Isolation of Norditerpenoid Alkaloids from Flowers of *Aconitum lycoctonum*

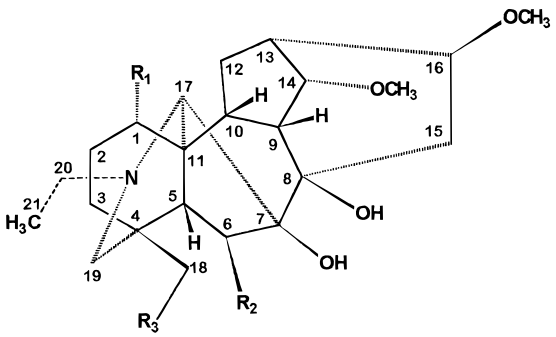
Ying Chen and Alfred Katz*

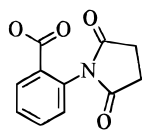
Natural Products Research Laboratory, Dr. Alfred Katz, Oberwilerstrasse 9, CH-4054 Basel, Switzerland

Received December 23, 1998

The structures of two new lycoctonine-type alkaloids having an acetoxy group at C-6, 6-*O*-acetyldemethylenedelcorine (**1**) and 6-*O*-acetyl-14-*O*-methyldelphinifoline (**2**), isolated from the flowers of *Aconitum lycoctonum*, were determined by spectroscopic analysis. In addition, 14-*O*-methyldelphinifoline (**4**), gigactonine (**5**), and lycoctonine (**6**) were obtained.

In the course of our continuing investigation of the European aconites and after having studied the alkaloids of seeds and roots of *Aconitum lycoctonum* L. (Ranunculaceae),[1] we now report the isolation of diterpenoid alkaloids from the flowers of this plant collected in the Engadin Valley (Switzerland) near Sils-Maria. As far back as 1884, Dragendorff and Spohn[2a,2b] isolated from the roots of this species the alkaloids lycoctonine (**6**), lycaconitine (**7**), and its dimer myoctonine. More recently Beul[1] found in the seeds and roots from plants collected in the Engadin Valley gigactonine (**5**), demethylenedelcorine (**3**), 14-*O*-methyldelphinifoline (**4**), and pseudokobusine, besides lycoctonine (**6**) and lycaconitine (**7**). She did not find myoctonine. Although



	R_1	R_2	R_3
1	OCH_3	OAc	OCH_3
2	OH	OAc	OCH_3
3	OCH_3	OH	OCH_3
4	OH	OH	OCH_3
5	OH	OCH_3	OH
6	OCH_3	OCH_3	OH
7	OCH_3	OCH_3	

aconitine, one of the main alkaloids of the European species *Aconitum napellus* L. was used medicinally as an antipyretic and analgesic until recent times, *A. lycoctonum* found medicinal use only in ancient times and became obsolete a long time ago.[3] The toxic effect of its main alkaloids is curariform, acting on the muscular endplates.[4] From the review article of Jacyno,[5] it is evident that the acute toxicity of the *A. lycoctonum* alkaloids is much lower than that of the diterpenoid alkaloids from *A. napellus*. In the present paper two new norditerpenoid alkaloids, 6-*O*-acetyldemethylenedelcorine (**1**) and 6-*O*-acetyl-14-*O*-methyldelphinifoline (**2**), along with the known alkaloids, 14-*O*-methyldelphinifoline (**4**), gigactonine (**5**), and lycoctonine (**6**), were isolated from the flowers of *A. lycoctonum* and identified by spectroscopic analysis. Gigactonine (**5**) was found to be the main base of the flowers. The three known alkaloids were identified by direct comparison (NMR, TLC) with authentic samples.[1]

Table 1. ^{13}C NMR Shifts of Compounds **1**−**4**

carbon	**1** (DEPT)	**3** (DEPT)[1]	**2**	**4** (DEPT)[1]
C-1	84.0 (d)	84.2 (d)	72.3	72.6 (d)
C-2	26.0 (t)	25.6 (t)	29.4	29.2 (t)
C-3	31.9 (t)	32.0 (t)	27.2	27.5 (t)
C-4	38.6 (s)	38.5 (s)	38.3	37.5 (s)
C-5	51.7 (d)	54.3 (d)	47.2	49.9 (d)
C-6	81.1 (d)	80.8 (d)	80.4	80.7 (d)
C-7	88.9 (s)	87.2 (s)	88.4	87.9 (s)
C-8	77.1 (s)	78.3 (s)	78.5	79.3 (s)
C-9	43.3 (d)	44.1 (d)	43.6	44.1 (d)
C-10	45.8 (d)	45.6 (d)	43.4	43.5 (d)
C-11	48.5 (s)	48.2 (s)	49.1	49.0 (s)
C-12	28.8 (t)	28.9 (t)	30.8	30.6 (t)
C-13	37.5 (d)	37.0 (d)	37.0	36.9 (d)
C-14	84.4 (d)	84.2 (d)	84.9	84.8 (d)
C-15	38.0 (t)	36.6 (t)	38.6	36.6 (t)
C-16	82.2 (d)	82.3 (d)	82.7	82.6 (d)
C-17	66.0 (d)	65.9 (d)	67.0	66.8 (d)
C-18	78.6 (t)	79.2 (t)	78.2	78.9 (t)
C-19	52.8 (t)	53.5 (t)	57.6	57.8 (t)
C-20	51.1 (t)	51.7 (t)	50.4	50.5 (t)
C-21	14.1 (q)	14.8 (q)	13.7	13.7 (q)
OCH_3-1	55.6 (q)	55.8 (q)		
OCH_3-14	57.7 (q)	57.8 (q)	57.4	57.7 (q)
OCH_3-16	56.3 (q)	56.3 (q)	56.3	56.2 (q)
OCH_3-18	59.4 (q)	59.6 (q)	59.4	59.6 (q)
$OCOCH_3$-6	172.4 (s)		*a*	
	21.5 (q)		21.6	

[a] Peak not observed.

The base peak $[M + Na]^+$ at *m*/*z* 532.63 recorded by LCQ MS, and the 27 signals exhibited by the ^{13}C NMR spectrum (Table 1) of **1** suggested the molecular formula $C_{27}H_{43}NO_8$ of a norditerpenoid alkaloid. The ^1H NMR spectrum indicated the presence of an *N*-ethyl group (δ 1.04, 3H, t, $J = 7.1$ Hz), an ester methyl group (δ 2.04, 3H, s), and four methoxyl groups (δ 3.25, 3.28, 3.32, 3.40, each 3H, s). DEPT experiments revealed the presence of four quaternary, one carboxyl, nine methine, seven methylene, four methoxyl,

* To whom correspondence should be addressed. Tel.: (++41-61) 2813417. Fax: (++41-61) 2813418. E-mail: katz@ubaclu.unibas.ch.

10.1021/np980576q CCC: $18.00
Published on Web 04/28/1999

and two methyl carbons. The four quaternary carbons at δ 38.6 (C-4), 48.5 (C-11), 77.1 (oxygenated C-8), and 88.9 (oxygenated C-7) suggested that this compound is a lycoctonine-type alkaloid.[6] A one-proton triplet (δ 3.69, $J = 4.7$ Hz) assigned to H-14β revealed that there was an α-OCH_3 group at C-14 and no oxygenated substituent at C-9 or C-13. The ^{13}C NMR chemical shifts of **1** are similar to those reported for demethylenedelcorine (**3**) except for C-5, C-7, C-8, and C-15, and the acetoxy group.[1] The difference in the C-15 chemical shifts of demethylenedelcorine (**3**) and its 6-*O*-acetyl derivative **1** is notable. A similar difference in the C-15 shifts has been reported for demethylenedeltamine and its 6-*O*-acetyl derivative demethylenedeltaline.[7] In the ^1H NMR spectrum, a one-proton singlet observed at δ 4.32 in **3** was shifted downfield to δ 5.31 in **1**. This provided evidence of an acetoxy group at C-6 in **1**, which hence was determined to be 6-*O*-acetyldemethylenedelcorine.

The new compound **2** was isolated as a colorless, oily alkaloid. The molecular formula, $C_{26}H_{41}NO_8$, was derived from MS and NMR spectra. The ^1H NMR spectrum exhibited the presence of an *N*-ethyl (δ 1.10, 3H, t, $J = 7.2$ Hz), three methoxy groups (δ 3.32, 3.35, 3.40, each 3H, s), and an ester methyl group (δ 2.05, 3H, s). The ^{13}C NMR signal at δ 72.3 ppm indicated an α-OH group at C-1.[6] Comparison of the ^{13}C NMR spectra of **2** and 14-*O*-methyldelphinifoline (**4**) revealed that compound **2** had an extra acetoxy group, because the chemical shifts of **2** were similar to those of **4** except for C-5, C-15, and an extra ester methyl carbon.[7] Due to the small amount of sample, the ester carbonyl carbon was not observed in the ^{13}C NMR spectrum of **2**. Similar to compound **1**, alkaloid **2** was also characterized by the appearance of the signals at δ 5.48 (1H, s, H-6α) and 3.74 (1H, t, $J = 4.6$ Hz, H-14β) in the ^1H NMR spectrum, which suggested an acetoxyl at C-6 and a methoxyl group at C-14. Therefore, the structure of compound **2** was found to be 6-*O*-acetyl-14-*O*-methyldelphinifoline.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ on a Bruker 200 spectrometer (200.13 MHz for ^1H NMR and 50.32 MHz for ^{13}C NMR) with TMS as internal standard. Mass spectra were obtained on a Finnigan LCQ-G2 spectrometer, positive ions were used (ESI). Chromatographic separations were carried out by preparative TLC on Si gel 60 F_{254} plates (0.25 mm) and Al_2O_3 60 F_{254} plates (0.25 mm).

Plant Material. The plant material was collected by one of us (A. K.) in the Engadin Valley near Sils-Maria, Switzerland, in August 1998. A voucher specimen (7688Va2) is deposited in the herbarium of the Natural Products Research Laboratory, Dr. Alfred Katz, Basel, Switzerland.

Extraction and Separation. The dried and powdered flowers (20 g) of *A. lycoctonum* were extracted as described earlier[8] to give 174 mg of a basic extract. Purification of this extract was accomplished by repeated preparative TLC on Si gel [cyclohexane−EtOH−MeOH (4:4:2), $CHCl_3$−CH_3OH (8.5:1.5)] and Al_2O_3 [cyclohexane−EtOAc−EtOH (6:3.5:0.5), cyclohexane−$CHCl_3$−EtOH (2.2:7.5:0.3)]. Five alkaloids, 6-*O*-acetyldemethylenedelcorine (**1**, 3.5 mg), 6-*O*-acetyl-14-*O*-methyldelphinifoline (**2**, 1.2 mg), 14-*O*-methyldelphinifoline (**4**, 3.8 mg), gigactonine (**5**, 11.8 mg), and lycoctonine (**6**, 3.5 mg), were isolated.

6-*O*-Acetyldemethylenedelcorine (1): colorless oil; ^1H NMR and ^1H−^1H COSY ($CDCl_3$, 200 MHz) δ 2.97 (1H, dd, $J = 6.8$, 11.8 Hz, H-1β), 2.12 (1H, m, H-2α), 1.94 (1H, m, H-2β), 1.70 (1H, m, H-3α), 1.35 (1H, m, H-3β), 1.66 (1H, br s, H-5), 5.31 (1H, s, H-6α), 2.99 (1H, t, $J = 4.6$ Hz, H-9), 1.98 (1H, m, H-10), 2.41 (1H, dd, $J = 6.0$, 12.6 Hz, H-12α), 1.86 (1H, m, H-12β), 2.37 (1H, dd, $J = 4.7$, 7.2 Hz, H-13β), 3.69 (1H, t, $J = 4.7$ Hz, H-14β), 1.52 (1H, dd, $J = 5.7$, 16.3 Hz, H-15α), 2.87 (1H, dd, $J = 9.3$, 16.3 Hz, H-15β), 3.17 (1H, dd, $J = 6.9$, 9.1 Hz, H-16α), 2.72 (1H, s, H-17), 3.33 (1H, d, $J = 12.6$ Hz, H-18a), 3.16 (1H, d, $J = 12.6$ Hz, H-18b), 2.69 (1H, d, $J = 11.0$ Hz, H-19a), 2.49 (1H, dd, $J = 1.8$, 11.0 Hz, H-19b), 2.94 (1H, dq, $J = 6.7$, 14.2 Hz, H-20a), 2.98 (1H, m, H-20b), 1.04 (3H, t, $J = 7.1$ Hz, H-21), 3.25 (3H, s, OCH_3-1), 3.40 (3H, s, OCH_3-14), 3.32 (3H, s, OCH_3-16), 3.28 (3H, s, OCH_3-18), 2.04 (3H, s, $OCOCH_3$-6); ^{13}C NMR data, see Table 1; LCQ MS (MeOH) *m/z* 533.60 $[M + H + Na]^+$ (73), 532.63 $[M + Na]^+$ (100), 510.64 $[M + H]^+$ (34), 509.66 $[M]^+$ (56), 508.73 (66), 450.69 $[M + H - AcOH]^+$ (6), 449.65 $[M - AcOH]^+$ (10), 448.73 (16).

6-*O*-Acetyl-14-*O*-methyldelphinifoline (2): colorless oil; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.10 (3H, t, $J = 7.2$ Hz, H-21), 2.05 (3H, s, 6-$OCOCH_3$), 1.43−2.10 (9H, m, 2H-2, 2H-3, H-5, H-10, 2H-12, H-15α), 2.46 (1H, t, $J = 5.4$ Hz, H-13β), 2.54 (1H, d, $J = 11.6$ Hz, H-19b), 2.67 (1H, d, $J = 11.6$ Hz, H-19a), 2.85−3.00 (4H, m, H-15β, H-17, 2H-20), 3.07−3.29 (4H, m, H-9, H-16α, 2H-18), 3.31 (3H, s, OCH_3-16), 3.35 (3H, s, OCH_3-18), 3.40 (3H, s, OCH_3-14), 3.70 (1H, m, H-1β), 3.74 (1H, t, $J = 4.6$ Hz, H-14β), 5.48 (1H, s, H-6α); ^{13}C NMR data, see Table 1; LCQ MS (MeOH) *m/z* 519.63 $[M + H + Na]^+$ (70), 518.61 $[M + Na]^+$ (100), 496.83 $[M + H]^+$ (6), 436.78 $[M + H - AcOH]^+$ (13), 435.69 $[M - AcOH]^+$ (5), 434.78 (8).

Acknowledgment. We are grateful to the Pharmaceutical Institute of the University of Basel, Switzerland, for providing the facilities to record NMR spectra; to Dr. Johannes Anklin of the same Institute, for his assistance in recording 2D NMR spectra; and to Dr. S. Gunzenhauser, Organic Institute of the University of Basel, Switzerland, for the measurement of mass spectra.

References and Notes

(1) Beul, M. Diterpenalkaloide der in der Schweiz vorkommenden Subspecies von *Aconitum lycoctonum* L., Ph.D. Thesis, Universität Basel, Basel, Switzerland, 1990.
(2) (a) Dragendorff; Spohn, H. *Pharm. Z. Russl.* **1884**, *23*, 313−323, 329−340, 345−355, 361−366, 377−384. (b) Schulze, H.; Bierling, E. *Arch. Pharm.* **1913**, *251*, 8−49.
(3) Hartwich, C. *Die Neuen Arzneidrogen aus dem Pflanzenreiche*; Julius Springer: Berlin, 1897; p 30.
(4) Benn, M. H.; Jacyno, J. M. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley and Sons: New York, 1983; Vol. 1, Chapter 4, p 205.
(5) Jacyno, J. M. In *Chemistry and Toxicology of Diverse Classes of Alkaloids*; Blum, M. S., Ed.; Alaken Inc.: Fort Collins, CO, 1996; Vol. 5, Chapter 2, p 301.
(6) Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley and Sons: New York, 1984; Vol. 2, Chapter 5, pp 205−462.
(7) Pelletier, S. W.; Desai, H. K.; Kulanthaivel, P.; Joshi, B. S. *Heterocycles* **1987**, *26*, 2835−2840.
(8) Hanuman, J. B.; Katz, A. *J. Nat. Prod.* **1993**, *56*, 801−809.

NP980576Q