

chromatographed on a Chromatotron using a 1 mm thick silica gel rotor. Elution was carried out using chloroform and then increasing concentrations of methanol (2-20%). Fractions 5-6 (40 mg) gave delsemine as an amorphous product. $[\alpha]_D^{31}$ +33.9° (c, 0.75, EtOH), MS: m/z 699 (M^+), ir (nujol) 3440, 3320, 1680, 1605, 1590 and 1255 cm^{-1}; ^{13}C nmr was identical with that of a synthetic sample (see below).

Preparation of delavaine (**10**) and methyl ester (**13**) from the reaction of methyllycaconitine (**3**) with sodium bicarbonate in methanol — Methyllycaconitine (**3**, 75 mg) in 2 ml of methanol was treated with sodium bicarbonate (20 mg). The mixture was kept at room temperature with occasional shaking for 20 h and then was filtered through a cotton plug and applied to a Chromatotron. A 1 mm thick alumina rotor was used and elution was carried out as in the following sequence: $CHCl_3$-Ether (1:1), 50 ml; $CHCl_3$, 50 ml; and $CHCl_3$-ether-MeOH (1:1:2%), 50 ml. Fraction 1 (60 mg) was rechromatographed on the same alumina rotor and eluted with $CHCl_3$-EtOAc (4:1). Fraction 1 gave 20 mg of methyl ester **13** as an oil, MS: m/z 279 (M^+, 14%); ir (nujol) 1745, 1692, 1605, 1590 and 1260 cm^{-1}; $[\alpha]_D^{29}$ -10.6 (c, 0.6, $CHCl_3$); ^1H nmr ($CDCl_3$): δ 11.10, 11.22 (-NH), 8.69 (1H, d, J = 8.3 Hz), 8.02 (1H, d, J = 8.3 Hz), 7.53 (1H, t, J = 6.8 Hz), 7.06 (1H, t, J = 6.8 Hz), 3.93 (3H, s, $COOCH_3$), 3.71 and 3.68 ppm ($COOCH_3$, two isomers); ^{13}C nmr ($CDCl_3$): δ 17.1, 17.9, 35.9, 37.7, 39.1, 41.5, 51.7, 51.9, 52.3, 114.5, 114.6, 130.8, 134.6, 141.6, 141.8, 168.0, 170.0, 172.5, 174.1, and 176.0 ppm. Fractions 2-3 gave 35 mg of delavaine (**10**), as an amorphous product: $[\alpha]_D^{29}$ +37.2° (c, 1.6, $CHCl_3$). HRMS, m/z: 683.354358. M^+-OCH_3 requires: 683.35436. Ir (nujol) 3460, 3310, 1735, 1690, 1605, 1590 and 1257 cm^{-1}; ^{13}C nmr was identical with that of natural delavaine (see above).

Preparation of delavaine (**10**) by refluxing methyllycaconitine (**3**) in methanol — Methyllycaconitine (**3**, 20 mg) in methanol (20 ml) was heated under reflux for 4 days. The solvent was evaporated under reduced pressure to give 21 mg of a yellow gummy residue. It was chromatographed on the Chromatotron using a 1 mm thick alumina rotor and eluted with chloroform-ethylacetate (4:1, 100 ml). Fraction 1 gave 4 mg of **13** (tlc and ^1H nmr spectrum). Fraction 2 afforded 8 mg of a homogeneous amorphous product (**10**). Fraction 3 (6 mg) was rechromatographed as above to give 2 mg of **10**. The fractions were combined. Tlc behavior and ^1H and ^{13}C nmr spectra were identical with those of authentic delavaine (**10**).

Preparation and Separation of delavaine A (**10A**) and B (**10B**): — A solution of methyllycaconitine (**3**, 58 mg) in methanol (50 ml) was treated with sodium acetate (30 mg) and the reaction mixture was heated under reflux for four days. The solvent was evaporated under reduced pressure and the residue was extracted with 3 x 50 ml of chloroform. The solvent was dried over anhydrous sodium sulfate and was evaporated under reduced pressure to give 55 mg of residue. The latter was chromatographed on a Chromatotron using a 2 mm thick alumina rotor and eluted with hexane-chloroform (2:1). Fractions 2-3 gave 16 mg of delavaine A (**10A**), as an amorphous product: $[\alpha]_D^{29}$ +39.4 (c, 0.8, $CHCl_3$); ^1H nmr ($CDCl_3$): δ 1.07 (3H, t, J = 7.0 Hz, $-NCH_2CH_3$), 1.28 (3H, d, J = 7.0 Hz, $-CH(CH_3)-$), 3.27, 3.35, 3.40 and 3.42 (each 3H, s, OCH_3), 3.68 (3H, s, $-COOCH_3$), four aromatic protons 7.10 (1H, t, J = 7.3 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz), 8.72 (1H, d, J = 8.3 Hz) and 11.17 (1H, broad s, -NH-CO-); ^{13}C nmr (table 1).

Fractions 11-14 gave 16 mg of delavaine B (**10B**), as an amorphous solid, $[\alpha]_D^{29}$ +31.7° (c, 0.8, $CHCl_3$); ^1H nmr ($CDCl_3$): δ 1.07 (3H, t, J = 7.0 Hz, $-NCH_2CH_3$), 1.3 (3H, d, J = 7.0 Hz, $-CH(CH_3)-$),

3.27, 3.35, 3.40 and 3.42 (each 3H, s, -OC$\underline{H}_3$), 3.71 (3H, s, -COOC$\underline{H}_3$), four aromatic protons 7.10 (1H, t, J = 7.3 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz), 8.72 (1H, d, J = 8.8 Hz) and 11.05 (1H, broad s, -NH-CO-); ^{13}C nmr (Table 1).

Fractions 4-10 gave 17 mg of amorphous mixture of delavaine A and delavaine B (^1H and ^{13}C nmr).

Preparation of (11) by treatment of methyllycaconitine (3) with sodium acetate in ethanol— Methyllycaconitine (3, 10 mg) in ethanol (10 ml) was treated with sodium acetate (10 mg). The mixture was kept at room temperature with occasional shaking for 2 weeks. The mixture was filtered through a cotton plug and the solvent was removed under reduced pressure. The residue was chromatographed on a ptlc plate of neutral alumina (ptlc), using acetone-chloroform (1:0 as an eluent. The major zone was extracted ^1H nmr: δ 1.02 (3H, t, J = 7.5 Hz, -NCH$_2$C$\underline{H}_3$), two overlapping doublets at 1.31 and 1.28 (each 3H, J = 6.5 Hz), four aromatic protons at 7.14 (1H, t, J = 7.8 Hz), 7.69 (1H, t, J = 7.3 Hz), 8.02 (1H, d, J = 7.3 Hz) and 8.75 (1H, d, J = 7.8 Hz) and 11.1 and 11.2 (each 1H, broad s, -N$\underline{H}$CO-). On another sample, HRMS, m/z: 728.39758. $C_{39}H_{56}N_2O_{11}$ requires 728.39748.

Preparation of delsemine (6) from the reaction of methyllycaconitine (3) with aqueous ammonium hydroxide — Methyllycaconitine (3, 95 mg) in ether (40 ml) was treated with aqueous ammonium hydroxide (1 ml, 10%) and the reaction mixture was kept at room temperature with continuous stirring for 89 h. The solvent was evaporated under reduced pressure to give 1 ml of aqueous suspension that was extracted with chloroform (three-10 ml portions). The residue after evaporating the solvent was chromatographed on a Chromatotron using a 1 mm thick alumina rotor. Elution was performed as in the folowing sequence: ether-$CHCl_3$ (1:1), 50 ml then ether-$CHCl_3$-MeOH (20:20:5), 45 ml. Fraction 4 gave 63 mg of delsemine (yield 66%) as a homogeneous amorphous product; $[\alpha]_D^{31}$ +33.5° (c, 3.0, $CHCl_3$); MS: m/z 699 (M^+); ir (nujol) 3440, 3320, 1680, 1605, 1590 and 1255 cm^{-1}; ^{13}C nmr (Table 1).

Preparation of delsemine (6) by treatment of methyllycaconitine with ammonia vapor — Methyllycaconitine (3, 9 mg) in chloroform (10 ml) was kept in a desiccator containing 20 ml of conc. ammonium hydroxide. After 2 weeks, the solvent was removed under reduced pressure and the residue was chromatographed on a ptlc plate of alumina using 4% MeOH in acetone-chloroform (1:1), as an eluent. The major zone was cut and extracted to give 4 mg of amorphous product, identical (^1H proton nmr and tlc) with delsemine (6) prepared as in A.

Treatment of methyllycaconitine with ammonium hydroxide in methanol: — Methyllycaconitine (3, 60 mg) in methanol (10 ml) was treated with aqueous ammonium hydroxide (10 ml, 5%) and the reaction mixture was kept at room temperature with continuous stirring for 5 h. The solvent was evaporated under reduced pressure to give 1 ml of aqueous suspension that was extracted with chloroform (three - 15 ml portions). The residue after evaporating the solvent was chromatographed on three plates of neutral alumina (ptlc), using MeOH-EtOAc-$CHCl_3$ (1:1:8) as eluent. The major zone was cut and extracted to give 45 mg of lycoctonine (characterized by tlc and ^1H and ^{13}C nmr).

Preparation and Separation of delsemine A (6A) and delsemine B (6B): — Methyllycaconitine (98 mg) was dissolved in ether (80 ml). Ammonium hydroxide (2 ml) was added and the reaction mixture was kept at room temperature with continuous stirring for 92 hours. The solvent was evapo-

rated under reduced pressure and the residue was chromatographed on a Chromatotron, using a 1 mm thick alumina rotor. Elution was performed using 1% MeOH in hexane-chloroform (1:1). Fractions 10-13 gave 15 mg of delsemine B (**6B**), as an amorphous solid, $[\alpha]_D^{30}$ +28.2° (c, 0.6, $CHCl_3$); ^1H nmr ($CDCl_3$): δ 1.07 (3H, t, J = 7.0 Hz, $-NCH_2CH_3$), 1.26 (3H, d, J = 7.0 Hz, $-CH(CH_3)-$), 3.26, 3.34, 3.38 and 3.41 (each 3H, s, $-OCH_3$), four aromatic protons 7.10 (1H, t, J = 7.8 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz), 8.68 (1H, d, J = 8.3 Hz) and 11.07 (1H, broad s, -NH-CO-); ^{13}C nmr (Table 1). On another sample, HRMS, m/z: 651.35127. (M^+-H_2O) requires 651.35133.

Fractions 19-20 gave 15 mg of delsemine A (**6A**), as an amorphous solid, $[\alpha]_D^{30}$ +36.8° (c, 0.7, $CHCl_3$); H nmr ($CDCl_3$): δ 1.07 (3H, t, J = 7.0 Hz), $-NCH_2CH_3$), 1.30 (3H, d, J = 7.0 Hz, $-CH(CH_3)-$), 3.26, 3.34, 3.38 and 3.41 (each 3H, s, OCH_3), four aromatic protons 7.10 (1H, t, J = 7.8 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.97 (1H, d, J = 7.8 Hz), 8.68 (1H, d, J = 8.3 Hz) and 11.17 (1H, broad s, -NHCO-); ^{13}C nmr (Table 1). On another sample, MS, m/z: 699 (M^+), 681 (M^+-H_2O), 668 (M^+-OCH_3), 651 (M^+-OCH_3-OH).

Fractions 14-18 gave 30 mg of amorphous solid of a mixture of delsemine A and delsemine B (^1H and ^{13}C nmr).

ACKNOWLEDGMENT

We thank Dr. Harridutt Desai for running several nmr spectra.

REFERENCES

1. J.A. Glinski, B.S. Joshi, S.Y. Chen and S.W. Pelletier, *Tetrahedron Letters*, 1984, **25**, 1211.
2. B.S. Joshi, J.A. Glinski, H.P. Chokshi, S.Y. Chen, S.K. Srivastava and S.W. Pelletier, *Heterocycles*, 1984, **22**, 2037.
3. S.W. Pelletier, B.S. Joshi and H.K. Desai, "Techniques for Isolation of Alkaloids" in *Advances in Medicinal Plant Research*, editors: A.J. Vlietinck and R.A. Dommisse, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1985, pp. 181-185.
4. H.K. Desai, B.S. Joshi, A.M. Panu and S.W. Pelletier, *J. Chromatography*, 1985, 322, 223.
5. S.W. Pelletier, R.S. Sawhney and A.R. Aasen, *Heterocycles*, 1979, **12**, 377.
6. S.W. Pelletier and J. Bhattacharyya, *Tetrahedron Letters*, 1977, **32**, 2735.
7. Alkaloid B has been incorrectly identified as delsemine,[6,8] an alkaloid first discovered by Yunusov and Abubakirov[9] and further studied by Kuzovkov.[10] A careful review of our original experimental records on the identification of alkaloid B from *D. tricorne* as delsemine reveals the following facts: 1. The record of the isolation of alkaloid B from *D. tricorne* is available. 2. There is no record describing the conversion of methyllycaconitine to delsemine. 3. The index of experiments on *D. tricorne* lacks any mention of such an experiment. 4. The ^{13}C nmr spectrum of alkaloid B is available, but there is no ^{13}C nmr spectrum of a delsemine sample prepared from methyllycaconitine. Thus we have been unable to locate any experimental documentation for the statements[6] "We treated pure methyllycaconitine (Alkaloid A) with NH_4OH following the procedure used by previous workers, but the resulting product gave a ^{13}C nmr spectrum identical with that of Alkaloid B..... Thus, the identity of

'delsemine' with Alkaloid B is established." Subsequent experiments show that treating methyllycaconitine with dilute NH_4OH under various conditions always leads to either delsemine mixture (**6**) or to lycoctonine.

8. S.W. Pelletier, N.V. Mody, R.S. Sawhney and J. Bhattacharyya, *Heterocycles*, 1977, **7**, 327.
9. S. Yu Yunusov and N.K. Abubakirov, *J. Gen. Chem.*, 1951, **21**, 173, 967; 1951, **22**, 1461; 1954, **24**, 735.
10. A.D. Kuzovkov, *J. Gen. Chem. USSR (English Translation)*, **25**, 2317 (1955).
11. The names delsemine B and delsemine A were assigned to parallel the structures of the side chain in delavaine B and delavaine A, respectively.
12. S.W. Pelletier, J.A. Glinski, B.S. Joshi and S.Y. Chen, *Heterocycles*, 1983, **20**, 1347.
13. S.W. Pelletier, A. Aneja and K.W. Copinath, *Phytochemistry*, 1968, **7**, 625.
14. D. Dvornik and O.E. Edwards, *Tetrahedron*, 1961, **14**, 54.
15. S.W. Pelletier and N.V. Mody, *J. Am. Chem. Soc.*, 1979, **101**, 492.
16. S.W. Pelletier, O.D. Dailey and N.V. Mody, *J. Org. Chem.*, 1981, **46**, 3284.
17. S.W. Pelletier, N.V. Mody and O.D. Dailey, Jr., *Can. J. Chem.*, 1980, **58**, 1875.
18. S.W. Pelletier, R.S. Sawhney, H.K. Desai and N.V. Mody, *J. Nat. Prod.*, 1980, **43**, 395.
19. A.S. Narzullaev, M.S. Yunusov, and S.Yu. Yunusov, *Khim. Prir. Soedin. (English Translation)*, 1973, **9**, 424.
20. O.E. Edwards and M. Przybylska, *Can. J. Chem.*, 1982, **60**, 2661.
21. A.J. Jones and M.H. Benn, *Can. J. Chem.*, 1973, **51**, 486.

Received, 27th December, 1985