*Tetrahedron* Vol. 48, No. 7, pp. 1183-1192, 1992
Printed in Great Britain

0040-4020/92 $3.00+.00


# New Norditerpenoid Alkaloids from *Aconitum Septentrionale*

Samir A. Ross+ and S. William Pelletier*
Institute for Natural Products Research and School of Chemical Sciences
The University of Georgia, Athens, GA 30602, U.S.A.

Arne Jørgen Aasen
Department of Pharmacy, University of Oslo, P. O. Box 1068, Blindern 0316 Oslo 3, Norway

(*Received in USA* 17 *December* 1991)

Abstract: A study of the alkaloids present in the roots of *Aconitum septentrionale* Koelle has led to the isolation of four new norditerpenoid alkaloids: 8-*O*-methyllycaconitine (**1**), 6-*O*-acetylacosepticine (**2**), acoseptrigine (**3**), and acoseptriginine (**4**), besides seven known alkaloids: lappaconine (**5**), *N*-acetylsepaconitine (**6**), puberaconitine (**7**), lappaconitine (**8**), *N*-deacetyllappaconitine (**9**), lycoctonine (**10**), and lapaconidine (**11**). The structures of these alkaloids were determined by spectral data and chemical correlation with alkaloids of established structures. Thus, 8-*O*-methyllycaconitine (**1**) was converted to septentrionine (**12**) by treatment with methanolic ammonia. Saponification of 6-*O*-acetylacosepticine (**2**) and acoseptrigine (**3**) with 5% ethanolic KOH yielded acoseptricine (**13**) and 14-*O*-methylforesticine (**14**), respectively. A synthetic sample of lappaconine (**5**) was prepared from lappaconitine (**8**) by alkaline hydrolysis. Acidic hydrolysis of *N*-acetylsepaconitine (**6**) afforded sepaconitine (**15**). *N*-acetylsepaconitine (**6**) and puberaconitine (**7**) have not been previously isolated from *A. septentrionale*. $^{13}C$ chemical shifts for C(5), C(10), C(13) and *N*-$CH_2$-$CH_3$ of lapaconidine (**11**) have been revised on the basis of DEPT measurements.

## Introduction

*Aconitum septentrionale* Koelle is a plant that is extraordinarily rich in alkaloids.[1-5] In continuation of our work on the alkaloidal constituents of this plant, we report the isolation of four new norditerpenoid alkaloids: 8-*O*-methyllycaconitine (**1**), 6-acetylacosepticine (**2**), acoseptrigine (**3**), and acoseptriginine (**4**)). Also seven known alkaloids have been isolated: lappaconidine (**5**), *N*-acetyllappaconitine (**6**), puberaconitine (**7**), lappaconitine (**8**), *N*-deacetyllappaconitine (**9**), lycoctonine (**10**) and lapaconidine (**11**). The alkaloids *N*-acetylsepaconitine (**6**) and puberaconitine (**7**) have not been reported previously from *Aconitum septentrionale*.

## Results and Discussion

The defatted powdered roots of *A. septentrionale* were extracted with 80% EtOH, and the crude alkaloidal mixture (5.05% W/W) was obtained as indicated in the Experimental section. Extensive chromatographic separations, involving vlc[6], preparative tlc and centrifugally accelerated, radial, thin-layer chromatography (Chromatotron),[7,8] afforded eleven alkaloids of which six have not been reported previously in this plant.

The new alkaloid 8-*O*-methyllycaconitine (**1**) was amorphous, $[\alpha]_D$ +22.6° ($CHCl_3$). The molecular formula $C_{37}H_{50}N_2O_{10}$ was derived on the basis of its eims m/z 682 $[M]^+$ and its $^{13}C$ nmr

+ On leave from the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

spectral data. Its ir spectrum showed absorptions at 3500 (OH), 1720 (CO), 1605 and 1495 (aromatic system) $cm^{-1}$. The proton noise – decoupled spectrum and DEPT studies indicated nine quaternary carbon singlets, thirteen methine carbon doublets, nine methylene carbon triplets and six methyl quartets (see the table). The $^1H$ nmr spectrum indicated the presence of an *N*-$CH_2$-$CH_3$ group (δ 1.04, 3H, *t*), five methoxyl groups (3.21, 3.41, 3.43 each 3H, *s* and 3.34, 6H, s), a 14-β proton (3.53, *t*), a 6-α proton (4.04, s) and resonances typical of a C-18 ester residue such as is found in lycaconitine (**16**)[9] [2.91 (4H, *m*), δ 7.23 and 8.06 (each 1 H, *d*, Ar-$H_3$' and $H_6$'), 7.51 and 7.65 (each 1H, *t*, Ar-$H_4$' and $H_5$')]. The $^{13}C$ nmr spectrum, which showed signals characteristic of the above functional groups, compares well with those of septentrionine (**12**)[2] and delvestidine (**17**)[10] except for the C(18)-ester which is *N*-(methylsuccinyl)anthranoyl in **12** and anthranoyl in **17** (see the table). The $^{13}C$ nmr chemical shifts of **1** also compare well with those of methyllycaconitine (**18**)[9] except for the replacement of the C(8)-OH in **18** by methoxyl in **1** and the methyl group in the C(18)-ester residue in **18** is absent in **1**. This fact is consistent with the mass spectrum of **1** which showed $[M]^+$ m/z 682, the same as that for methyllycaconitine (**18**) and 14 mass units more than that of lycaconitine (**16**). The location of a methoxyl group on C(8) is also deduced by the presence of a downfield singlet at 80.6 ppm and quartet at 54.2 ppm, which are analogous to those found for septentrionine (**12**) and delvestidine (**17**) (see the table). Finally the structure was confirmed as 8-*O*-methyllycaconitine (**1**) by treating the new alkaloid with methanolic ammonia to afford septentrionine (**12**). The mp, mass, proton and $^{13}C$ nmr spectra of synthetic septentrionine (**12**) compare very favorably with those published for natural septentrionine.[2] Septentrionine (**12**) had been previously isolated from the roots of *Aconitum septentrionale*.[2]

The alkaloid 6-*O*-acetylacosepticine (**2**), $C_{25}H_{39}NO_7$, was isolated in a crystalline form (from ether), mp 168.5–170.5°C, $[\alpha]_D$ -1.2° ($CHCl_3$); eims m/z 465 $[M]^+$. Its ir spectrum showed absorption at 3540 (OH) and 1730 (CO) $cm^{-1}$. The $^1H$ nmr spectrum indicated the presence of a methyl group of an *N*-ethyl group at 1.02 ppm (3H, *t*, J = 7 Hz), an acetyl group at 2.02 ppm (3H, *s*), three methoxyl groups at 3.23, 3.30 and 3.38 ppm (each 3H, *s*) and a 14-β proton at 3.69 ppm (1H, *t*, J = 4.5 Hz). Its $^{13}C$ nmr spectrum exhibited twenty-five signals for twenty five carbon atoms present in the molecule and DEPT experiments revealed four quaternary carbons, ten methines, six methylenes and five methyl groups. Alkaline hydrolysis of **2** afforded a product that was identical with acosepticine (**13**),[5] thus establishing the nature of the parent alkanolamine of alkaloid **2**. The acetate group in alkaloid **2** could be attached to C(6), C(7) or C(8). Acetylation of acosepticine (**13**) with acetic anhydride and pyridine gave **2**. This result confirms that the acetate group in **2** must be attached to C(6). Therefore, this alkaloid is assigned the structure of 6-acetylacosepticine (**2**). Acosepticine (**13**) which is the hydrolytic product of alkaloid **2** had been recently isolated from the same plant.[5]

The structure of acoseptrigine (**3**), $C_{27}H_{43}NO_7$, was deduced on the basis of the $^1H$ and $^{13}C$ nmr spectral data. The ir spectrum exhibited bands at 3530 (OH), and 1740 (CO) $cm^{-1}$. The $^1H$ nmr spectrum indicated the presence of an *N*-$CH_2$-$CH_3$ group at 1.05 ppm (3H, *t*, J = 7 Hz), an acetate group at 2.02 ppm (3H, *s*), four methoxyl groups at 3.27, 3.28, 3.31 and 3.40 ppm (3H each, *s*) and also showed one proton triplet at 3.68 ppm (J = 4.5 Hz) attributed to C(14)-β-H and one proton doublet at 5.30 ppm (J = 7 Hz) assigned to C(6)-α-H. The $^{13}C$ nmr spectrum of **3** ex-

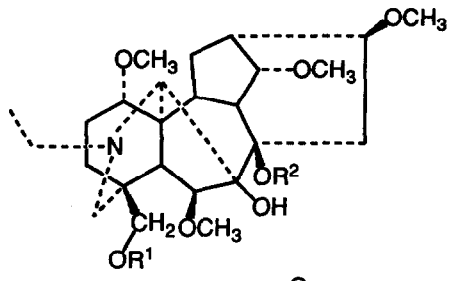
hibited twenty-seven lines for twenty-seven carbon atoms in the molecule. The DEPT spectra showed the presence of four quaternary carbons, ten methines, seven methylenes and six methyl carbons. Alkaline hydrolysis of acoseptrigine (**3**) with 5% ethanolic KOH afforded 14-*O*-methylforesticine (**14**). 14-*O*-methylforesticine (**14**) was identified by tlc behavior and comparison of ir and proton nmr spectral data with those of an authentic sample. 14-*O*-methylforesticine (**14**) had been recently isolated in our laboratory from the roots of *A. septentrionale*.[5] Thus the structure of acoseptrigine could be 6-acetyl-14-O-methylforesticine or 8-acetyl-14-O-methylforesticine. In the aconitine-type alkaloids,[9] the chemical shifts of C(8) bearing a β-OH is from 72.5 to 74.5 ppm, whereas the chemical shifts for C(8) bearing an acetate group is from 85.5 to 86.0 ppm. Therefore, the quaternary carbon at 74.1 ppm in the $^{13}C$ nmr spectrum of acoseptrigine is assigned to C(8)-β-OH and the structure of acoseptrigine consequently is **3**.

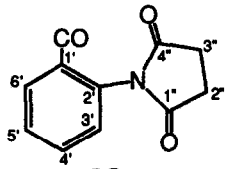
Structure **4** was deduced for acoseptriginine from its spectral data and comparison of its data with neolinine (**19**),[11] nuttalline (**20**)[12] and acoseptrigine (**3**). Acoseptriginine (**4**) has the composition $C_{25}H_{41}NO_6$ (eims m/z 451) with prominent high-mass ions at m/z 436, 421, 420 (base peak) and 418 corresponding to the loss of one methyl, two methyls, one methoxyl and one methyl and water, respectively. The $^1H$ nmr spectrum contained signals for methyl of an ethyl group (δ 1.05, 3H, *t*, J = 7 Hz), and four methyoxyls (δ 3.28, 3.33, 3.41 and 3.43, each 3H, *s*). These data suggested that **4** had a norditerpenoid alkaloid skeleton. The $^{13}C$ nmr spectrum exhibited 25 lines for the 25 carbon atoms of the molecule. The DEPT spectra of **4** exhibited 3 singlets at 38.5, 48.6 and 74.4 ppm. The two upfield signals (38.5 and 48.6 ppm) are assigned to the non-oxygenated quaternary carbons 4 and 11, respectively. The signal at 74.4 ppm is assigned to the only oxygenated quaternary C(8). The four methoxyl signals in the proton nmr spectrum correspond to C(1), C(6), C(14) and C(16) as indicated by four doublet signals at 84.6, 83.0, 84.2 and 82.0 ppm and four quartet signals at 56.1, 57.5, 57.7 and 56.2 ppm for C(1)-$OCH_3$, C(6)-$OCH_3$, C(14)-$OCH_3$ and C(16)-$OCH_3$, respectively. The location of a methoxyl group at C(1) was also indicated by the prominent M-31 ion (base peak) in the eims of **4**. The location of an OH group on C(18) was deduced by the change of chemical shifts of the only oxygenated C(18) methylene from ~ 80.0 to 68.0 ppm (triplet) and the absence of the $OCH_3$ signal at ~ 59.0 ppm in the $^{13}C$ nmr spectrum. The triplet at 68.0 ppm is analogous to that found for nuttalline (**20**)[12] at 68.1 ppm. Acoseptriginine thus has structure **4**. This compound has not previously been described.

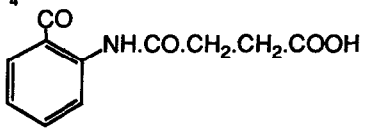
Lappaconine (**5**) was obtained crystalline from $Et_2O$-hexane, mp 93–95°C, $[\alpha]_D$ +26.4° ($CHCl_3$). Its molecular formula $C_{23}H_{37}NO_6$ was deduced from its eims, $[M]^+$ m/z 423, $^1H$ and $^{13}C$ nmr spectra. Its ir spectrum showed absorption at $\gamma_{max}$ 3360-3420 $cm^{-1}$ (OH). The $^1H$ nmr spectrum exhibited signals at δ 1.07 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$) and 3.28, 3.30, 3.40 (each 3H, *s*, 3 X $OCH_3$). The $^{13}C$ nmr spectrum showed twenty-three lines for twenty-three carbon atoms in the molecule (see the table). The DEPT spectra revealed the presence of four quaternary carbons, eight methine carbons, seven methylene carbons and four methyl carbons. The $^{13}C$ chemical shifts assignments are consistent with structure **5**. This compound proved to be identical with a synthetic sample of lappaconine (prepared from lappaconitine by alkaline hydrolysis with 5% ethanolic KOH) by tlc behavior, mp, mmp, ir, proton and $^{13}C$ nmr spectra. Lappaconine was prepared earlier from lappaconitine[1,13,14,19,20] by saponification.

All the six known alkaloids isolated from the roots of *A. septentrionale* [*N*-acetylsepaconitine (**6**),[15,16] puberaconitine (**7**),[9] lappaconitine (**8**),[1,14] *N*-deacetyllappaconitine (**9**),[9] lycoctonine (**10**)[9] and lapaconidine (**11**)[14,17]] were identified by comparing their mps, tlc, mass, $^1H$ and $^{13}C$ nmr spectra with those of authentic samples. *N*-acetylsepaconitine (**6**) and puberaconitine (**7**) have not been previously isolated from this plant.

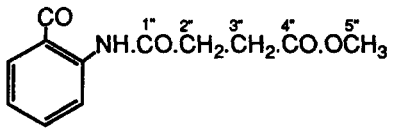
The $^{13}C$ nmr and DEPT spectra for lapaconidine (**11**) showed that the chemical shifts assigned to C(5), C(10), C(13) and *N*-$CH_2$-$CH_3$ in the literature[14] should be revised (see the table).



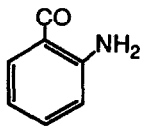
**1** $R^1$ =  , $R^2 = CH_3$

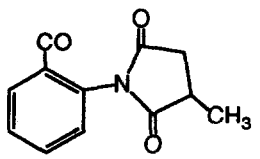
**7** $R^1$ =  , $R^2$ = H Puberaconitine

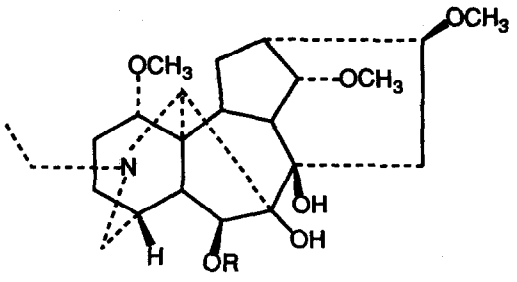
**10** $R^1 = R^2$ = H Lycoctonine

**12** $R^1$ =  , $R^2 = CH_3$ Septentrionine

**16** $R^1$ = as in **1**, $R^2$ = H Lycaconitine

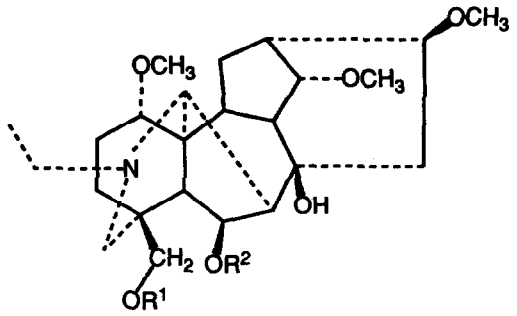
**17** $R^1$ =  , $R^2 = CH_3$ Delvestidine

**18** $R^1$ =  , $R^2$ = H Methyllycaconitine



**2** R = Ac

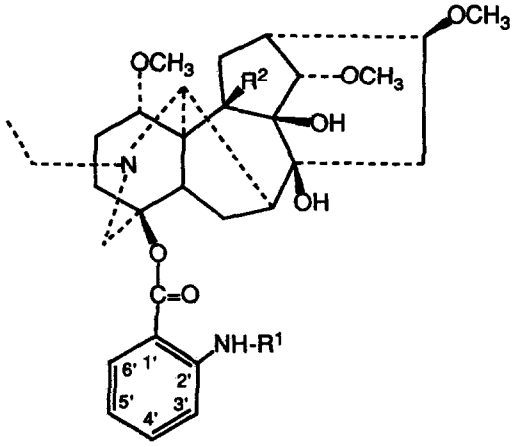
**13** R = H Acosepticine



**3** $R^1 = CH_3$, $R^2$ = Ac

**4** $R^1$ = H, $R^2 = CH_3$

**14** $R^1 = CH_3$, $R^2$ = H 14-*O*-Methylforesticine

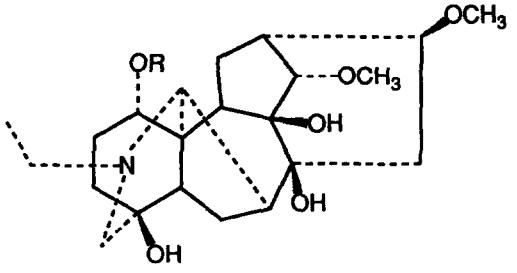


**6** $R^1$ = Ac, $R^2$ = OH *N*-acetylsepaconitine

**8** $R^1$ = Ac, $R^2$ = H Lappaconitine

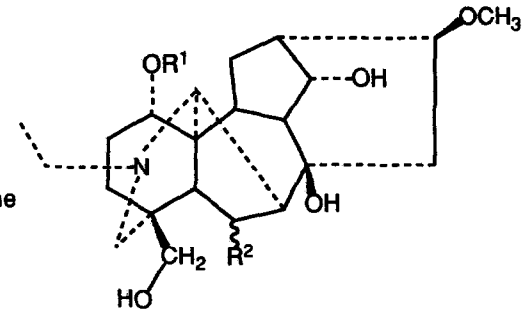
**9** $R^1 = R^2$ = H *N*-deacetyllappaconitine

**15** $R^1$ = H, $R^2$ = OH Sepaconitine



**5** R = $CH_3$

**11** R = H Lapaconidine



**19** $R^1$ = H, $R^2$ = α-$OCH_3$ Neolinine

**20** $R^1 = CH_3$, $R^2$ = β-$OCH_3$ Nuttalline

$^{13}C$ nmr chemical shifts and assignments for 8-*O*-methyllycaconitine (**1**), 6-acetylacosepticine (**2**), acoseptrigine (**3**), acoseptriginine (**4**), lappaconine (**5**), *N*-acetylsepaconitine (**6**), lapaconidine (**11**), septentrionine (**12**)[2] and delvestidine (**17**)[10].

| Carbon | 1[a] | 2 | 3 | 4 | 5 | 6[b] | 11 | 12[c] | 17[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 83.3 d | 84.2 d | 84.6 d | 84.5 d | 85.0 d | 77.7 d | 72.2 d | 83.1 | 83.4 |
| 2 | 25.4 t | 26.0 t | 26.1 t | 26.2 t | 26.4 t | 26.6 t | 29.6 t | 25.6 | 25.6 |
| 3 | 31.5 t | 29.1 t | 32.0 t | 29.6 t | 37.1 t | 31.6 t | 33.2 t | 31.9 | 31.9 |
| 4 | 37.6 s | 37.5 d | 38.6 s | 38.5 s | 70.9 s | 84.7 s | 70.2 s | 37.7 | 37.7 |
| 5 | 40.5 d | 50.6 d | 46.0 d | 46.4 d | 50.7 d | 44.5 d | 46.2 d | 46.7 | 40.5 |
| 6 | 91.2 d | 84.5 d | 72.7 d | 83.0 d | 26.8 t | 24.5 t | 27.2 t | 91.5 | 91.3 |
| 7 | 90.0 s | 89.2 s | 51.3 d | 51.5 d | 47.5 d | 46.7 d | 46.7 d | 90.4 | 90.1 |
| 8 | 80.6 s | 76.9 s | 74.1 s | 74.4 s | 75.6 s | 74.6 s | 76.0 s | 80.9 | 80.7 |
| 9 | 51.9 d | 45.6 d | 48.5 d | 49.5 d | 78.5 s | 78.8 s | 77.3 s | 51.9 | 51.9 |
| 10 | 46.6 d | 43.4 d | 44.2 d | 44.5 d | 49.5 d | 79.6 s | 48.2 d | 40.6 | 46.6 |
| 11 | 47.4 s | 48.6 s | 47.8 s | 48.6 s | 50.8 s | 56.4 s | 50.1 s | 47.6 | 47.5 |
| 12 | 27.8 t | 29.1 t | 29.0 t | 29.2 t | 23.5 t | 37.4 t | 22.9 t | 27.9 | 27.9 |
| 13 | 37.8 d | 35.2 d | 36.7 d | 37.8 d | 36.1 d | 34.4 d | 36.1 d | 37.7 | 37.9 |
| 14 | 82.9 d | 84.3 d | 84.4 d | 84.2 d | 90.1 d | 87.8 d | 90.1 d | 83.5 | 83.0 |
| 15 | 27.9 t | 37.9 t | 42.9 t | 39.4 t | 44.7 t | 44.9 t | 44.7 t | 27.9 | 28.0 |
| 16 | 82.7 d | 82.3 d | 82.4 d | 82.1 d | 82.9 d | 82.7 d | 82.7 d | 82.8 | 82.8 |
| 17 | 66.1 d | 66.3 d | 64.3 d | 63.4 d | 61.7 d | 61.6 d | 62.7 d | 66.2 | 66.2 |
| 18 | 70.3 t | – | 78.7 t | 68.0 t | – | – | – | 70.6 | 69.5 |
| 19 | 53.0 t | 49.7 t | 53.8 t | 53.7 t | 57.8 t | 55.4 t | 60.2 t | 53.2 | 53.3 |
| N-$CH_2$ | 51.7 t | 51.2 t | 49.2 t | 49.3 t | 48.9 t | 48.9 t | 48.0 t | 51.9 | 51.8 |
| $CH_3$ | 14.8 q | 14.2 q | 13.5 q | 13.4 q | 13.4 q | 13.5 q | 12.9 q | 14.8 | 14.8 |
| 1' | 55.5 q | 55.9 q | 56.0 q | 56.1 q | 56.0 q | 56.2 q | – | 55.7 | 55.6 |
| 6' | 59.8 q | – | – | 57.5 q | – | – | – | 60.0 | 59.8 |
| 8' | 54.2 q | – | – | – | – | – | – | 54.4 | 54.3 |
| 14' | 57.6 q | 57.6 q | 57.7 q | 57.7 q | 57.9 q | 58.0 q | 57.8 q | 57.7 | 57.6 |
| 16' | 56.3 q | 56.2 q | 56.1 q | 56.2 q | 56.5 q | 56.3 q | 56.0 q | 56.5 | 56.4 |
| 18' | – | – | 59.4 q | – | – | – | – | – | – |
| C(6)-CO | – | 172.4 s | 171.1 s | – | – | – | – | – | – |
| $CH_3$ | – | 21.6 q | 21.7 q | – | – | – | – | – | – |

a $^{13}C$ shifts of $R^1$: (CO) 164.3 s, C(1') 127.2 s, C(2') 132.6 s, C(3') 129.8 d, C(4') 131.5 d, C(5') 133.5 d, C(6') 129.4 d, C(1") 176.6 s, C(2") 28.8 t, C(3") 28.8 t, C(4") 176.6 s.

b $^{13}C$ shifts of *N*-acetylanthranoyl moiety: (CO) 167.4 s, C(1') 115.7 s, C(2') 141.7 s, C(3') 120.2 d, C(4') 134.4 d, C(5') 122.3 d, C(6') 131.0 d, NH-$COCH_3$ (169.1 s and 25.6 q).

c $^{13}C$ shifts of *N*-(methylsuccinyl)anthranoyl moiety: (CO) 168.4, C(1') 115.1, C(2') 141.8, C(3') 120.6, C(4') 134.9, C(5') 122.7, C(6') 130.8, C(1") 170.6, C(2") 29.0, C(3") 32.7, C(4") 173.3, C(5") 51.9.

d $^{13}C$ shifts of anthranoyl moiety: (CO) 167.9, C(1') 110.7, C(2') 150.6, C(3') 116.7, C(4') 134.1, C(5) 116.3, C(6') 131.0.

## Experimental

### General Procedures

Melting points are corrected. Spectra were recorded on the following instruments: ir: Perkin-Elmer model 1420; $^1H$ and $^{13}C$ nmr (in $CDCl_3$) on a Bruker AC-300 (300 MHz for $^1H$, 75.0 MHz for $^{13}C$); and Bruker AC-250 (250 MHz for $^1H$, 62.5 MHz for $^{13}C$); and ms: Finnegan Quadrupole model 4023. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Chromatographic separations were carried out using vacuum liquid chromatography (VLC)[6] [alumina H basic, type E (EM Art no. 1085) or silica gel 60 H (EM Art no. 7736)] and on a "Chromatotron"[7,8] with a rotor of 1 mm thickness coated with alumina (EM Art. no. 1104-3) or silica gel (EM Art no. 7741); for ptlc, silica gel PF-254 (EM Art no. 7747) or alumina 60 HF-254 (EM Art no. 1094).

### Plant material

The roots of *Aconitum septentrionale* Koelle were collected on the 27th of October and 4th of November, 1990 in Sørkedalen, 10 miles north of Oslo, Norway. The plant was identified by Professor Arne J. Aasen, Department of Pharmacy, University of Oslo, Norway. A voucher specimen carrying the number AJAA/901027/1 has been deposited in the Herbarium of the Department of Pharmacy, University of Oslo.

### Extraction of plant material

The air-dried and powdered roots of *Aconitum septentrionale* (495 g) were defatted with hexane (3 l) and then exhaustively extracted at room temperature with 80% EtOH (6 l). Evaporation of the EtOH extract gave 75.23 g of residue which was partitioned between $CHCl_3$ (1.5 l) and 2% aqueous $H_2SO_4$ (2 l). Evaporation of the $CHCl_3$ gave a neutral fraction (0.79 g, fraction 1). Basification of the acidic layer (NaOH, pH 12) and extraction with $CHCl_3$ (6 l) gave a crude alkaloidal fraction (24.2 g, fraction 2).

### Isolation of lappaconitine (8)

Fraction 2 (24.2 g) was dissolved in $Me_2CO$ (50 ml) and cooled for 2 hrs, when white crystals separated (13.95 g). These crystals were crystallized twice from $CHCl_3$–$Me_2CO$ to give 7.59 g of lappaconitine (**8**)[1], mp 227.5–229.5°C.

The mother liquors after the separation of lappaconitine were combined and chromatographed (VLC) on alumina. Elution was performed with hexane, $Et_2O$ and EtOH in order of increasing polarity. Four groups were collected: group 1 (3.50 g, eluted with 40%, 60% and 70% $Et_2O$–hexane), group 2 (2.02 g, 80% $Et_2O$–hexane), group 3 (3.60 g, $Et_2O$ and 5% EtOH–$Et_2O$), group 4 (2.40 g, EtOH).

### Isolation of 8-*O*-methyllycaconitine (1), 6-acetylacosepticine (2), lappaconitine (8), and *N*-deacetyllappaconitine (9) from group 1

Group 1 (3.50 g) was crystallized twice from $CHCl_3$–$Me_2CO$ to give 549 mg of lappaconitine (**8**), mp 229.5–231.5°C. The mother liquor (2.95 g) was chromatographed (VLC) on alumina. Elution was carried out with hexane, $Et_2O$ and EtOH in order of increasing polarity. In all, twelve fractions (150 ml each) were collected. Fractions eluted with 50% $Et_2O$–hexane were combined (599 mg) and fractionated twice on an alumina rotor to give 63 mg of 8-O-methyllycaconitine (**1**) and 61 mg of 6-acetylacosepticine (**2**). Fractions eluted with 60% $Et_2O$–hexane were combined (807 mg) and fractionated three times on an alumina rotor to afford 462 mg of lappaconitine (**8**)[1] and 16 mg of *N*-deacetyllappaconitine (**9**).[9]

### 8-*O*-Methyllycaconitine (1)

63 mg, amorphous; $[\alpha]_D$ +22.6° (*c*, 0.32, $CHCl_3$); ir (nujol): 3500 $cm^{-1}$ (OH), 1720 $cm^{-1}$ (CO), 1605 and 1495 $cm^{-1}$ (C=C); mass m/z: 682 ($M^+$, $C_{37}H_{50}N_2O_{10}$, 0.2), 667 ($M^+$ -$CH_3$, 1), 651 ($M^+$ -$OCH_3$, 65.9), 636(22.7), 619(8.3), 202(40.8), 174(18.3), 75(34.3), 71(54.7), 55(94.3), 45(60.1), 43(100), 41(88.4); $^1H$ nmr ($CDCl_3$): δ 1.04 (3H, *t*, J = 7 Hz, *N*-$CH_2$-C$H_3$), 2.91 (4H, *m*), 3.21, 3.41, 3.43 (3H each, *s*, 3 X OC$H_3$), 3.34 (6H, *s*, 2 X OC$H_3$), 3.53 (1H, *t*, J = 5 Hz, C(14)-β-H), 4.04 (1H, *s*, C(6)-α-*H*), 7.23 and 8.06 (1H each, *d*, J = 8 Hz, Ar-$H_{3'}$ and $H_{6'}$), 7.51 and 7.65 (1H each, *t*, J = 8 Hz, Ar-$H_{4'}$ and $H_{5'}$): for $^{13}C$ nmr data see the table.

**6-Acetylacosepticine (2)**

61 mg, mp 168.5–170.5°C (from $Et_2O$); $[\alpha]_D$ -1.2° (*c*, 0.2, $CHCl_3$); ir (nujol): 3540 $cm^{-1}$ (OH), 1730 $cm^{-1}$ (CO); mass m/z: 465 ($M^+$, $C_{25}H_{39}O_7N$, 1.5), 450 ($M^+$ -$CH_3$, 0.4), 435(3.6), 434 ($M^+$ -$OCH_3$, 18.9), 406(17), 390(3.3), 121(12.5), 108(6.2), 71(17.8), 58(51.3), 45(17.5), 43(100); $^1H$ nmr ($CDCl_3$): δ 1.02 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$), 2.02 (3H, *s*, OCOC$H_3$), 3.23, 3.30, 3.38 (3H each, *s*, 3 X OC$H_3$), 3.69 (1H, *t*, J = 4.5 Hz, C(14)-β-H), 5.15 (1H, *s*); for $^{13}C$ nmr data see the table.

**Isolation of *N*-acetylsepaconitine (6), lappaconitine (8) and lycoctonine (10) from group 2**

Group 2 (2.02 g) was crystallized from $Et_2O$–hexane to give 480 mg of crystals (a mixture of $C_{20}$ alkaloids). The mother liquor (1.512 g) was chromatographed (VLC) on alumina. Elution was performed with hexane, $Et_2O$ and EtOH in order of increasing polarity. In all, ten fractions (150 ml each) were collected. Fractions eluted with 50% and 60% $Et_2O$-hexane were combined (210 mg) and fractionated twice on an alumina rotor to afford 26 mg of lappaconitine (**8**)[1], mp 227.5–229.5°C. Fractions eluted with 70% and 80% $Et_2O$-hexane were combined (775 mg) and fractionated three times on an alumina rotor to give 59 mg of lycoctonine (**10**)[9], mp 96–98°C (from $Me_2CO$) and 37 mg of *N*-acetylsepaconitine (**6**).[15,16]

**N-acetylsepaconitine (6)**

37 mg, amorphous, $[\alpha]_D$ +20.5° (*c*, 0.53, $CHCl_3$); ir (nujol): 3460, 3400, 3300 $cm^{-1}$ (OH), 1680 and 1675 $cm^{-1}$ (CO), 1620, 1583 and 1520 $cm^{-1}$ (C=C); mass m/z: 600 ($M^+$, $C_{32}H_{44}N_2O_9$, 0.2), 585(0.4), 569(1.9), 423(4.7), 421 ($M^+$ -HOOC-$C_6H_4$-NH-$COCH_3$, 27.2), 406(12.7), 390(8), 361(11.7), 178(44.4), 137(7.5), 120(19.3), 119(13.2), 92(9.8), 71(19.3), 58(19.6), 44.6(21.5), 43(100), 41(17.1); $^1H$ nmr ($CDCl_3$): δ 1.13 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$), 2.24 (3H, *s*, OCOC$H_3$), 3.32, 3.33, 3.43 (3H each, *s*, 3 X OC$H_3$), 3.78 (1H, *d*, J = 4.5 Hz, C(14)-β-H), 7.30 and 7.49 (1H each, *dt*, J = 1.3 and 8 Hz, Ar-$H_{4'}$ and $H_{5'}$), 7.92 and 8.68 (1H each, *dd*, J = 1.3 and 8 Hz, Ar-$H_{3'}$ and $H_{6'}$), 11.05 (1H, br *s*, N*H*); for $^{13}C$ nmr data see the table.

**Isolation of acoseptriginine (4), puberaconitine (7), lycoctonine (10) and lapaconidine (11) from group 4**

Group 4 (2.40 g) was chromatographed (VLC) on silica. Elution was carried out with hexane, $CHCl_3$ and EtOH in order of increasing polarity. In all, nine fractions (150 ml each) were collected. The fraction eluted with 8% EtOH–$CHCl_3$ (709 mg) was purified on a silica rotor, then on an alumina rotor to afford 135 mg of puberaconitine (**7**) and 103 mg of lapaconidine (**11**)[14,17] [for $^{13}C$ nmr data see the table]. Fractions eluted with 15% EtOH–$CHCl_3$ and EtOH were combined (930 mg) and fractionated four times on an alumina rotor to give 125 mg of puberaconitine (**7**)[9], 28 mg of lycoctonine (**10**)[9], mp 96–98°C (from $Me_2CO$) and 3 mg of acoseptriginine (**4**).

**Acoseptriginine (4)**

3 mg (amorphous); ir ($CHCl_3$): 3420 $cm^{-1}$ (OH); mass m/z 451 ($M^+$, $C_{25}H_{41}NO_6$, 3.6), 437(5.7), 436 ($M^+$-$CH_3$, 30.1), 434 ($M^+$ -OH, 8.3), 421 ($M^+$ -2 $CH_3$, 20.1), 420 ($M^+$ -$OCH_3$, 100), 418 ($M^+$ -$CH_3$, -$H_2O$, 32.2), 388(14.5), 91(23.8), 85(39.2), 75(41.9), 71(63.8), 58(78.3), 45(68.6), 43(88.3), 41(87.5); $^1H$ nmr ($CDCl_3$): δ 1.05 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$), 3.28, 3.33, 3.41, 3.43 (each 3H, *s*, 4 X $OCH_3$), 3.60 (1H, *t*, J = 4.5 Hz, C(14)-β-H), 3.84 (1H, *d*, J = 7 Hz, C(6)-β-H); for $^{13}C$ nmr data see the table.

**Isolation of acoseptrigine (3), lappaconine (5) and lappaconitine (8)**

All the impure fractions from all the above separations were combined (2.5 g) and chromatographed (VLC) on alumina. Elution was performed with hexane, $Et_2O$ and EtOH in order of increasing polarity. In all, fifteen fractions (150 ml each) were collected. Fractions eluted with 30%, 40% and 50% $Et_2O$-hexane were combined (136 mg) and subsequent fractionation on an alumina rotor and ptlc (alumina) afforded 25 mg of acoseptrigine (**3**). Fractions eluted with 60% $Et_2O$-hexane were combined (101 mg) and crystallized from $CHCl_3$–$Me_2CO$ to give 31 mg of lappaconitine (**8**)[1], mp 227.5–229.5°C. Fractions eluted with 5% EtOH–$Et_2O$ and EtOH were combined (951 mg). The residue was partitioned between $CHCl_3$ (150 ml) and 2% aqueous $H_2SO_4$

(150 ml). Basification of the acidic layer ($Na_2CO_3$, pH 10, ice cooled) and extraction with $CHCl_3$ (600 ml) gave 489 mg of residue. Subsequent purification of that residue on an alumina rotor and ptlc (alumina) gave 39 mg of lappaconine (**5**).

**Acoseptrigine (3)**

25 mg, amorphous, $[\alpha]_D$ +20.4° (*c*, 0.35, $CHCl_3$); ir(nujol): 3530 $cm^{-1}$ (OH), 1740 $cm^{-1}$ (CO); mass m/z 493($M^+$, $C_{27}H_{43}NO_7$, 1.8), 492($M^+$ -H, 0.8), 478($M^+$ -$CH_3$, 1.4), 463((28.9), 462($M^+$ -$OCH_3$, 100), 450(2.5), 433(1.7), 432(6.0), 418(3.3), 402(5.0), 128(1.7), 85(3.8), 71(6.3), 58(10.9), 45(8.0), 43(10.5); $^1H$ nmr ($CDCl_3$): δ 1.05 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$), 2.02 (3H, *s*, $OCOCH_3$), 3.27, 3.28, 3.31, 3.40 (each 3H, *s*, 4 X $OCH_3$), 3.68 (1H, *t*, J = 4.5 Hz, C(14)-β-H), 5.30 (1H, *d*, J = 7 Hz, C(6)-β-H): for $^{13}C$ nmr data see the table.

**Lappaconine (5)**

39 mg, mp 93–95° (Lit. [1,13] 96°C), $[\alpha]_D$ +26.4° (*c*, 0.7, $CHCl_3$) [Lit. [1,13] 27°, $CHCl_3$]; ir (nujol): 3360–3420 $cm^{-1}$ (OH); mass m/z 423($M^+$, $C_{23}H_{37}NO_6$, 2.4), 408($M^+$ -$CH_3$, 1.8), 394(4), 393(24.1), 392($M^+$ -$OCH_3$, 100), 367(1.8), 71(3.7), 58(6.8); $^1H$ nmr ($CDCl_3$): δ 1.07 (3H, *t*, J = 7 Hz, *N*-$CH_2$-$CH_3$), 3.28, 3.30, 3.40 (each 3H, *s*, 3 X $OCH_3$); for $^{13}C$ nmr data see the table.

**Conversion of 8-*O*-methyllycaconitine (1) to septentrionine (12)**

To 15 mg of **1** in 4 ml of $CH_3OH$ was added 2 drops of ammonia solution (28–30% conc.). The reaction was kept at room temperature for 20 hrs. The $CH_3OH$ was distilled off and the residue (14 mg) was crystallized from $Me_2CO$-hexane to give 7.9 mg of septentrionine (**12**)[2,18], mp 123–125°C. The mp, mass, proton and $^{13}C$ nmr spectral data of the synthetic septentrionine (**12**) compare favorably with those published for a natural sample.[2,18]

**Conversion of 6-*O*-acetylacosepticine (2) to acoseptricine (13)**

To 12 mg of **2** in 1 ml of EtOH was added 1 ml of 5% ethanolic KOH solution. The reaction mixture was kept at room temperature for 20 hrs. The EtOH was distilled off and 10 ml of ice water was added; the mixture was extracted with $CHCl_3$ (5 x 10 ml each) to afford 10.3 mg of acoseptricine (**13**).[5] Acoseptricine (**13**) was identified by tlc behavior, and comparison of ir, mass, proton and $^{13}C$ nmr spectral data with those of an authentic sample.

**Conversion of acoseptrigine (3) to 14-*O*-methylforesticine (14)**

To 10 mg of **3** in 1 ml of EtOH was added 1 ml of 5% ethanolic KOH solution. The reaction mixture was kept at room temperature for 20 hrs. Usual workup afforded 8.5 mg of 14-O-methylforesticine (**14**)[5] which was identified by tlc behavior, and comparison of ir and proton nmr spectral data with those of an authentic sample.

**Acid hydrolysis of *N*-acetylsepaconitine (6)**

To 8 mg of **6** was added 5 ml of 8% HCl solution and the reaction mixture was refluxed at 100°C for 2 hrs. Basification with $NaHCO_3$ (pH 8) and extraction with $CHCl_3$ (5 x 10 ml) afforded 7 mg of sepaconitine (**15**), mp 247–249°C (from $Me_2CO$-hexane) [Lit.[3,16], mp 250–253°C]. Synthetic sepaconitine (**15**) was identified by comparison of tlc behavior and mp, mass, ir and $^1H$ nmr spectra with those of an authentic sample.

**Saponification of lappaconitine (8)**

To 25 mg of **8** in 5 ml of EtOH was added 5 ml of 5% ethanolic KOH solution. The reaction mixture was kept at room temperature for 20 hrs. Usual workup afforded 19.2 mg of lappaconine (**5**), mp 93–95°C (from $Et_2O$-hexane).

**Acknowledgement**

We thank Dr. H. K. Desai for constructive suggestions for this paper.

## References


1. Marion, L.; Fonzes L.; Wilkins, C. K. Jr.; Boca, J. P.; Sandberg, F.; Thorsen, R.; Lindén, E. *Can J. Chem.* **1967**, *45*, 969.
2. Pelletier, S. W.; Sawhney, R. S.; Aasen, A. J. *Heterocycles* **1979**, *12*, 377.
3. Usmanova, S. K.; Tel'nov, V. A.; Yunusov, M. S.; Abdullaev, N. D.; Shreter, A. L.; Filippova, G. B. *Khim. Prir. Soedin.* **1987**, 879.
4. Sirotenko, E. G.; Rashkes, Y. V.; Usmanova, S. K.a *Khim. Prir. Soedin.* **1989**, *25*, 538.
5. Sayed, H. M.; Desai, H. K.; Ross, S. A.; Pelletier, S. W. *Heterocycles*, unpublished work.
6. Pelletier, S. W.; Chokshi, H. P.; Desai, H. K. *J. Nat. Prod.* **1986**, *49*, 892.
7. Desai, H. K.; Joshi, B. S.; Panu, A. M.; Pelletier, S. W. *J. Chromatogr.* **1985**, *322*, 223.
8. Desai, H. K.; Trumbull, E. R.; Pelletier, S. W. *J. Chromatogr.* **1986**, *366*, 439.
9. Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. *Alkaloids: Chemical and Biological Perspectives*, Ed. Pelletier, S. W., John Wiley, New York, **1984**, vol. 2, pp. 302, 416, 418, 421, 435.
10. Desai, H. K.; Joshi, B. S.; Pelletier, S. W. *Heterocycles* **1985**, *23*, 2483.
11. Ross, S. A.; Desai, H. K.; Pelletier, S. W. *Heterocycles* **1987**, 26, 11, 2895.
12. Bai, Y.; Benn, M.; Majak, W. *Heterocycles* **1990**, *31*, 7, 1233.
13. Mollov, N.; Tada, M.; Marion L. *Tetrahedron Letts* **1969**, 2189.
14. Pelletier, S. W.; Mody, N. V.; Sawhney, R. S. *Can. J. Chem.* **1979**, *57*, 1652.
15. Zhamierashvili, M. G.; Tel'nov, V. A.; Yunusov, M. S.; Yunusov, S. Y.; Nigmatullaev, A.; Taizhanov, K. *Khim. Prir. Soedin.* **1980**, 658.
16. Tel'nov, V. A.; Yunusov, M. S.; Abdullaev, N. D.; Zhamierashvili, M. G. *Khim. Prir. Soedin.* **1988**, 556.
17. Tel'nov, V. A.; Yunusov, M. S.; Rashkes, Y. V.; Yunusov, S. Y. *Khim. Prir. Soedin.* **1971**, 622.
18. Pelletier, S. W.; Mody, N. V.; Varughese, K. I.; Maddry, J. A.; Desai, H. K. *J. Am. Chem. Soc.* **1981**, *103*, 6536.
19. Tel'nov, V. A.; Yunusov, M. S.; Yunusov, S. Y. *Khim. Prir Soedin.* **1970**, 583.
20. Plugar, V. N.; Rashkes, Y. V.; Zhamierashvili, M. G.; Tel'nov, V. A.; Yunusov, M. S.; Yunusov, S. Y. *Khim. Prir. Soedin.* **1982**, 80.