THE DITERPENOID ALKALOIDS OF *CONSOLIDA AMBIGUA*

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ABSTRACT.—A detailed study of the basic components of the seeds of *Consolida ambigua* (L) (formerly known as *Delphinium ajacis*) has led to the isolation of seven new diterpenoid alkaloids as well as ten known alkaloids. Of the seven new alkaloids, five, viz. 14-acetylbrowniine (**6**), ajacusine (**17**), ajadine (**15**), ambiguine (**5**) and dihydroajaconine (**2**), are fully characterized: two of them designated as alkaloids F and G are partially characterized. The ten known diterpenoid alkaloids are 14-acetyldelcosine (**7**), ajacine (**4**), ajaconine (**1**), anthranoyl-lycoctonine (**10**), browniine (**11**), delcosine (**13**), delphatine (**12**), delsoline (**9**), lycoctonine (**3**) and methyl-lycaconitine (**14**). The methods of their isolation and characterization are discussed in detail in this paper. The coexistence of C_{19}- and C_{20}-diterpenoid alkaloids in this plant is worth noting.

Consolida ambigua, formerly known (1) as *Delphinium ajacis*, is an ornamental plant with the common name of garden larkspur. Some of the *Consolida* (earlier reported as *Delphinium*) plants are known to possess insecticidal (2) and growth inhibiting (3) activities. The seeds of the plant have been known for a long time to contain alkaloids. In 1914 Keller and Völker (4) reported the isolation of ajaconine for the first time from the seeds of garden larkspur. Subsequently Dvornik and Edwards established (5) the structure of ajaconine as **1** in 1960. Since then several papers concerning the isolation and structure elucidation of lycoctonine-type diterpenoid alkaloids from the seeds of this plant have been reported (6).

In 1972, Sastry and Waller (7a) reported the presence of five components when the deuterated trimethylsilyl derivative of pure ajaconine (**1**) was analyzed by the gc-ms system. Recently, the same laboratory reported (7b) the isolation and identification of alkaloids from the "common larkspur" (*D. ajacis*) by tlc, gc and gc-ms techniques. They detected the presence of four known alkaloids, ajaconine (**1**), delcosine (**13**), acetyldelcosine (**7**), delsoline (**9**), and four unknown alkaloids by tlc analyses. The identity of four known alkaloids was established only by the mass spectral data. On the basis of the mass spectral data and biosynthetic considerations, the structures of four new alkaloids, delosine, delcoline, dimethylacetyldelcosine and trimethylacetyldelcosine, were proposed. None of the alkaloids reported by Waller and Lawrence were isolated in crystalline form and fully characterized. To our knowledge, a systematic investigation of the alkaloid content of the seeds of garden larkspur (*C. ambigua*) has not been reported. We have carefully reexamined the basic extract from the seeds of *C. ambigua* with the object of isolating new and structurally interesting compounds. This paper reports the isolation of seven new alkaloids and ten known alkaloids. Of these ten known alkaloids, only four alkaloids have been reported previously to be present in the seeds. During our investigation, we did not encounter any of the four "new alkaloids" reported by Waller and Lawrence (7b).

RESULTS AND DISCUSSION

A 4.55 kg batch of finely powdered *C. ambigua* seeds was defatted with ligroin and then thoroughly extracted with 85% ethanol by percolation at room temperature. The extracts were evaporated to dryness under reduced pressure at room

temperature. The alkaloids were isolated from these extracts by a combination of gradient pH separation, column chromatography, and preparative thick-layer chromatographic (ptlc) techniques.

The alkaloids were divided into two main fractions: the weakly basic fraction extracted at pH 7.5 to 8, and the strongly basic fraction extracted at pH-12. Ligroin and 85% ethanol extracts were processed separately for the weak and strong base fractions and then combined, respectively.

The total weak-base fraction yielded 58.0 g (1.27%) of crude alkaloid mixture and the total strong-base fraction yielded 8.8 g (0.19%) of crude alkaloid mixture. Each fraction was processed separately for the isolation of the alkaloids.

ALKALOIDS OF THE "STRONG-BASE" FRACTION.—The strong-base fraction was shown to consist mainly of ajaconine (**1**). This fraction (8.8 g), on crystallization, afforded 3.9 g of pure ajaconine (5). (See table 1 for physical constants). The

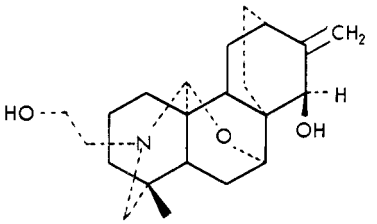
TABLE 1. Physical constants of the alkaloids of *Consolida ambigua*.

Name	mp °C (cor.)	$[\alpha]_D$
14-Acetylbrowniine (**6**)	123–124	+27.8° ($CHCl_3$)
14-Acetyldelcosine (**7**)	193–194	+30.4° (EtOH)
Ajacine (**4**)	142–143	+50.0° (EtOH)
Ajaconine (**1**)	165–166	−99.7° (EtOH)
Ajacusine (**17**)	158–161	+65.2° (EtOH)
Ajadine (**15**)	134–136	+43.9° (EtOH)
Ambiguine (**5**)	106–108	+38.0° ($CHCl_3$)
Anthranoyl-lycoctonine (**10**)	130–133	+46.3° (EtOH)
Browniine (**11**)	amorphous	—
Browniine perchlorate	210–211	+25.4° (EtOH)
Delcosine (**13**)	203–204	+53.6° ($CHCl_3$)
Delsoline (**9**)	212–213	+54.3° ($CHCl_3$)
Delphatine (**12**)	amorphous	+38.2° ($CHCl_3$)
Delphatine perchlorate	205–207	—
Delsemine (**16**)	85–92	+46.3° (EtOH)
Dihydroajaconine (**2**)	99–100	−35.0° (EtOH)
Lycoctonine (**3**)	103–120	+48.3° ($CHCl_3$)
Lycoctonine perchlorate	206–207	+27.1° (EtOH)
Methyl-lycaconitine (**14**)	100–115	+41.2° (EtOH)

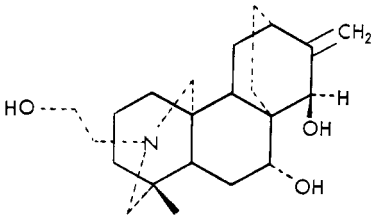
mother liquor left after ajaconine crystallized showed the presence of 4 spots on tlc. A portion of the mother liquor was separated into three different fractions by preparative thick-layer chromatography. Crystallization of the least polar fraction (Rf 0.60) gave an alkaloid, mp 99–100°, which proved to be dihydroajaconine (**2**) as shown by its identity (mp, ir, ^1H and ^{13}C spectra) with an authentic sample prepared by sodium borohydride reduction of ajaconine. This is the first time that dihydroajaconine has been isolated in a pure form as a component of *C. ambigua* (8). Sastry and Waller reported (7a) the presence of dihydroajaconine in gc-ms studies of ajaconine isolated from this plant. A sample of what had been earlier identified as pure ajaconine furnished a mixture of five components when the deuterated trimethylsilyl derivative was analyzed on the gc-ms. The temperature on the gc column (215°) and the time required for elution (12.7–27.8 min.) may have given rise to some rearrangement products.

The second fraction (Rf 0.55) on crystallization gave lycoctonine (**3**). The identity of this alkaloid was established by comparison with an authentic sample (source *Delphinium tricorne*) (13), and by its ^{13}C nmr spectrum (9). The most polar fraction yielded ajaconine upon crystallization from acetone.

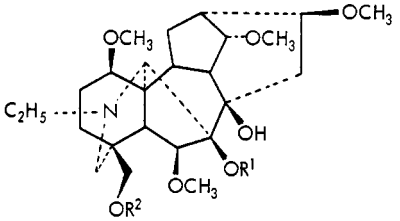
ALKALOIDS OF THE "WEAK-BASE" FRACTION.—The weak-base fraction was dissolved in chloroform, and the solution was extracted with 3% hydrochloric acid. The aqueous acidic layer was basified with sodium bicarbonate (pH 7.5 to 8.0) and extracted with ether. The ether extract was designated as E–1 (32.5g). The aqueous layer was further extracted with chloroform and the chloroform extract was designated as Ch-1 (6.1g). The original chloroform layer was evaporated to dryness, and the residue containing very weak bases was dissolved in 1% hydrochloric acid and processed as above to give the ether extract E–2 (7.25g). The chloroform extract gave a negligible residue which was discarded (see scheme 1 in Experimental section).



1 Ajaconine



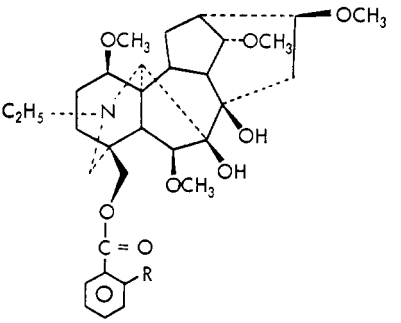
2 Dihydroajaconine



3 $R^1 = R^2 = H$ Lycoctonine

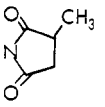
12 $R^1 = H$; $R^2 = CH_3$ Delphatine

20 $R^1 = R^2 = CH_3$

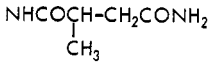


4 R = $NHCOCH_3$ Ajacine

10 R = NH_2 Anthranoyl-lycoctonine

14 R =  Methyl-lycaconitine

16A R = $NHCOCH_2$-CH(CH_3)-$CONH_2$ Delsemine

16B 

ALKALOIDS OF FRACTION E–1.—Fraction E–1 on tlc showed a mixture of several alkaloids. This fraction was concentrated to about 50 ml and kept at room temperature when a solid separated. This solid, on purification by ptlc and repeated crystallization, afforded a white crystalline alkaloid, mp 140–141°, which was identified as ajacine (4) by its spectral properties and its correlation with

lycoctonine (**3**). Basic hydrolysis of ajacine yielded lycoctonine in quantitative yield. The structure of ajacine also was established independently by ^{13}C nmr analysis (9).

The mother liquor of fraction E–1, on concentration, yielded a gummy residue, which was subjected to column chromatography over alumina. The fractions obtained from this chromatography were monitored by tlc and appropriate fractions were combined. Tlc of 5.02 g of the combined fractions 24–29 (table 2) revealed the presence of several alkaloids; the sample was chromatographed again on a column of neutral alumina (table 3). The residue from the combined fractions 21–25 (table 3) was separated by ptlc into fractions A and B.

Fraction A, on crystallization from aqueous ethanol, afforded white needles of ambiguine, $C_{28}H_{45}NO_8$ (elemental analysis and ms), mp 106–108° (corrected), $[\alpha]^{25}D+38.0°$. Infrared absorption at 3560, 1735 and 1090 cm^{-1} indicated the presence of hydroxyl, acetate, and ether groups, respectively. The ^1H nmr spectrum of ambiguine in $CDCl_3$ exhibited the presence of $N-CH_2-CH_3$ (3H, *t*, *J* 7.5 Hz) centered at δ 1.03; $-OCOCH_3$ (3H, *s*) at δ 2.05, and aliphatic methoxy groups (each 3H, *s*) at δ 3.28, 3.35, 3.48 and 3.55. A doublet of doublets observed at δ 4.72 is typical of a C(14)β-proton when a α-hydroxy group at C(14) is esterified. On the basis of these data and the nature of other C_{19}-diterpenoid alkaloids isolated from *C. ambigua*, we conclude that ambiguine has a lycoctonine-type skeleton. Alkaline hydrolysis of ambiguine (5% potassium hydroxide in methanol) gave the amino-alcohol (**8**) which, on acetylation (acetic anhydride/pyridine), regenerated ambiguine. Treatment of ambiguine with Ac_2O/BF_3 or acetic anhydride/*p*-toluenesulfonic acid did not acetylate the tertiary C(7)-hydroxy group, probably because of steric factors. The fragmentation pattern of ambiguine in the mass spectrum also indicated the presence of a lycoctonine-type skeleton. The base peak at M^+-31 revealed the presence of a methoxy group at C(1)[6]. The ^{13}C nmr spectrum of ambiguine was consistent with strucuture **5**. The complete structure elucidation of ambiguine by ^{13}C nmr spectroscopy has been presented in detail in a recent paper (8).

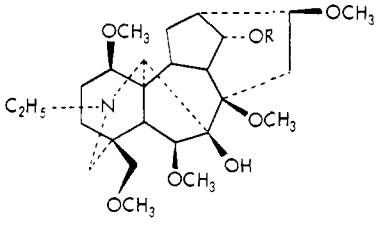
Fraction B on crystallization from hexane gave an acetylbrowniine. Alkaline hydrolysis of this compound afforded the known alkaloid browniine (**11**). Acetylation of browniine gave a compound identical with our "acetylbrowniine" (**6**), a fact which indicates that the acetate group in this compound is present at the C(14) position. Although this compound has been known (10) as a synthetic derivative of browniine, this isolation (9) demonstrates its first natural occurrence. Subsequently acetylbrowniine also has been reported as occurring in *D. brownii* (11) and *D. oreophilum* (12).

The mother liquor obtained during the crystallization of acetylbrowniine was further separated into fractions A and B by preparative tlc. The amorphous alkaloid obtained from fraction A was designated as alkaloid F. Analytical and spectroscopic data, including the ^{13}C nmr spectrum of alkaloid F, suggest that the compound has a lycoctonine-type skeleton (see experimental section). The ^1H nmr spectrum shows a 3H triplet at δ 1.03 for an $N-CH_2-CH_3$ group, a 3H singlet at δ 2.01 for an acetate group and several singlets at δ 3.36, 3.40 and 3.45 for methoxyl groups. Fraction B on tlc showed two spots which were separated by ptlc. The one with higher Rf was identified as 14-acetylbrowniine; the other is an unstable base which was designated as alkaloid G and crystallized from hexane, mp 235–241°. The ^1H nmr spectrum of alkaloid G reveals the presence of an *N*-ethyl group and several methoxyl groups on the lycoctonine-skeleton. Alkaloid

G decomposes in solution at room temperature. Because of an insufficient amount of these alkaloids, the complete structure elucidation of alkaloids F and G has been interrupted.

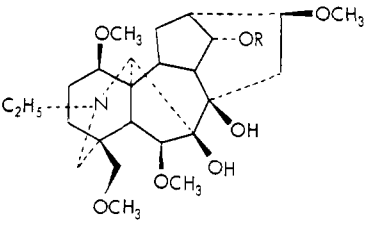
Fractions 35 to 44 (table 3) were combined and crystallized from ether-petroleum ether to give cubes of 14-acetyldelcosine (**7**), mp 193–194°. 14-Acetyldelcosine and delsoline (**9**) were also isolated in crystalline form from fractions 30 to 61 (table 2) by preparative tlc. Fractions 62 to 104 yielded the known alkaloid, ajacine (**4**).

Continued elution of the column bearing fraction E–1 with hexane-ethanol (95:5) gave fractions 105 to 130 from which anthranoyl-lycoctonine (**10**) and browniine (**11**) were obtained. The combined fractions 131 to 146 gave lycoctonine as a white solid.



5 R = Ac Ambiguine

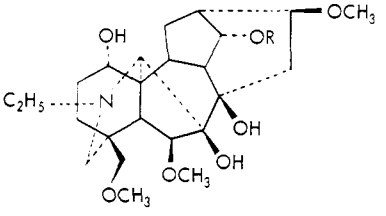
8 R = H



6 R = Ac Acetylbrowniine

11 R = H Browniine

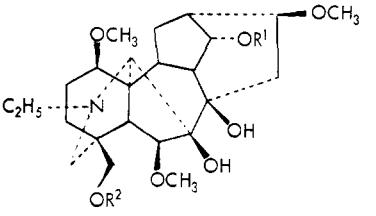
19 R = Bz Benzoylbrowniine



7 R = Ac Acetyldelcosine

9 R = CH_3 Delsoline

13 R = H Delcosine



15 R^1 = Ac; R^2 = CO-C_6H_4-o-NHAc Ajadine

17 R^1 = Bz; R^2 = CO-C_6H_4-o-N(succinimide-CH_3) Ajacusine

18 R^1 = R^2 = H Delectinine

Delphatine (**12**) was obtained in an amorphous form from the combined fractions 147–153 (table 2). Delphatine formed a perchlorate, mp 205–207°. The structure of delphatine was further supported by its ^{13}C nmr analysis (9). Finally, the identity of this alkaloid was confirmed by direct comparison with synthetic material prepared by methylation of lycoctonine with methyl iodide and sodium hydride.

ALKALOIDS OF FRACTION E–2.—This fraction (7.25 g), on crystallization from 70% ethanol, afforded 2.3 g of a white crystalline solid. Repeated crystallization

from the same solvent yielded silky needles of ajacine (**4**). This alkaloid was isolated earlier from fraction E–1. The mother liquor obtained during the crystallization of ajacine was separated by preparative tlc to yield amorphous methyl-lycaconitine (**14**).

Examination of fraction E–2 mother liquors accumulated during the isolation of ajacine and methyl-lycaconitine revealed the presence of several alkaloids. These closely related alkaloids were separated by repeated chromatography over alumina and fractional crystallization to give two new alkaloids: ajadine (**15**), mp 134–136°, and ajacusine (**17**), mp 158–161°, and two known aklaloids, ajacine (**4**) and delsemine (**16**). Recently we reported (13), that the alkaloid delsemine is an apparently inseparable mixture of the closely related compounds **16A** and **16B**, which may be artifacts resulting from the use of NH_4OH during the isolation procedure. We feel that delsemine may have been formed because of the use of diethylamine (contaminated with ammonia) during the preparative tlc separation. Reaction of methyl-lycaconitine with ammonia would give rise to delsemine.

Ajadine, $C_{35}H_{48}N_2O_{10}$, crystallized from acetone-hexane, mp 134–136° (d), $[\alpha]_D$+43.9° (c 1.0, abs. EtOH), showed ir bands (Nujol) at 3465 (OH), 1733, 1700, 1685 (carbonyls), 1600 (aromatic) and 1080 (ether) cm^{-1}. The ^1H nmr spectrum of ajadine exhibits signals at δ 1.07 (3H, *t*, N–CH_2–CH_3), 2.07 (3H, *s*, OCOCH_3), 2.24 (3H, *s*, NHCOCH_3), 3.28, 3.34, 3.38 (each 3H, *s*, –OCH_3), 4.77 (1H, *d* of *d*, 14β–*H*), 7.13, 7.59, 7.97, 8.72 (aromatic protons) and 11.0 (broad *s*, N*H*COCH_3).

Ajacusine, $C_{43}H_{52}N_2O_{11}$, crystallized from methylene chloride-hexane, mp 158–161° (d), $[\alpha]^{24}_D$+65.2° (c 0.98, abs. EtOH), showed ir absorption (Nujol) at 3475 (OH), 1715, 1705, 1695 (carbonyl), 1605 (aromatic) and 1087 (ether) cm^{-1}. The ^1H nmr spectrum of ajacusine indicates the presence of an N–CH_2–CH_3 (3H, *t*) at δ 1.07 and 3 aliphatic methoxy groups at δ 3.27, 3.29 and 3.32 ppm. The spectrum also shows a doublet of doublets at δ 5.03 ppm which is typical of a C(14)β-proton. A group of signals appearing in the region δ 7.22–8.16 ppm are indicative of the aromatic protons of benzoyl and anthranoyl groups.

Ajadine and ajacusine both afforded the known amino alcohol, delectine (**18**), when saponified with 5% methanolic potassium hydroxide at room temperature. The structure of delectine (**18**) was confirmed by comparison of its ^{13}C nmr spectrum with those of browniine (**11**) and lycoctonine (**3**). The presence of a benzoyl group at C(14) in ajacusine was confirmed by comparison of the observed chemical shifts in ajacusine with those of 14-benzoylbrowniine (**19**) (14).

DELCOSINE (13) FROM FRACTION CH–1.—The dark brown residue (1.8g) was dissolved in chloroform and filtered. Addition of ether to the filtrate caused separation of a yellow material (400 mg). Purification of this sample by ptlc furnished a single alkaloid which crystallized from alcohol and was identified as delcosine (**13**), mp 198–200°; $[\alpha]^{25}_D$ = +52.5° ($CHCl_3$) and $[\alpha]^{8}_D$ = +53.6° ($CHCl_3$) [reported (6) mp 203–204°; $[\alpha]_D$+57° ($CHCl_3$)]. The perchlorate salt showed mp 215–216°. The identity was further supported by its ir, ^1H nmr and ^{13}C nmr[9] spectral analysis.

EXPERIMENTAL SECTION

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected and were taken on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Rotations were taken in $CHCl_3$, unless otherwise noted, on a Perkin-Elmer polarimeter, model 141. Infrared spectra were recorded on a Perkin-Elmer model 297 spectrophotometer. Proton nmr measurements were taken in $CDCl_3$ solutions, unless otherwise mentioned, on JEOL JNM-PST-100 and Varian T-60 spectrometers with TMS as an internal standard. The following abbreviations

are used to express the multiplicity of the signals: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets and m = multiplet. Carbon-13 nmr spectra were taken at 25.03 MHz using a JEOL-PFT-100 spectrometer and at 15.03 MHz with a JEOL-FX-60 spectrometer. ^{13}C chemical shifts are reported in ppm downfield from TMS. Spectra of the compounds were determined in $CDCl_3$ solution (which also provided the lock signal). Thin layer chromatography (tlc) of the alkaloids was carried out on Merck aluminium oxide GF-254 (type E or 60/E) and the compounds were visualized in uv light and by spraying with Dragendorff's reagent. Column chromatography was conducted on Merck neutral aluminium oxide (90 active) (activity state III) 70-230 mesh ATSM. Preparative thick-layer chromatography (ptlc) was carried out on 20 x 40 plates coated with a 2.5 mm thick layer of Merck aluminium oxide, 150 PF-254-366 (Type T), and compounds were visualized in uv light. Solvent systems used for tlc and ptlc were mostly either benzene: ethylacetate: diethylamine (7:2:1), designated as SSA, or hexane:ethanol (9:1), designated as SSB.

PROCESSING OF *Consolida ambigua* SEEDS.—The dry seeds (4.55 kg) were percolated at room temperature with ligroin (bp 63-75°) (3 x 8 liters). After partial defatting it was easier to grind the seeds. The powdered seeds were again percolated at room temperature with ligroin (3 x 7 liters) and then with 85% ethyl alcohol (15 x 9 liters) at room temperature. The extracts were concentrated under reduced pressure at room temperature. Residues from both extracts were worked up separately for the separation of the crude basic material and then the crude bases were combined for further processing.

The concentrated extract was treated with 5% hydrochloric acid under ice-cold conditions. The combined acid extracts were basified with solid sodium bicarbonate to pH 7.5-8 and extracted with chloroform. The chloroform extract was concentrated to give a total weak basic fraction of 58.0 g (1.27%). The aqueous layer at pH 7.5-8 was further basified to pH ~12 with 25% sodium hydroxide solution with cooling and then extracted exhaustively with chloroform to furnish a total strong base fraction of 8.8 g (0.19%).

ALKALOIDS OF THE STRONG-BASE FRACTION: AJACONINE (**1**), DIHYDROAJACONINE (**2**) AND LYCOCTONINE (**3**).—The crude mixture (8.8 g) on crystallization from acetone afforded colorless crystals (3.9 g; 0.085%), mp 140-144°, which were collected and washed with cold acetone. Recrystallization of a sample afforded ajaconine (**1**), mp 165-166°, (reported (5) mp 162-163°); $[\alpha]^{24}D$ −99.7° (c 1.0 EtOH); ir, νmax (nujol) 3370, 3285 (OH), 1660, 935 (>C=CH_2) and 1118 (ether) cm^{-1}; nmr, δ 0.75 (3H, *s*, C(4)-C*H*$_3$), 4.18 (1H, broad *s*, O*H*), 4.57 (1H, *s*, NC*H*-O) and a two-proton doublet centered at δ 5.06 for exocyclic methylene protons.

The mother liquor left after isolating ajaconine, on tlc (SSB) examination showed the presence of 4 spots, of which the most polar one corresponded to ajaconine. A portion of the residue (0.43 g) was resolved on ptlc using SSB solvent system and 3 bands were isolated. Band 1 (Rf 0.60) on exhaustive extraction with chloroform-ethanol (1:1) gave a gum (0.062 g; 0.015%) which crystallized from aqueous alcohol as needles of dihydroajaconine (**2**), mp 99-100°, (reported (5) mp 99-101°); $[\alpha]^{24}D$ −35.0° (c 1.0 abs. EtOH); ir, νmax (KBr) 3350 (OH) and 1660, 920 (>C=CH_2) cm^{-1}; nmr, δ 0.8 (3H, *s*, C(4)-C*H*$_3$), and 5.12 (2H, *m*, >C=CH_2).

Band 2 (Rf 0.55) on similar extraction gave a gum (0.084 g) which on crystallization from aqueous alcohol gave lycoctonine (**3**), mp 103-120°; $[\alpha]D$ +48.3° (c 0.925). The melting point was not improved on recrystallization and drying in vacuum. Tlc (SSA) showed it to be a single compound. Lycoctonine exhibited the following spectral properties: ir, νmax (nujol) 3500, 3423, 3310 (OH) and 2740 (N-CH_2-) and 1085 (ether) cm^{-1}; ^1H nmr, δ 1.04 (3H, *t*, N-CH_2-C*H*$_3$), 3.25, 3.33, 3.41, and 3.44 ppm (each 3H, *s*, OC*H*$_3$). Lycoctonine formed a $HClO_4$ salt which crystallized from methanol-ether; mp 206-207° (dec); (reported (15) mp 206°); $[\alpha]^{27}D$ +27.1° (c 1.0 abs. EtOH).

PREPARATION OF DIHYDROAJACONINE.—To a solution of 50 mg of ajaconine in 10 ml of 90% methanol was added 50 mg of sodium borohydride. The reaction mixture was stirred at room temperature for 1 hour and the solution was evaporated to dryness *in vacuo*. After adding 25 ml of water to the resulting solid mass, the base was extracted with methylene chloride. Evaporation of the solvent and crystallization afforded dihydroajaconine, mp 99-100°, in quantitative yield. The physical and spectral data of the synthetic and natural samples were identical.

ALKALOIDS OF THE WEAK-BASE FRACTION.—The crude mixture of the weak-base fraction (58 g) was dissolved in chloroform (700 ml) and processed according to scheme 1.

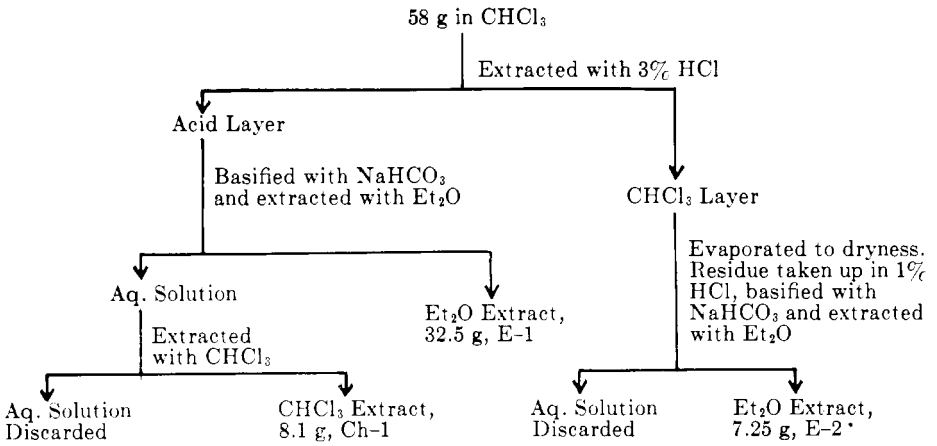
Thus the weak base fraction was separated into 3 main fractions, viz. E-1 (32.5 g), E-2 (7.25 g), and Ch-1 (8.1 g). Each of these fractions was investigated separately.

EXAMINATION OF FRACTION E-1: AJACINE (**4**).—Fraction E-1 on tlc showed a mixture of several alkaloids. This fraction was concentrated *in vacuo* to about 50 ml and kept at ambient temperature when 350 mg of solid separated. The latter on purification by ptlc (SSA) and then repeated crystallization from 70% ethanol gave silky needles of ajacine (**4**), mp 140-141° [reported (2) mp 140-142°]; ir νmax (KBr) 3474 (OH), 1694, 1682 (>C=O) and 1606, 1594

(>C=C<) cm^{-1}; ^1H nmr, δ 1.08 (3H, *t*, NCH_2CH_3), 2.24 (3H, *s*, $NHCOCH_3$), 3.27, 3.35, 3.38 and 3.42 (each 3H, *s*, OCH_3). The ^{13}C nmr spectrum was in agreement with structure **4**.

HYDROLYSIS OF AJACINE.—Ajacine (1.25 g) was hydrolyzed by boiling with 5% ethanolic potassium hydroxide. The usual work up gave a gum which crystallized from aqueous alcohol as white needles of lycoctonine (0.73 g), mp 101–112°. Physical data for the hydrolyzed product are in agreement with those of lycoctonine.

SCHEME 1



COLUMN CHROMATOGRAPHY OF FRACTION E-1.—The remaining fraction E-1 (32 g) was dissolved in a minimum volume of methylene chloride and placed on a column of neutral alumina (1.8 kg, activity III). The following fractions were collected and combined together on the basis of their tlc pattern (table 2).

FRACTIONS 24–29: AMBIGUINE (**5**), ACETYLBROWNIINE (**6**), ACETYLDELCOSINE (**7**), ALKALOIDS F AND G.—The fraction (5.02 g) on tlc (SSA and SSB) examination, showed the presence of several bases; it was chromatographed on a column of neutral alumina (300 g). Fractions containing the same components were combined and are presented in table 3.

The mixture from the combined fractions 21–25 was further separated by ptlc (SSB) into

TABLE 2. Column chromatography data of fraction E-1.

Fraction No.	Eluent	Volume (lit.)	Weight (g)
1–9	Hexane	7.70	—
10–14	Hexane:Ethanol (99.5:0.5)	2.30	—
15–23	Hexane:Ethanol (99:1)	4.25	0.18
24–25	" "	0.70	3.60
26–29	" "	0.70	1.42
30–35	" "	0.90	2.30
36–40	" "	0.75	0.97
41–44	" "	0.75	0.74
45–49	" "	0.75	0.68
50–55	" "	0.90	1.18
56–61	" "	0.90	0.30
62–104	" "	8.40	1.25
105–130	Hexane:Ethanol (95:5)	7.50	1.70
131–146	" "	3.00	1.30
147–153	" "	1.40	0.66
154–178	" "	4.80	1.57
179–204	" "	7.45	1.27
205–240	" "	8.75	1.17
241–266	Hexane:Ethanol (85:15)	6.50	0.35
267–274	Methanol	4.00	0.45

TABLE 3. Column chromatography for fractions 24–29.

Fraction No.	Eluent	Volume (lit.)	Weight (g)
1–8	Hexane	1.50	—
9–20	Hexane:Ethanol (99:1)	1.76	0.06
21–25	" "	0.15	0.70
26–34	" "	0.24	2.15
35–37	" "	0.09	0.25
38–44	" "	0.35	1.34

fraction A (0.39 g) and fraction B (0.35 g). Fraction A was dissolved in CH_2Cl_2 and passed through a small column of alumina. The gum obtained was crystallized from aqueous alcohol as clusters of needles (0.225 g). Recrystallization of the sample gave ambiguine, mp 106–108°: $[\alpha]^{25}D+38.0°$ (c 1.0); $C_{25}H_{45}NO_8$ requires C 64.22; H 8.66 and N 2.67%. Found C 64.20, H 8.66 and N 2.65%; ir, ν max (nujol) 3560 (OH), 1735 (>C=O) and 1090 (ether) cm^{-1}; ^1H nmr, δ 1.03 (3H, *t*, NCH_2CH_3), 2.05 (3H, *s*, $OCOCH_3$), 3.28, 3.35, 3.38, 3.48 and 3.55 (each 3H, *s*, OCH_3) and 4.72 (1H, *dd*, C(14)β-*H*).

Fraction B on crystallization from hexane gave acetylbrowniine (0.115 g); mp 123–124° (reported (11) mp 115–116°); $[\alpha]^{27}D+27.8°$ (c 1.0). Acetylbrowniine showed: ir, ν max (nujol) 3440 (OH), 1725 (>C=O) and 1080 (ether) cm^{-1}; ^1H nmr, δ 0.97 (3H, *t*, NCH_2CH_3), 1.97 (3H, *s*, $OCOCH_3$), 3.20, 3.23, 3.25 and 3.37 (each 3H, *s*, OCH_3) and 4.72 (1H, *dd*, C(14)β-*H*).

The mother liquor obtained during the crystallization of acetylbrowniine showed three major spots on tlc (ethyl acetate-ether, 1:1). The residue (0.233 g) was further resolved by ptlc (ethyl acetate-ether, 1:1) into fractions A and B. Fraction A (higher Rf) (0.03 g) showed only one spot on tlc and did not crystallize. Fraction A is designated as alkaloid F and gave the following spectral data: ^1H nmr, δ 1.03 (3H, *t*, NCH_2CH_3), 2.01 (3H, *s*, O–CO–CH_3), 3.36, 3.40 (each 3H, *s*, OCH_3) and 3.45 (6H, *s*, $2OCH_3$). ^{13}C nmr: 170.6, 91.0, 90.8, 82.3, 81.7, 78.7, 75.4, 72.2, 66.3, 59.4, 59.3, 57.3, 56.2, 50.7, 50.4, 49.2, 48.7, 44.5, 38.2, 37.1, 36.7, 30.3, 29.7, 29.4, 27.0, 21.4, and 13.8 ppm. Fraction B (lower Rf) (0.2 g) on silica gel tlc (methylene chloride-carbon tetrachloride-methanol, 6:3:1) showed two spots. These were resolved by silca gel ptlc using the same solvent system. One compound (0.07 g) was found to be acetylbrowniine and the other was designated as alkaloid G. The latter is very unstable and decomposes in solution. Alkaloid G crystallized from hexane: the crystals sintered at 227°, mp 235–241°; ^1H nmr, δ 1.07 (3H, *t*, NCH_2CH_3) and 3.20, 3.26, 3.36, 3.43, 3.50, 3.60 (each 3H, *s*, OCH_3); ^{13}C nmr 92.4, 89.3, 82.9, 82.6, 82.4, 81.1, 76.8, 75.6, 74.6, 73.7, 66.9, 59.6, 56.7, 56.1, 53.3, 50.8, 50.1, 46.5, 45.6, 42.7, 37.1, 29.8, 27.8, 27.3, 26.5, 24.2, 22.7, and 13.2 ppm.

ALKALINE HYDROLYSIS OF AMBIGUINE.—A solution of ambiguine (0.05 g) in 5% methanolic potassium hydroxide, was kept at room temperature for 16 hr. The solvent was removed *in vacuo* and the product in water was extracted with chloroform. Evaporation of the dried extract, *in vacuo*, gave a gum (0.042 g) of the aminoalcohol (**8**). The gum could not be induced to crystallize. Ir, ν max (nujol) 3500 (OH) and 1090 (ether) cm^{-1}; ^1H nmr δ 1.03 (3H, *t*, NCH_2CH_3), 3.26, 3.33, 3.43, 3.50, 3.56 ppm (each 3H, *s*, OCH_3).

ACETYLATION OF THE AMINOALCOHOL (**8**).—The amino alcohol (0.040 g) was dissolved in an equal mixture of acetic anhydride and pyridine (1 ml) and the solution was allowed to stand for 10 days at room temperature. The usual work up and crystallization gave ambiguine (mp, mmp, and ir the same as an authentic sample.).

ATTEMPTED ACETYLATION OF AMBIGUINE.—Attempts to acetylate ambiguine with various acetylation agents (viz. Ac_2O/Py, Ac_2O/TsOH, Ac_2O/BF_3, CH_3COCl) in the usual manner, met with failure.

ALKALINE HYDROLYSIS OF ACETYLBROWNIINE.—Acetylbrowniine (50 mg) was treated with 10 ml of 5% methanolic potassium hydroxide solution for 12 hours at room temperature. After the usual reaction work-up, browniine was obtained in quantitative yield. The identity of browniine was established by comparison with an authentic sample.

FRACTIONS 26–44: 14-ACETYLBROWNIINE (**6**) and 14-ACETYLDELCOSINE (**7**).—The combined fractions 26 to 34 (table 3) on further purification and crystallization afforded the previously identified compound, 14-acetylbrowniine (**6**), as a major alkaloid of this fraction.

The combined fractions 35 to 44 (table 3) on purification through a small alumina column column and crystallization from ether-petroleum ether afforded cubes of 14-acetyldelcosine (**7**), mp 193–194°; $[\alpha]^{24}D+30.4°$ (c 1.0 EtOH) [reported (6) mp 193–195°; $[\alpha]D+34°$]; ir, ν max (nujol), 3470, 3430, (OH), 1735 (>C=O) and 1075 (ether) cm^{-1}; ^1H nmr δ 1.1 (3H, *t*, NCH_2CH_3), 2.07 (3H, *s*, $OCOCH_3$), 3.29, 3.33 and 3.34 (each 3H, *s*, OCH_3) and 4.79 (1H, *dd*, C(14)β-*H*).

FRACTIONS 30–61: 14-ACETYLDELCOSINE (**7**) AND DELSOLINE (**9**).—The mixture (6.17 g) on tlc (SSB) showed two major spots (Rf=0.45 and 0.42). Partial resolution of the residue (1.0 g) by ptlc (SSB) gave compound A (0.32 g) with the higher Rf and compound B (0.41 g) with the lower Rf. Compound A on further purification through a small column of alumina and crystallization from ether-petroleum ether gave cubes of 14-acetyldelcosine, mp 193–194°; $[\alpha]_D$+30.4° (c 1.0, EtOH). Compound B on further purification through a small column of alumina and crystallization from methanol gave delsoline, mp 212–213°; $[\alpha]^{24}_D$+54.3° (c 1.0) (reported (12) mp 215–216°; $[\alpha]_D$+53.4°); ir, ν max (nujol) 3510, 3470, 3330 (OH), 2720 (NCH_2–) and 1075 (ether) cm^{-1}, ^1H nmr, δ 1.09 (3H, *t*, NCH_2CH_3), 3.33, 3.41 (each 3H, *s*, OCH_3) and 3.38 (6H, *s*, 2 OCH_3).

FRACTIONS 62–104: AJACINE (**4**).—The mixture (1.25 g) from fractions 62–104 on tlc (SSB) showed 1 major spot (Rf 0.4) along with traces of earlier reported bases. Further purification by ptlc afforded a gum (0.73 g) which crystallized from 70% alcohol, mp 137–139°. This compound was identified as ajacine on the basis of mp, specific rotation, and ^{13}C nmr spectral data.

FRACTIONS 105–130: ANTHRANOYL-LYCOCTONINE (**10**) AND BROWNIINE (**11**).—A fraction (1.7 g) on tlc (SSA) examination showed mainly two spots, one of them (Rf 0.55) showing a strong blue fluorescence under uv light. Partial resolution of a sample (0.4 g) on ptlc gave two products.

The gum (0.175 g), obtained from the fluorescent band, could not be obtained in a crystalline form but gave a brownish amorphous solid of anthranoyl-lycoctonine, mp 130–133° (d); $[\alpha]^{24}_D$+46.3° (c 0.55 EtOH) [reported (2) mp 145–154°, $[\alpha]_D$+51°]; ir, ν max (nujol) 3460 (OH), 3350 (NH_2), 1690 (>C=O), 1615, 1590 (C=C) and 1080 (ether) cm^{-1}; ^1H nmr, δ 1.07 (3H, *t*, NCH_2CH_3), 3.26, 3.34, 3.37, 3.42 (each 3H, *s*, OCH_3) and 6.72, 7.29, 7.28, 7.75 and 7.83 (aromatic protons).

The more polar fraction (0.13 g) could not be induced to crystallize and was found to be a single compound by tlc (SSA), and spectral examination. It formed a crystalline perchlorate salt (0.08 g), which on recrystallization from a mixture of methanol and ethyl acetate, was identified as browniine perchlorate, mp 210–211°; $[\alpha]^{24}_D$+25.4° (c 0.55 EtOH) [reported (10), mp 212°; $[\alpha]_D$+25°]; ir, ν max (nujol) 3410 (OH), 2840 and 1080 (ether) cm^{-1}. Browniine liberated from the perchlorate was amorphous and exhibited the following spectral properties: ir, ν max (nujol) 3470 (OH) and 1085 (ether) cm^{-1}; ^1H nmr, δ 1.04 (3H, *s*, NCH_2CH_3), 3.24, 3.30, 3.36 and 3.40 (each 3H, *s*, OCH_3).

FRACTIONS 131–146: LYCOCTONINE (**3**).—The fraction (1.30 g) on tlc (SSA) showed one major spot (Rf 0.45) along with 3 trace impurities. Purification of the gum (0.5 g) by ptlc (SSA) gave lycoctonine as a white crystalline solid, mp 102–117°. The melting point was not improved by recrystallization. Other data were the same as those reported earlier in this paper.

FRACTIONS 147–153: DELPHATINE (**12**).—The residue (0.66 g) on tlc (SSA) showed one major spot (Rf 0.75). Purification by ptlc (SSB) gave a gum (0.21 g) which could not induced to crystallize. It gave a perchlorate salt, mp 205–207° (reported (6), mp 212–214°). The base liberated from the salt gave an amorphous solid, $[\alpha]^{29}_D$+38.2° (c 2.95); [reported (6), $[\alpha]_D$+38.5°]. The spectral data were in agreement with the published data for delphatine.

PREPARATION OF DELPHATINE FROM LYCOCTONINE.—A well dried sample of lycoctonine (0.25 g) was methylated with methyliodide in the presence of sodium hydride in a sealed tube at 110° for 26 hr. Workup gave a gum which showed the presence of four compounds on tlc (SSA). The gum was resolved by ptlc (SSA) and the two major bands were isolated. The less polar compound from band 1 was obtained as a gum (0.057 g) of 7,18-di-*O*-methyllycoctonine (**20**). The second compound which was isolated as a gum (0.072 g) had the same ^1H and ^{13}C nmr data observed for delphatine.

FRACTIONS 154–204: 14-ACETYLBROWNIINE (**6**).—The combined fractions 154–204 showed only one major spot on tlc in different solvent systems. Crystallization of this fraction from hexane afforded 14-acetylbrowniine (**6**), mp 123–124°. All the data for 14-acetylbrowniine were identical with those reported earlier in this section.

FRACTIONS 205–240: DELCOSINE (**13**).—The fraction (1.67 g) on tlc showed mainly a single spot (Rf 0.20). The combined fractions on repeated crystallization from alcohol gave fine crystals of delcosine (0.78 g), mp 203–204°; $[\alpha]^{25}_D$+53.6° (c 1.0); [reported (2), mp 203–204°, $[\alpha]_D$+57°]; ir, ν max (nujol) 3510, 3464, 3405 and 3343 (OH), 2730 (NCH_2) cm^{-1}; ^1H nmr, δ 1.09 (3H, *t*, NCH_2CH_3), 3.33, (3H, *s*, OCH_3) and 3.36 (6H, *s*, 2 OCH_3).

ALKALOIDS OF FRACTION E-2: AJACINE (**4**) AND METHYL-LYCACONITINE (**14**).—The mixture (7.25 g) from this fraction on crystallization from 70% ethanol, gave a white crystalline solid (2.3 g). Tlc (SSB) of the solid showed it to be a mixture of 3 compounds. Repeated crystallization from 70% ethanol gave silky needles of ajacine (**4**), mp 142–143°; $[\alpha]^{21}_D$+50.0° (c 1.0 abs EtOH).

The residue (0.95 g out of 4.95 g) from the mother liquor of ajacine was partially resolved by ptlc (SSA). Band 2 (Rf 0.6) gave a gum (0.14 g), which could not be induced to crystallize, but was obtained as an amorphous solid of methyl-lycaconitine, mp 100–115°; $[\alpha]^{26}D$ +41.2° (c 1.8 EtOH); ir, ν max (nujol) 3470 (OH), 2720 (NCH_2), 1600 and 1585 (>C=C<) cm^{-1}; ^1H nmr, δ 1.08 (3H, *t*, NCH_2CH_3), two doublets centered at δ 1.19 (3H, J=7, $CHCH_3$) and δ 1.26 (2H, *J*=7, $COCH_2CH$), 3.26 (3H, *s*, OCH_3), 3.34 (6H, *s*, 2 OCH_3), 3.40 (3H, *s*, OCH_3), and δ 7.54, 7.62, 7.64 and 8.01 (aromatic protons).

INVESTIGATION OF THE MOTHER LIQUOR OF FRACTION E-2.—The mother liquor of fraction E-2, after removing a white crystalline solid, on tlc (SSA and SSB) examination showed the presence of several bases which were separated by column chromatography. The residue (6.0 g) was dissolved in a minimum volume of methylene chloride and loaded on a column of neutral alumina (300 g). The fractions shown in table 4 were collected.

TABLE 4. Column chromatography of the mother liquor of fraction E-2.

Fraction No.	Eluent	Volume	Weight (g)	Remarks
1–26	Hexane	200 ml each	0.07	Non-alkaloidal
27–47	Hexane:EtOH (99.5:0.5)	50 ml each	1.73	Ajacine
48–101	" "	100 ml each	1.37	Mixture of alkaloids
102–150	" "	" "	0.27	" "
151–190	Hexane:EtOH (98:2)	" "	0.39	" "
191–200	Hexane:EtOH (92:8)	" "	0.04	" "

FRACTIONS 48–101: AJACINE (**4**), AJADINE (**15**), DELSEMINE (**16**) AND AJACUSINE (**17**).—The residue (0.95 g) from these fractions was further divided into fractions A, B and C by ptlc (SSA). Fraction A (Rf 0.65:0.25 g) on crystallization from 70% ethanol afforded ajacine (**4**), mp 140–141°. Tlc of fraction B (Rf 0.70; 0.32 g) gave a single spot in system SSA, but a mixture of two compounds in system SSB. Fraction B was further purified by ptlc on silica gel (benzene-methanol, 4:1) to give bands 1 and 2. Crystallization of band 1 (0.14 g) from hexane-acetone gave needles (0.08 g), mp 130–135° (d) (sintering at 115°). Recrystallization from the same solvent gave needles of ajadine: mp 134–136° (d) (sintering at 115°): $[\alpha]^{21}D$ +43.9° (c 1.0, abs. EtOH): $C_{35}H_{45}N_2O_{10}$ requires C: 64.01; H: 7.37 and N: 4.27%. Found: C: 63.12; H: 7.43 and N: 4.18%; ir, ν max (nujol) 3465 (OH), 1733, 1700, 1685 (>C=O), 1600 (-C=C-) and 1080 (ether) cm^{-1}; ^1H nmr, δ 1.07 (3H, *t*, NCH_2CH_3), 2.07 (3H, *s*, $OCOCH_3$), 2.24 (3H, *s*, $NHCOCH_3$), 3.28, 3.34, 3.38 (each 3H, *s*, OCH_3), 4.77 (1H, *dd* 14*β-H*), 7.13, 7.59, 7.97, 8.72 (aromatic protons) and 11.0 (1H, broad *s*, $NHCOCH_3$). Band-2 (Rf 0.85) was dissolved in a minimum amount of chloroform and the solution was loaded on a small column of neutral alumina. Elution with hexane-ethanol (9:1) gave a colorless gum of delsemine (**16**) which crystallized from 70% alcohol, mp 85–92°: $[\alpha]^{25}D$ +46.3° (c 1.5, EtOH) [reported (6), mp 125° (hydrate); $[\alpha]D$ +43° (EtOH)].

The residue from fraction C was further purified first by passing its chloroform solution through a small column of neutral alumina and then by ptlc (SSB). The gum (0.13 g) thus obtained, on repeated crystallization first from abs alcohol and finally from methylene chloride-hexane, gave crystalline ajacusine, mp 158–161°: $[\alpha]^{24}D$ +65.2° (c 0.98, abs EtOH): ir, ν max (nujol) 3475 (OH), 1715, 1705, 1695 (>C=O), 1605 (aromatic) and 1087 (ether) cm^{-1}; ^1H nmr, δ 1.07 (3H, *t*, NCH_2CH_3), 3.27, 3.29, and 3.32 (each 3H, *s*, OCH_3), 5.03 (1H, *dd*, C(14)*β-H*) and 7.22–8.16 (aromatic protons).

ALKALINE HYDROLYSIS OF AJADINE.—Ajadine (0.051 g) was treated with 5% methanolic potassium hydroxide solution (1.5 ml) at room temperature for 15 hr. Work up gave a gum (0.03 g) which was further purified by ptlc to furnish the aminoalcohol (**18**) (0.01 g) which had an Rf value on tlc corresponding to that of a sample obtained by alkaline hydrolysis of ajacusine.

ALKALINE HYDROLYSIS OF AJACUSINE.—The base (0.094 g) was dissolved in 5% methanolic potassium hydroxide solution (3 ml) and the reaction mixture was left at room temperature for 16 hr. After removal of the solvent the residue was taken up in water and extracted with chloroform. The dried (Na_2SO_4) extract was passed through a small column of neutral alumina and the eluant was evaporated to give amorphous delectinine (**18**) (0.045 g) which could not be crystallized. Delectinine was identified by ^1H and ^{13}C nmr spectral data (14).

FRACTION CH-1: DELCOZINE (**13**).—Addition of ether to a chloroform solution of a portion (1.8 g) of the residue precipitated a pale yellow, solid material which on tlc (SSA) showed mainly one spot, but could not be crystallized. A small portion (0.4 g) of this fraction was resolved by ptlc and the major band gave a gummy residue (0.09 g) which on repeated crystal-

lization from alcohol gave crystalline delcosine, mp 203–204° (reported (6) mp 203–204°). The ^{13}C nmr spectrum was in agreement with structure **13**.

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