THE DITERPENOID ALKALOIDS OF DELPHINIUM STAPHISAGRIA

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The structure determination and chemistry of four C_{19}-diterpenoid alkaloids, delphinine (1), delphisine (9), delphidine (24) and delphirine (12), isolated from the seeds of Delphinium staphisagria by a combination of gradient pH extractions and chromatographic techniques, is summarized. The unusual ultraviolet absorption of pyrodelphonine (5) is discussed from the point of view of a photoreduction reaction and the ^{13}C and ^{1}H nmr spectra. On the basis of the hydrolysis product of delphisine (9), the structures of neoline, chasmanine and homochasmanine have been revised to 11, 13 and 15, respectively. The structural elucidation of a new class of eight bis-diterpenoid alkaloids isolated from the mother liquors of D. staphisagria is discussed. The structure of staphisine as 27 has been established by a single-crystal X-ray analysis of the monomethiodide. Application of ^{13}C and ^{1}H nmr spectroscopy to the remaining seven alkaloids led to the assignment of structures for staphidine (28), staphinine (29), staphimine (30), staphigine (31), staphirine (32), staphisagnine (33) and staphisagrine (34). These bis-diterpenoid alkaloids occur as methoxyl and demethoxyl pairs in D. staphisagria.

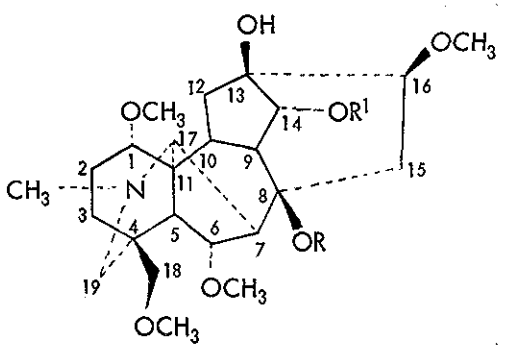
The diterpenoid alkaloids isolated from plants of the Delphinium and Aconitum genera of Ranunculaceae have long been of interest because of their pharmacological properties and complex structures. Biogenetically, these alkaloids are possibly derived from tetracyclic or pentacyclic diterpenoids in which the nitrogen atom of a molecule of β-aminoethanol, methyl amine, or ethyl amine is linked to carbons 19 and 20 or carbons

17 and 19 to form a heterocyclic ring. In this review, recent developments in the chemistry of diterpenoid alkaloids isolated from the seeds of Delphinium staphisagria will be discussed.

DELPHININE

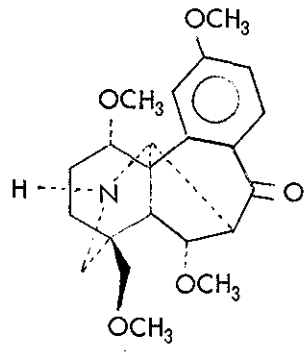
Periodically since early 1800, the seeds of *D. staphisagria*, commonly known as stavesacre, have been under investigation to account for their insecticidal activity. The seeds of this plant have been found on extraction with ligroin to yield an appreciable amount of alkaloidal faction, which consists mainly of the alkaloid delphinine (1). The latter was first isolated in 1819 by Lassaigne and Feneulle.[1] Waltz[2] revised the previous formula reported for delphinine by earlier workers to $C_{34}H_{47}NO_9$ on the basis of the analysis of the free base and its oxalate. This formula was also supported by Keller[3] and later by Markwood.[4]

In 1939, Jacobs and Craig[5] modified the formula of delphinine to $C_{33}H_{45}NO_9$ and assigned the nine oxygen atoms to four methoxyl groups, one acetyl group, one benzyl group and a hydroxyl group. In addition to this, they also indicated the presence of an N-methyl group in delphinine. Subsequently, delphinine became the subject of intensive chemical studies,[6-14] and only recently Professor Wiesner's research group at New Brunswick[15] has assigned a revised structure for delphinine (1) on the basis of an elegant synthesis of the aromatization product (3) obtained from the degradation of delphinine.[16]



1 R = Ac, R^1 = Bz

2 R = R^1 = H

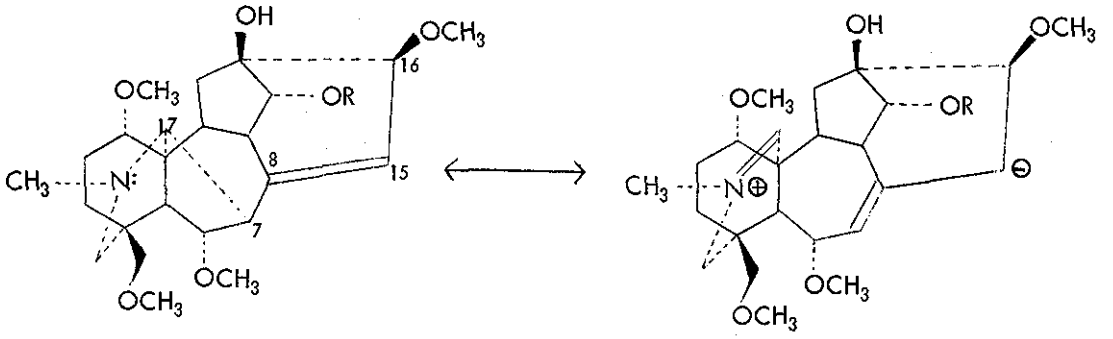


3

The parent amino alcohol of delphinine, delphonine (2), is claimed to have been isolated from the roots and aerial parts of Delphinium rotundifolium Afan by Russian investigators.[17] When the N-dealkylated derivative of this new alkaloid was realkylated with ethyl iodide, the product was found to be identical with the isolated alkaloid. Since delphonine has an N-methyl group, this result indicates that the alkaloid isolated from D. rotundifolium cannot be delphonine. No further explanation of these anomalous results have been forthcoming.

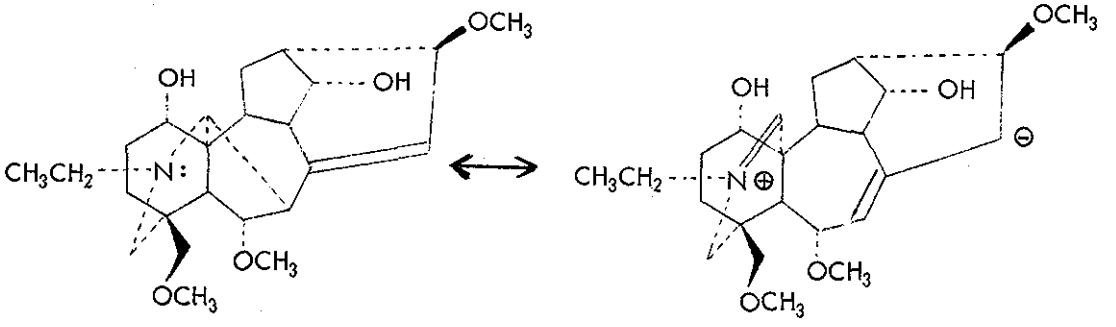
STUDY OF THE PYRODELPHONINE CHROMOPHORE

The pyrolysis product of delphinine, pyrodelphinine (4), on basic hydrolysis gives a pyroamino alcohol known as pyrodelphonine (5). Wiesner and coworker[18] reported



4 R = Bz
5 R = H

4A R = Bz
5A R = H

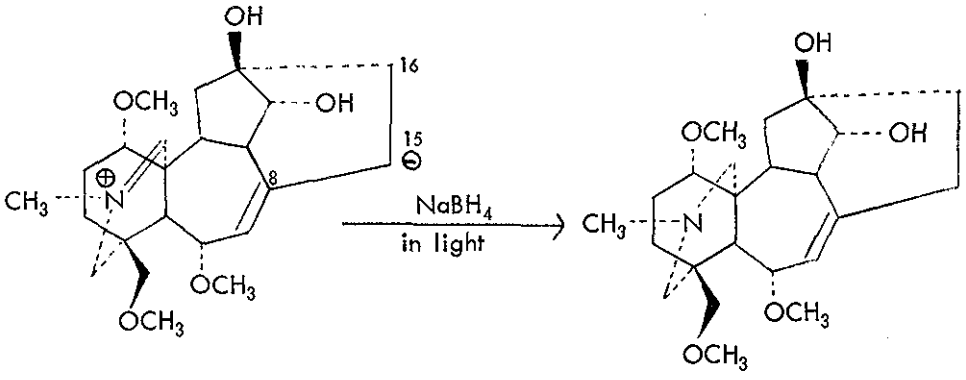


6 Pyroneoline

6A

an unexpected ultraviolet absorption (λ max 245 mμ, ϵ max 6300) for pyrodelphonine (5) and pyroneoline (6), which disappears upon acidification. To explain this phenomenon, they posulated the participation of the free electron pair on the nitrogen with the C-7 —— C-17 σ bond and the π system of the double bond between C-8 and C-15 in an excited state resembling structure 5A. Later, the same phenomenon was also explained by Cookson et al.[19] using valence bond theory.

Wiesner and Inaba[20] reported an unusual photoreduction of the C-17 —— C-7 bond in 16-desmethoxy pyrodelphonine (7) by sodium borohydride to compound 8. They also repeated the same reduction reaction using sodium borodeuteride to support the proposed nature of this chromophore. The high resolution mass spectrum of the reaction product indicated that it was monodeuterated, a result which suggested that one of the new hydrogen atoms in compound 8 came from the reagent and the other from the solvent (methanol). These results support the postulated nature of the chromophore.



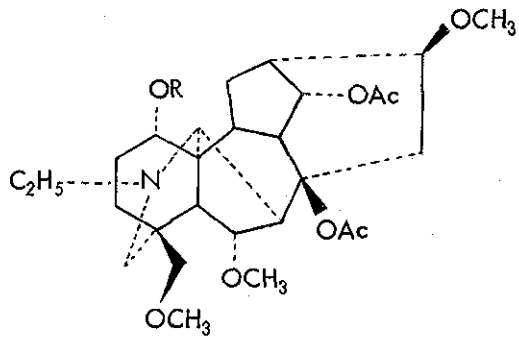
7 8

Recently we studied the pyrodelphinine chromophore in the electronic ground state of the molecule.[21] Our results are based on the differences in the ^{13}C and ^{1}H nmr spectra of pyrodelphinine caused by the protonation of the nitrogen atom. The ^{13}C and ^{1}H nmr spectra of pyrodelphinine were recorded during the stepwise acidification with deuterated acetic acid as well as the N-oxidation by m-chloro perbenzoic acid. A large upfield shift of the signal for C-15 (5.5 ppm) and the downfield shift of the signal for C-8 (4.1 ppm) in pyrodelphinine was observed upon acidification or N-oxidation. On the

basis of these observations, we postulated that the free electron pair of the nitrogen, the C-17 —— C-7 σ bond, and the π electron pair of the C-8 —— C-15 double bond are conjugated in the electronic ground state of the molecule, and the structure of pyrodelphinine may be portrayed as a resonance hybrid between the contributing structures 4 and 4A. Delocalization of the lone pair electron of the nitrogen will build up a negative charge on C-15 and result in an upfield shift of its resonance which can be seen upon protonation (acidification) or oxidation of the nitrogen atom. The same results were also confirmed by observing the change in chemical shift of the C-15 proton in ^{1}H nmr upon acidification or oxidation of the nitrogen atom.

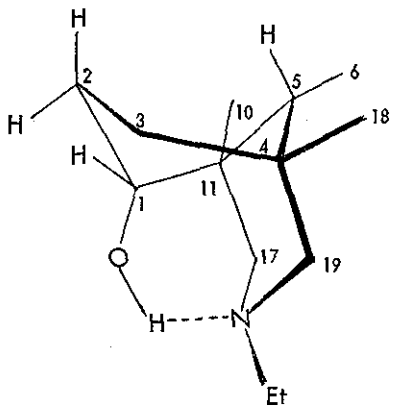
THE STRUCTURE AND STEREOCHEMISTRY OF DELPHISINE, NEOLINE, CHASMANINE AND HOMOCHASMANINE

Recently, we isolated a new diterpenoid alkaloid named delphisine[22,23] by a combination of gradient pH extractions and chromotographic techniques from the mother liquors accumulated during the isolation of a large quantity of delphinine (1). The structure and absolute configuration of delphisine (9) was established by an X-ray crystallographic analysis of the hydrochloride as 1S, 4S, 5R, 6R, 7R, 8R, 9R, 10R, 11S, 13R, 14S, 16S and 17R. Ring D in delphisine is in a boat conformation, flattened at C-15. Ring A exists in the boat form as shown in structure 9A and is stabilized by intramolecular-N · · · H — O hydrogen bonding. This latter observation was also indicated by a careful ^{1}H nmr study of delphisine in deuterochloroform solution at room temperature. The infrared spectrum of delphisine displays a broad absorption band between 3640 and 3000 cm^{-1} with a small peak at 3600 and 3235 cm^{-1}, indicative of a hydrogen bonded hydroxyl group and the boat conformation for ring A. When the 1α-hydroxyl in delphisine is acetylated (10), ring A exists in a chair conformation as shown in 10A with the absence of the intramolecular hydrogen bonding.

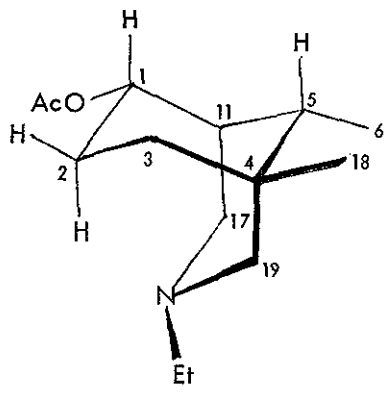


9 R = H

10 R = Ac



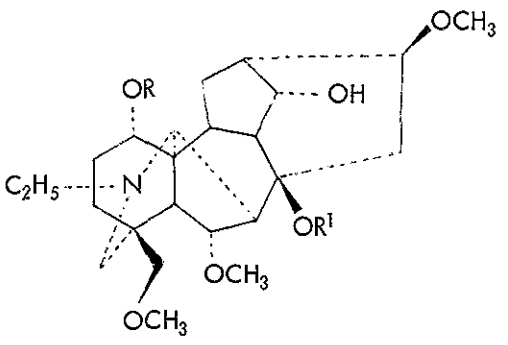
9A

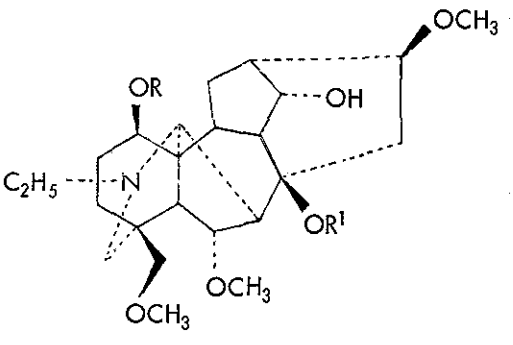


10β

Upon basic hydrolysis, delphisine afforded the parent amino alcohol, $C_{24}H_{39}NO_6$, mp. 160 - 161°, which was shown to be identical with neoline. The latter alkaloid was first isolated from amorphous aconitine (Aconitum napellus) by Freudenberg and Rogers.[24] On the basis of extensive chemical studies, Wiesner and his coworkers[18] assigned the structure of neoline as 11, with a 1α-hydroxyl. Marion et al.[25] subsequently correlated neoline with chasmanine, an alkaloid isolated from the roots of Aconitum chasmanathum and reported to have structure 14.[26,27] On the basis of this correlation, they

assigned structure 12 with a 1β-hydroxyl group to neoline.





11 Neoline R = R¹ = H

13 Chasmanine R = CH_3, R¹ = H

15 Homochasmanine R = R¹ = CH_3

12 R = R¹ = H

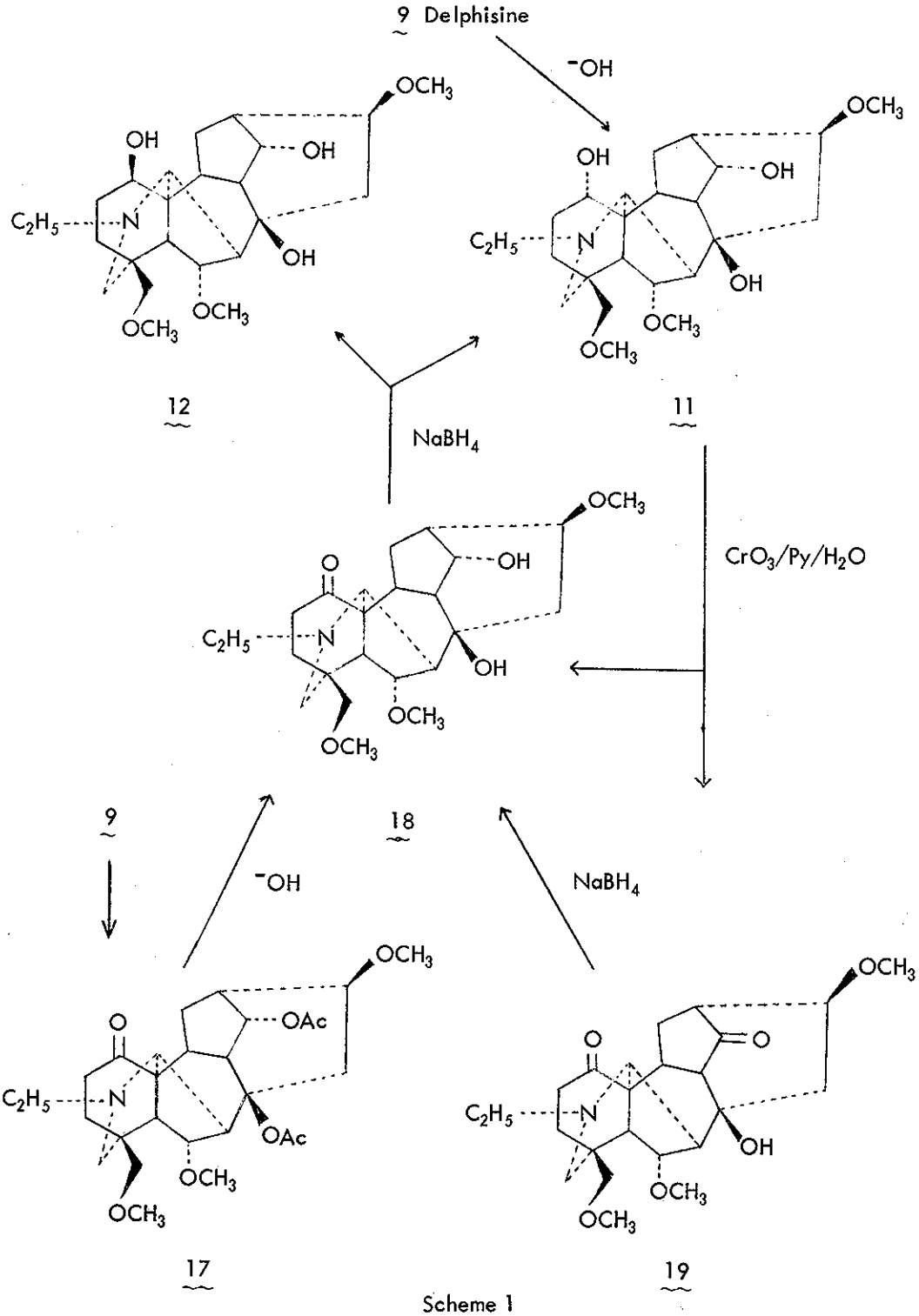
14 R = CH_3, R¹ = H

16 R = R¹ = CH_3

This discrepancy in the structures assigned to neoline has been resolved recently.[28] Delphisine was converted to a pair of C-1 hydroxyl epimers, 11 and 12, by three different routes as shown in scheme 1. Also the mild basic hydrolysis of delphisine gave epimer 11, which proved to be identical with natural neoline. This simple correlation proves that Wiesner's original assignment for neoline (11) is correct and that Marion's revised structure 12 is in error.

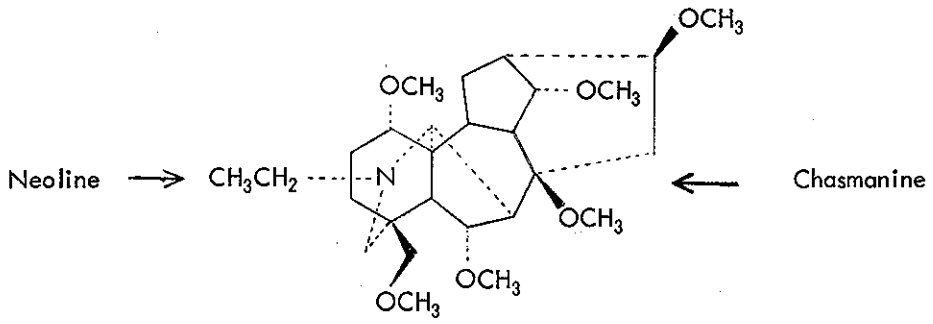
Oxidation of delphisine with Cornforth reagent gave 1-ketodelphisine(17). The latter on hydrolysis with alkali afforded the 1-keto-8,14-diol derivative (18). Compound 18 was also prepared from delphisine by the reverse sequence. Alkaline hydrolysis of delphisine gave the corresponding triol 11; the latter on oxidation with Cornforth reagent gave derivative 18 as well as the 1,14-diketo derivative (19). Compound 19 was reduced selectively to compound 18 with 1 equivalent of sodium borohydride. The stereospecific reduction of the 14-keto group to an α-oriented 14-hydroxyl group was anticipated because the β-side of the 14-keto group is less hindered.

Reduction of derivative 18 with sodium borohydride afforded a mixture of epimers 11 and 12 in a ratio of 1:2, respectively. These epimers were separated by preparative silica gel tlc. The less polar epimer 12, mp 95 - 100°C, was not identical with natural


9 Delphisine
⁻OH
OCH3
OH
OH
C2H5
N
OH
OCH3
OCH3
12
11
NaBH4
CrO3/Py/H2O
O
18
9
⁻OH
NaBH4
OAc
OAc
17
19

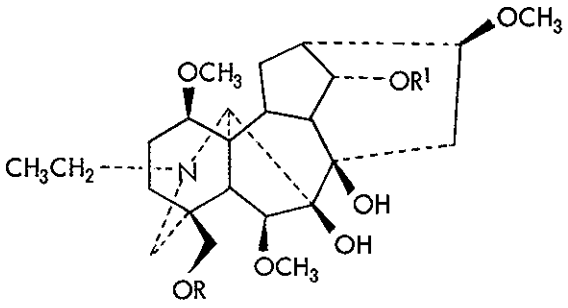
Scheme 1

neoline, while the more polar epimer 11, mp 160 - 161°C, was shown to be identical with natural neoline as well as with the hydrolysis product of delphisine. Treatment of epimer 11 with acetic anhydride and p-toluenesufonic acid at 100°C formed a triacetate (10). The identical triacetate was also obtained by treating delphisine with acetic anhydride with pyridine at room temperature. On the basis of this chemical correlation, structure 11 has been assigned to neoline.[28]



20

Since Marion and his coworkers have correlated chasmanine with neoline, the structures previously assigned to chasmanine,[27] and homochasmanine[29] (14 and 16, respectively) must be reconsidered. They converted chasmanine and neoline to 8,14-di-O-methyl chasmanine and 1,8,14,tri-O-methylneoline (20), respectively, by treatment of each alkaloid with sodium hydride and methyl iodide in refluxing dioxane. The products obtained from chasmanine and neoline were found to be identical by the usual criteria. On the basis of this chasmanine-neoline correlation, chasmanine must also have a 1α substituent as present in neoline, and we therefore assigned structure 13 to chasmanine. Because chasmanine diacetate is convertable to homochasmanine by treatment with methanol under pressure, followed by saponification,[29] the structure of homochasmanine was revised to 15.[28]

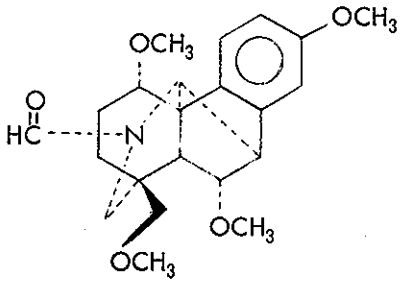


21 Browniine $R = CH_3$, $R^1 = H$

22 Lycoctonine $R = H$, $R^1 = CH_3$

A recent ^{13}C nmr study of twenty-six diterpene alkaloids[30] also supports the revised structures 11 and 13 for neoline and chasmanine, respectively. The structure of chasmanine as 13 was confirmed by an X-ray analysis[31] of chasmanine 14α-benzoate hydrochloride. With the structure of chasmanine now certain, the reported chemical correlation[27] between browniine and chasmanine was also found to be in error by a ^{13}C nmr study of browniine (21), lycoctonine (22) and their derivatives. All but two resonances for browniine and lycoctonine are essentially identical. The data of the study are consistent only with identical stereochemistry in ring A in browniine and lycoctonine and provide evidence that the procedure used in the reported correlation of browniine and chasmanine did not proceed as expected.

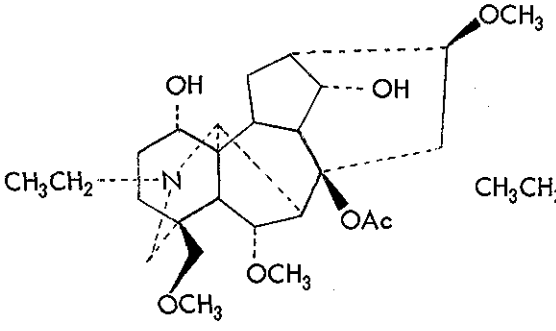
An elegant stereospecific total synthesis of the aromatic intermediate 23 of chasmanine (with a 1α methyl group) has been reported by Wiesner's research group[32] at New Brunswick. The conversion of this intermediate to chasmanine is envisaged.



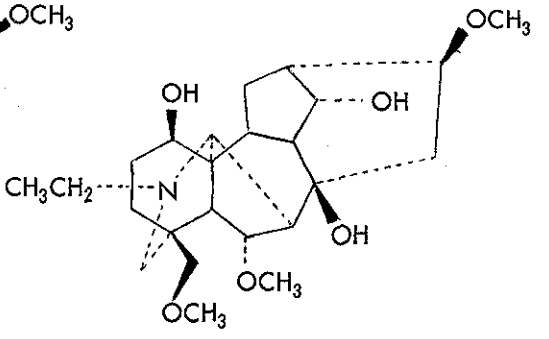
23

DELPHIDINE AND DELPHIRINE

During the isolation of delphisine, very small amounts of two new diterpenoid alkaloids designated as delphidine, $C_{26}H_{41}NO_7$, and delphirine, $C_{24}H_{39}NO_6$, were isolated by a combination of gradient pH extractions and chromatographic techniques.[33,34] On the basis of chemical studies and ^{13}C nmr analysis, structure 24 was assigned to delphidine. The known alkaloid delphisine (9) was converted to delphidine (24) by selective hydrolysis of the 14α-acetate of delphisine on an alumina column (Activity III). This result suggests the possibility that delphidine (24) may be an artefact formed during the chromatographic separation of the alkaloid mixture.

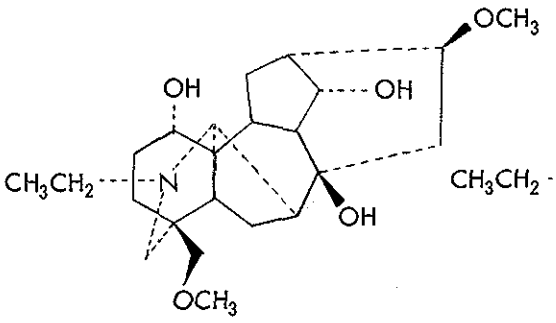


24 Delphidine

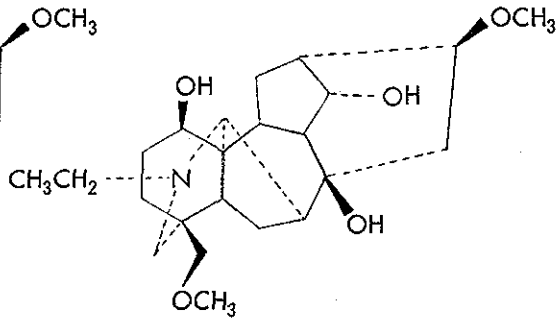


12 Delphirine

Delphirine, a very minor alkaloid of D. staphisagria was identified as 1-epineoline (12) by a comparison with a synthetic compound prepared from delphisine as shown earlier in scheme 1. The pKa values of neoline (11) (pKa = 7.5) and delphirine (12) (pKa = 6.7) are in reverse of the order which would be predicted from hydrogen bonding considerations, i.e. the 1α-hydroxyl in neoline (11) can form a hydrogen bond, while the 1β-hydroxyl in delphirine (12) cannot. Although such hydrogen bonding may be a factor in the basicity of this type of alkaloid, it clearly does not provide an explanation for the order of basicity of neoline and delphirine.



25 Isotalatizidine



26 Talatizidine

Biogenetically, it is interesting to note that the neoline-delphirine pair is the second example reported of C-1 hydroxyl epimers in the diterpenoid alkaloids. The first example reported is the pair, isotalatizidine (25) - talatizidine (26), isolated from Aconitum talassicum popov[35] and Delphinium denudatum wall.[36]

Table 1. Alkaloids of Delphinium staphisagria

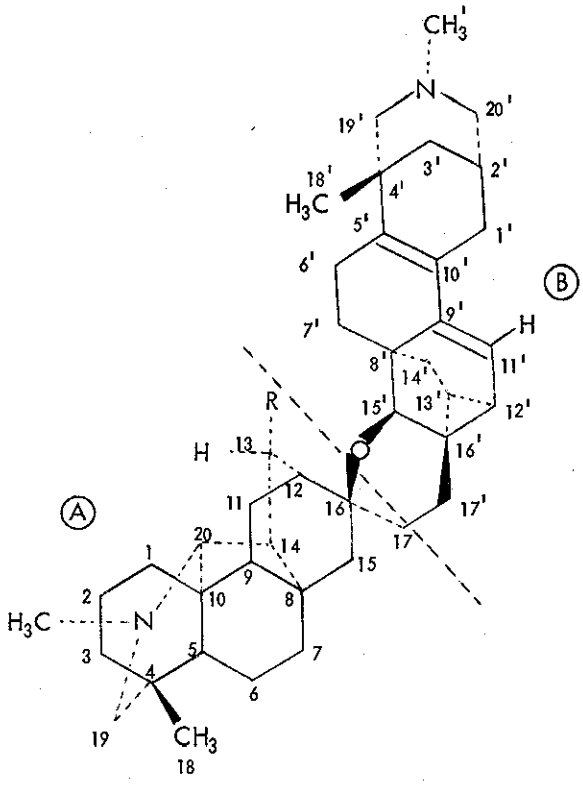
Name	Formula	MP (°C)	[α] D		References
Delphinine	$C_{33}H_{45}NO_9$	191.8°	+ 25°	(Ethanol)	15, 16
Delphisine	$C_{28}H_{43}NO_8$	122 - 123°	+ 7.1°	(Ethanol)	22, 23
Delphidine	$C_{26}H_{41}NO_7$	98 - 100°	+ 16.6°	(Ethanol)	33
Delphirine	$C_{24}H_{39}NO_6$	90 - 95°	+ 3.8°	(Ethanol)	34
Staphisine	$C_{43}H_{60}N_2O_2$	211 - 213°	-148.4°	(Benzene)	40, 41
Staphidine	$C_{42}H_{58}N_2O$	213 - 216°	-160°	(Benzene)	41
Staphinine	$C_{42}H_{56}N_2O_2$	Amorphous	- 57.5°	(Benzene)	41
Staphimine	$C_{41}H_{54}N_2O$	Amorphous	- 58.5°	(Benzene)	41
Staphigine	$C_{43}H_{58}N_2O_3$	225 - 227°	-116°	(Benzene)	42
Staphirine	$C_{42}H_{56}N_2O_2$	222 - 225°	-126°	(Benzene)	42
Staphisagnine	$C_{44}H_{62}N_2O_3$	Resin	-104.5°	(Benzene)	43
Staphisagrine	$C_{43}H_{60}N_2O_2$	229 - 231°	-105.6°	(Benzene)	43

BIS-DITERPENOID ALKALOIDS

In 1941 Jacobs and Craig at the Rockefeller Institute isolated a diterpenoid alkaloid named staphisine from the mother liquors accumulated during the isolation of delphinine from the seeds of D. staphisagria.[37] On the basis of chemical studies,[37,38] they postulated that staphisine is a diterpenoid alkaloid dimer with molecular formula $C_{44}H_{60}N_2O$ (later revised to $C_{42}H_{60}N_2O$),[39] which contains two N-methyl groups and no methoxyl group (despite the presence of 1.36% OCH_3). During the chromatographic separation, Jacobs and Craig found that the combustion analysis data of several samples of staphisine fluctuated between the limits of 82.13 and 82.85% for carbon and 9.47 and 9.77% for hydrogen. They also tried unsuccessfully to separate staphisine by crystallization of its salts. They cautioned that "the so-called staphisine could still be a persistent mixture of alkaloids which are very difficult to separate".

From these mother liquors we recently isolated by chromatography and triangular crystallization a methoxyl-containing bis-diterpenoid alkaloid, which we designated as staphisine. The structure of staphisine was determined as 27 by a single crystal X-ray analysis of the monomethiodide.[40] Chemical studies of staphisine were hindered by its instability and by the fact that attempted degradation led to complex changes involving numerous unstable products. High resolution mass spectrum and combustion analysis established the molecular formula of staphisine as $C_{43}H_{60}N_2O_2$. ^{13}C and ^1H nmr[41], ir, and uv spectral data indicated the presence of two N-methyl groups, a methoxyl group, a cyclopropyl ring, and a conjugated diene system. Catalytic hydrogenation experiments also supported the presence of two double bonds.[37] Jacobs' selenium dehydrogenation experiments had yielded pimanthrene and 1,3-dimethyl-7-isopropylphenanthrene,[39] degradation products which are compatible with structure 27. Thus, the structure of staphisine (27) determined by an X-ray analysis is consistent with observed chemical and spectral data.

Recently, we found that Jacobs "staphisine" is actually a mixture of alkaloid 27 (our staphisine) and a companion non-methoxyl bearing alkaloid designated as staphidine.[41] In addition, we have isolated two novel imine-containing bis-diterpenoid alkaloids named staphinine and staphimine (Table 1).



27 Staphisine R = OCH_3

28 Staphidine R = H

The structure of staphidine (28) was determined by a comparison of its ^1H and ^{13}C nmr spectra with those of staphisine (Table 2 and 3). The mass spectrum of staphidine showed a molecular ion peak at m/e 606 corresponding to the formula $C_{42}H_{58}N_2O$. Comparison of its ^1H nmr spectrum with staphisine revealed the absence of a methoxyl singlet at δ 3.30 and an upfield shift of one N-methyl group from δ 2.27 to δ 2.21 in staphidine. The observed change of 0.06 ppm in the chemical shift of the N-methyl was explained by the steric interaction between the $N-CH_3$ and $-OCH_3$ group in the A unit of staphisine. On the basis of this argument, the chemical shift at δ 2.13 was assigned to the N-methyl group in unit B of the molecule and that at δ 2.27 and δ 2.21 to the

N-methyl group in unit A in staphisine (27) and staphidine (28), respectively. Further comparison of staphidine with staphisine was made through their respective ^{13}C nmr spectra (Table 3). As expected, the absence in staphidine of absorption at 57.8 ppm for methoxyl and at 89.4 ppm for a methine group (C-13) was observed. Based on these data, structure 28 was assigned to staphidine.

The structures of staphinine (29) and staphimine (30) were also assigned on the basis of ^{13}C and ^{1}H nmr analysis. The ^{1}H nmr spectrum of staphinine indicated the presence of two angular methyl groups at δ 0.94 and δ 1.00, one N-methyl group at δ 2.13, a methoxyl group at δ 3.30, a vinyl proton at δ 5.85 and an imine proton at δ 7.30. The ^{1}H nmr spectrum of staphimine was similar except for the absence of a methoxyl singlet at δ 3.30 (Table 2). The presence of an imine (——CH=N——) group in these alkaloids was established by comparison with ^{13}C nmr chemical shifts of known atisine derivatives containing an imine group. The presence of an imine group in the A unit of each alkaloid is consistent with the downfield shift of the C-4 carbon and the upfield shift of the C-20 carbon in staphinine (29) and staphimine (30) relative to the known alkaloids staphisine and staphidine, respectively. Also an N-methyl singlet is present at δ 2.13 in the ^{1}H nmr spectra of staphinine and staphimine.

Table 2. ^{1}H NMR Chemical Shifts of Staphisine (27), Staphidine (28), Staphinine (29), Staphimine (30), Staphigine (31) and Staphirine (32)[a]

Carbon	27	28	29	30	31	32
$-C-CH_3^{18}$	0.91	0.91	1.00	1.00	1.12	1.12
$-C-CH_3^{18'}$	0.91	0.91	0.94	0.94	0.94	0.94
$N-CH_3'$	2.13	2.13	2.13	2.13	2.13	2.13
$N-CH_3$	2.27	2.21	-	-	2.98	2.92
$O-CH_3$	3.30	-	3.30	-	3.30	-
-C=CH-	5.85	5.85	5.85	5.85	5.85	5.85

a) ^{1}H NMR spectra were determined in $CDCl_3$ and shifts are given on the δ scale relative to TMS.

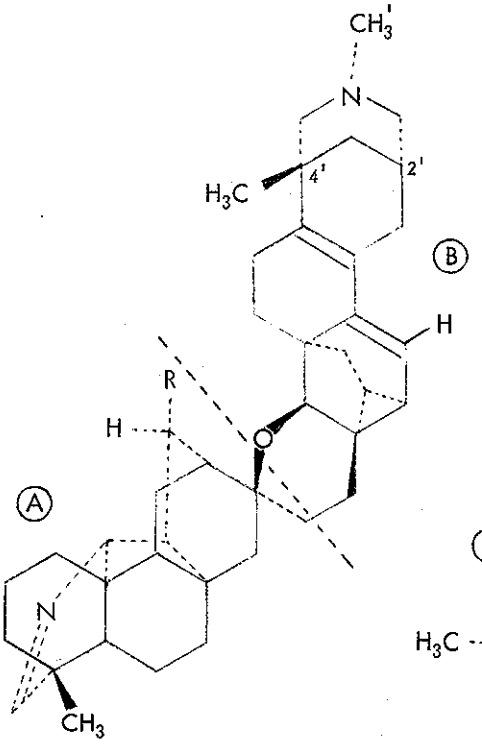
Table 3. Carbon-13 Chemical Shifts of Staphisine 27, Staphidine 28, Staphinine 29, Staphimine 30, Staphigine 31 and Staphirine 32[a]

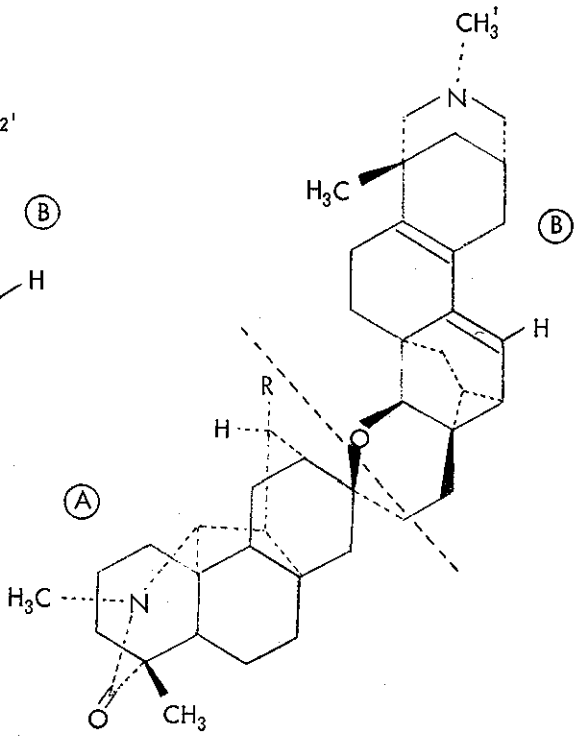
Carbon	27	28	29	30	31	32
C-4	34.2(s)	34.2(s)	41.5(s)	41.5(s)	44.6(s)	44.7(s)
C-8	37.4(s)	37.6(s)	38.1(s)	38.3(s)	38.4(s)	38.7(s)
C-10	46.0(s)	45.5(s)	44.3(s)	43.7(s)	44.6(s)	44.3(s)
C-13	89.4(d)	-	91.2(d)	-	90.3(d)	-
C-16	72.2(s)	73.6(s)	72.3(s)	73.8(s)	72.2(s)	73.5(s)
C-19	60.7(t)	60.4(t)	168.1(d)	167.6(d)	175.1(s)	175.0(s)
C-20	74.4(d)	77.0(d)	73.1(d)	75.8(d)	72.9(d)	77.0(d)
N-CH_3	43.9(q)	43.5(q)	-	-	46.9(q)	46.9(q)
C-OCH_3	57.8(q)	-	56.4(q)	-	57.0(q)	-
C-4'	34.5(s)	34.4(s)	34.4(s)	34.5(s)	34.5(s)	34.5(s)
C-5'[b]	135.6(s)	135.6(s)	135.5(s)	135.7(s)	135.6(s)	136.1(s)
C-8'	41.8(s)	41.6(s)	41.6(s)	41.6(s)	41.8(s)	41.9(s)
C-9'[b]	127.6(s)	127.7(s)	127.7(s)	127.9(s)	128.2(s)	128.1(s)
C-10'[b]	135.6(s)	135.8(s)	135.5(s)	135.7(s)	136.1(s)	136.4(s)
C-11'	112.9(d)	112.7(d)	112.9(d)	113.3(d)	113.7(d)	113.1(d)
C-15'	78.1(d)	77.6(d)	78.5(d)	77.9(d)	78.5(d)	78.1(d)
C-16'	29.5(s)	29.3(s)	29.5(s)	29.4(s)	29.7(s)	29.4(s)
C-19'[c]	62.5(t)	62.4(t)	62.5(t)	62.3(t)	62.5(t)	62.7(t)
C-20'[c]	64.7(t)	64.5(t)	64.7(t)	64.4(t)	64.7(t)	64.8(t)
N-CH_3'	46.6(q)	46.3(q)	46.3(q)	46.4(q)	46.4(q)	46.6(q)

[a] Carbon-13 spectra were taken at 25.03 MHz in the Fourier mode using a JEOL-PFT-100 spectrometer in conjuction with a EC-100-20K memory computer. Samples were dissolved in $CDCl_3$ containing TMS as an internal standard. Concentrations were about 0.3-0.8M. Multiplicities indicated are those obtained by off resonance decoupling techniques.

[b,c] These assignments may be interchanged.

Besides the four bis-diterpenoid alkaloids mentioned above, two new lactam-containing alkaloids designated as staphigine and staphirine have been isolated from the seeds of D. staphisagria.[42] The structures of staphigine (31) and staphirine (32), were determined on the basis of their ^{13}C and ^{1}H nmr analysis (Table 2 and 3).





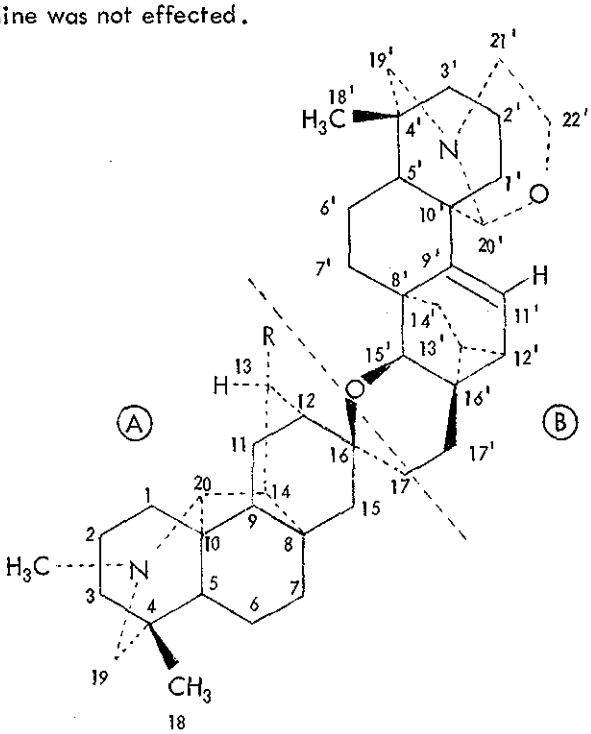
29 Staphinine R = OCH_3
30 Staphimine R = H

31 Staphigine R = OCH_3
32 Staphirine R = H

The ir spectra of these alkaloids indicated the presence of a lactam ring. The ^{1}H nmr spectrum of staphigine revealed the presence of two angular methyl groups at δ 0.94 and δ 1.12, two N-methyl groups at δ 2.13 and δ 2.98, a methoxyl group at δ 3.30 and a vinyl proton at δ 5.85. The ^{1}H nmr spectrum of staphirine was identical with that of staphigine except for the absence of a methoxyl singlet at δ 3.30 (Table 2).

The presence of the lactam ring in the A unit of these alkaloids was established by the appearance of an N-methyl singlet at δ 2.13 in the ^1H nmr spectrum and the constant carbon-13 chemical shifts shown by the C-19', C-20' and N-CH_3^1 carbons of staphigine and staphirine in comparison with the known alkaloids 27 to 30 (Table 3).

Staphinine, staphimine, staphigine and staphirine occur in extremely small amounts in the seeds of D. staphisagria in comparison with staphisine (27) and staphidine (28). All of these alkaloids (27 to 32) are closely related in structure and occur as methoxyl and desmethoxyl pairs in D. staphisagria. It is interesting to note that though these six alkaloids are obviously related to each other, at this time, there is no basis for predicting the sequence in which these alkaloids are biogenetically related. Because the nitrogen bridge connects carbons 4 and 10 in the A unit, and carbons 4' and 2' in the B unit, it is unlikely that these alkaloids are synthesized in the plant by dimerization of two identical atisine-type units. Due to the instability of the imine and lactam containing alkaloids toward different reagents, chemical correlation of these compounds with staphisine and staphidine was not effected.

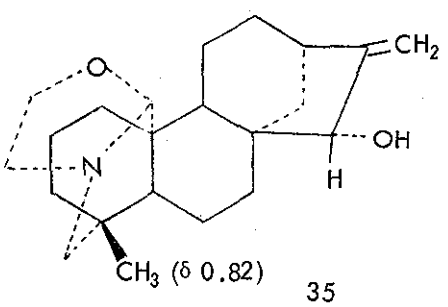


33 Staphisagnine R = OCH_3

34 Staphisagrine R = H

Staphisagnine and staphisagrine, two closely related bis-diterpenoid alkaloids were isolated from the seeds of D. staphisagria by chromatography and crystallization.[43] These alkaloids are unusual in containing an oxazolidine ring of the atisine- and veatchine-type in addition to many of the uncommon features of the staphisine skeleton.

The ir, ^{1}H, and ^{13}C nmr spectra of these alkaloids showed some similarity with the known alkaloids, staphisine (27) and staphidine (28). The ^{1}H nmr spectrum of staphisagnine indicated the presence of two angular methyl groups at δ 0.82 and δ 0.93, one N-methyl group at δ 2.27, a methoxyl singlet at δ 3.30, an N-CH-O proton as part of an oxazolidine ring at δ 4.06 and a vinyl proton at δ 5.93. The ^{1}H nmr spectrum of staphisagrine is identical with that of staphisagnine except for the absence of a methoxyl singlet at δ 3.30. The presence of the oxazolidine ring in the B unit of these alkaloids was established by ^{1}H nmr analysis. To establish the presence of the A unit in these alkaloids, staphisagnine (33) and staphisagrine (34) were related to the known bis-diterpenoid alkaloids (27) to (32) through a study of their ^{13}C nmr spectra. The presence of a normal type oxazolidine ring in unit B of staphisagnine and staphisagrine was confirmed through a comparison of their ^{13}C chemical shifts with those of the known alkaloids, veatchine (35), garryine (36), atisine (37) and isoatisine (38).[44,45]



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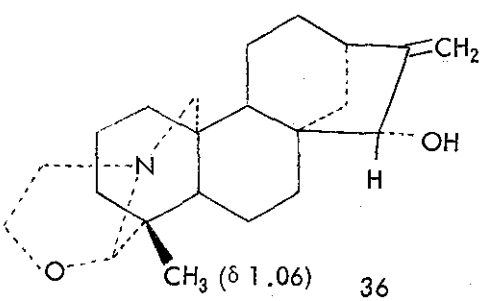
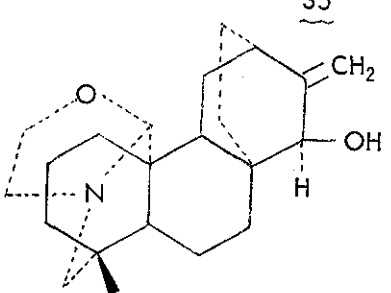
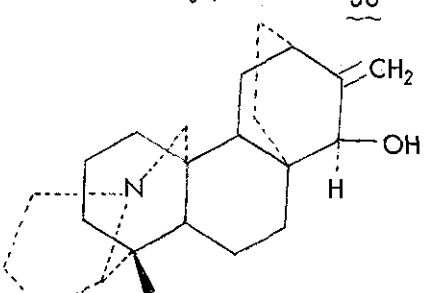


36



37



38

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