LAMARCKININE, A NEW BISNORDITERPENOID ALKALOID FROM *ACONITUM LAMARCKII* REICHENB.

Gabriel de la Fuente*, Tomás Orribo, José A. Gavín, and Rafael D. Acosta

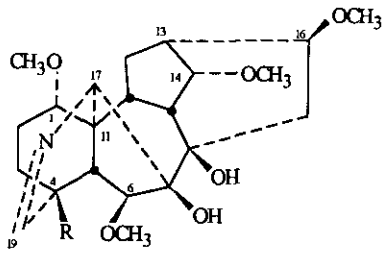
Instituto de Productos Naturales y Agrobiología de Canarias, CPNO "Antonio González", CSIC-Universidad de La Laguna, La Laguna, Tenerife, Spain

Abstract - A new bisnorditerpenoid alkaloid lamarckinine (**1**) containing a C(19) = N- azomethine group was isolated from Aconitum lamarckii Reichenb., besides neoline, 1,14-diacetylneoline, 8-O-ethylbenzoylaconine, columbianine, delcosine, lycoctonine, and 18-methoxygadesine. The structure of the new alkaloid was elucidated by 2D nmr spectroscopy and its conversion into deoxymethylenelycoctonine.

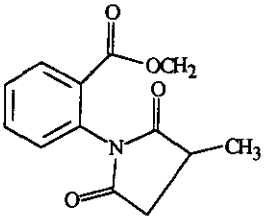
The species Aconitum lamarckii Reichenb., syn. A. vulparia Reichenb. subsp. neapolitanum (Ten.) Muñoz *Garmendia, is distributed in the mountainous areas of Southern Europe and Northern Africa.* The dried ethanolic extract of the aerial parts of the plants (5.4 kg), collected during the flowering period in loco dicto Barranco Gallinas, Saravillo, Huesca Province, Spain, was partitioned between 5% sulphuric acid and chloroform. The acid solution was then made alkaline with ammonium hydroxide (pH 10), extracted with chloroform, and the solvent was removed. The crude alkaloid mixture (52 g, 0.96%) was chromatographed on basic alumina using hexane-ethyl acetate step gradient followed by ethyl acetate-methanol step gradient. Further column chromatography and ptlc yielded the known alkaloids neoline[1] (1.25 g, 23.10^{-3}%), 1,14-diacetylneoline[2] (115 mg, $2.1.10^{-3}$ %), 8-O-ethylbenzoylaconine[3] (1.53 g, 28.10^{-3}%), columbianine[4] (213 mg, $3.9.10^{-3}$%), delcosine[1] (106 mg, $1.9.10^{-3}$%), lycoctonine[1] (154 mg, $2.8.10^{-3}$%), and 18-methoxygadesine[5] (80 mg, $1.5.10^{-3}$%), which were identified by their mp, ms, and ^{1}H and ^{13}C-nmr spectra, and a new alkaloid lamarckinine (**1**) (65. mg, $1.2.10^{-3}$%).

The new alkaloid was isolated as a resin, $[\alpha]_D$+76.6° (c 1.1, $CHCl_3$), and its molecular formula $C_{22}H_{33}NO_6$ was settled by hrms (M^+ 407.2291, calcd 407.2308) and ^{13}C-nmr. Ir (KBr), 3435 (br, OH), 2940, 2824, 1640 (C=N), 1462, 1385, 1304, 1259, 1118, 1090, 1038, 763, and 719 cm^{-1}. The eims revealed ions at m/z, 407 (9%) M^+, 392 (24%) M^+-CH_3, 376 (74%) M^+-OCH_3, 361 (12%) [M^+-CH_3]-OCH_3, 360 (22%) [M^+-CH_3]-CH_3OH, 359 (27%) [M^+-OH]-OCH_3, 358 (100%) [M^+-OCH_3]-H_2O, and 326 (18%). The ratio of the intensities of the M^+, M^+-CH_3 and M^+-OCH_3 ions strongly indicated that lamarckinine was a lycoctonine-type alkaloid with methoxy groups at C-1 and C-6.[6]

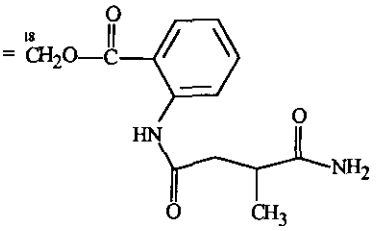
The nmr spectra (Tables 1 and 2) also exhibited characteristic features of a lycoctonine-type alkaloid,[1] and the assignments were made with the aid of proton decoupling, DEPT, HMQC,[7] and HMBC[8] techniques. The ^{1}H-nmr spectrum did not show signals for angular methyl, N-ethyl, or N-methyl groups, but for four methoxy groups. The proton signals at δ 3.52*s* and 3.65*t* (*J*=4.4 Hz), and the quaternary carbon resonances at 86.7 and 72.2 ppm readily disclosed the presence in the molecule of two methoxy groups at C-6β and C-14α, and two tertiary hydroxy groups at C-7 and C-8, respectively.[1] The location of the C(19)=N- azomethine group was inferred from the following facts. Double resonance experiments involving the broadened singlets at δ 3.82 (H-17) and 7.66 (H-19) proved their long range coupling since they became a doublet (*J*= 2.5 Hz) and a broad



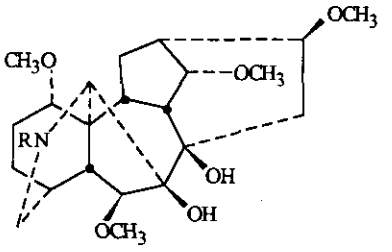
1 R=H



2 R=



3 R=



4 R=H

5 R=C_2H_5

triplet (J = 2.5 Hz), respectively; moreover, these protons showed one-bond correlation with the methine carbon resonances at 64.5 and 165.2 ppm, respectively, in the HMQC spectrum of lamarckinine. On the other hand, in a HMBC experiment, the proton at δ 3.82 (H-17) presented three-bond connectivities with the carbon resonances at δ 43.7 (C-5), 96.0 (C-6), 77.2 (C-8), and 165.2 (C-19), as did the broad singlet at δ 7.66 (H-19) with the methine carbon at δ 43.7 (C-5). Resonances at 82.0 and 82.5 ppm were assigned to C-1αOCH$_3$ and C-16β OCH$_3$, on account of the similarity of the ^{13}C-nmr spectrum of lamarckinine with those of anhweidelphinine (**2**),[9] bulleyanitine A (**3**),[10,11] and other structurally related alkaloids,[1] and the three-bond connectivity observed between the proton at δ 3.65 (H-14β) and the methine carbon resonance at 82.5 ppm.

The expounded spectroscopic data enable us to arrive at the structure depicted for lamarckinine, which was confirmed by chemical correlation of lamarckinine with deoxymethylenelycoctonine (**5**). Catalytic hydrogenation of **1** in ethyl acetate on 10% Pd/C at room temperature gave the dihydroderivative (**4**), M$^+$ 409 (4%); ^{1}H-nmr, δ 3.27, 3.30, 3.34, and 3.39 (3H each, *s*, four OCH$_3$), 3.58 (1H, *t*, J = 4.5 Hz, H-14β), and 3.88 (1H, *s*, H-6α); ^{13}C-nmr in Table 1. Treatment of **4** with ethyl bromide and potassium carbonate in dry acetone[12] at 40° for 33 h yielded an N-ethyl derivative (**5**) as a resin; ms, m/z 437 (5%) M$^+$, 422 (16%) M$^+$-CH$_3$, 406 (100%) M$^+$-OCH$_3$, 404 (16%) [M$^+$-CH$_3$]-H$_2$O, and 388 (5%) [M$^+$-OCH$_3$]-H$_2$O; ^{1}H-nmr, δ 1.03 (3H, *t*, 7.1 Hz, N-CH$_2$-C<u>H</u>$_3$), 3.24, 3.33, 3.38, and 3.40 (3H each, *s*, four OCH$_3$), 3.59 (1H, *t*, J= 4.5 Hz, H-14β), and 3.85 (1H, *s*, H-6α). Its ^{13}C-nmr spectrum was identical with that of deoxymethylenelycoctonine (Table 1).[13] The assigments for certain of the carbon atoms have been revised on the basis of this work and that of reference 11.

Until now only four norditerpenoidalkaloids, barbeline,[14] anhweidelphinine (**2**),[15] bulleyanitine A (**3**),[10] and pacifidine,[16] and a bisnor one, liconosine A,[17] containing a C(19) = N- azomethine group, have been isolated, but lamarckinine is the first example of a lycoctonine-type bisnorditerpenoid alkaloid with such a function.[18]

Table 1. ^{13}C-nmr assignments for lamarckinine (**1**), anhweidelphinine (**2**), bulleyanitine A(**3**), derivative **4**, and deoxymethylenelycoctonine (**5**).

Carbon	1	2	3	4	a	5	b
1	82.0	82.2	84.2	83.4	84.0		85.0
2	20.8	21.0	20.9	25.4	26.1		26.1
3	21.5	24.6	25.0	27.4	29.6		29.0
4	45.9 (2.59)	46.9	43.4	35.6	36.9		36.8
5	43.7	45.5	43.1	47.4	49.0		49.0
6	96.0 (3.52)	91.6	91.6	94.1	94.5		94.5
7	86.7	86.5	86.7	87.4	88.5		87.5
8	77.2	77.3	77.4	~77	~77		~77
9	43.4 (2.77)	43.0	50.6	43.8	43.8		43.4
10	44.1	43.5	38.2	44.8	46.2		46.2
11	48.5	50.5	46.8	45.5	48.7		48.9
12	29.6	30.3	30.5	28.8	29.0		29.7
13	38.6 (2.39)	38.4	45.9	38.7	38.2		38.3
14	84.3 (3.65)	84.2	82.4	84.1	84.8		84.0
15	33.2 (2.87)	33.1	33.3	33.2	33.4		33.4
16	82.5	81.4	81.4	82.6	82.8		82.8
17	64.5 (3.82)	64.5	64.5	60.7	65.0		65.3
18		66.7	67.2				
19	165.2 (7.6)	162.9	163.9	52.8	51.0		50.1
$^{+}N-CH_2$					50.2		51.3
CH_3					14.0		14.2
1'	56.3 (3.17)	56.3	56.4	56.0	55.8		56.1
6'	59.1 (3.38)	58.7	57.9	58.5	58.3		58.4
14'	57.7 (3.43)	57.8	58.8	57.8	57.6		57.8
16'	56.2 (3.35)	56.3	56.4	56.2	56.1		56.2

Chemical shifts in ppm downfield from TMS. Solvent deuteriochloroform. Multiplicities were determined by DEPT data. In parentheses, one-bond connectivities observed in an HMQC experiment. (a) Published values, (b) this work.

Table 2. ^{1}H-^{13}C long-range connectivities observed in the HMBC spectrum of lamarckinine (**1**).

Proton	δ	Carbon	Proton	δ	Carbon
13	2.38 dd (4.4, 6.8)	9,10,12, 14,15,16	6'	3.38s	6'
4	2.59m ($W_{1/2}$=12.5)	-	14'	3.43s	14'
9	2.77t (6.1)	8,10,12, 13,14,15	6α	3.52s	4,7,8,10,6'
15β	2.87dd (14.9, 8.7)	7,8,9, 13,16	14β	3.65t (4.4)	8,9,13, 16,14'
1'	3.17s	1'	17	3.82brs ($W_{1/2}$=6.7)	5,6,7, 8,19
16'	3.35s	16'	19	7.66brs ($W_{1/2}$=6.5)	4,5

^{1}H-Nmr spectrum was recorded in deuterochloroform at 400 MHz. The coupling constants in parentheses are in Hz.

ACKNOWLEDGEMENTS

We thank the Spanish CICYT for financial support through SEUI Grant PB91-0131.

REFERENCES AND NOTES

1. S. W. Pelletier, N.V. Mody, B. S. Yoshi, and L. C. Schramm, "Alkaloids: Chemical and Biological Perspectives", Vol. 2, ed. by S.W. Pelletier, Wiley-Interscience, N.Y., 1984, p. 205.

2. G. de la Fuente, M. Reina, E. Valencia, and A. Rodríguez-Ojeda, *Heterocycles*, 1988, **27**, 1009.

3. G. de la Fuente, M. Reina, and E. Valencia, *Heterocycles*, 1989, **29**, 1577.

4. V. Boido, O. E. Edwards, K. L. Handa, R. J. Kolt, and K. K. Purushothaman, *Can. J. Chem.*, 1984, **62**, 778.

5. A. G. González, G. de la Fuente, and O. Munguía, *Heterocycles*, 1983, **20**, 409.

6. M. S. Yunusov, Ya. V. Rashkes, B. T. Salimov, E. F. Ametova, and G. V. Fridlyanskii, *Chem. Nat. Compd.* (Engl. Transl.), 1985, 492.

7. A. Bax and S. Subramanian, *J. Magn. Reson.*, 1986, **67**, 565.

8. A. Bax and M. E. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.

9. F. Sun, Y. Bai, and M. Benn, *Heterocycles*, 1991, **32**, 1137.

10. X. Wei, S. Y. Chen, B. Wei, and J. Zhou, *Acta Bot. Yunnanica*, 1989, **11**, 453.

11. B. S. Joshi and S. W. Pelletier, *J. Nat. Prod.*, 1990, **53**, 1028.

12. S. W. Pelletier and M. M. Badawi, *J. Nat. Prod.*, 1987, **50**, 381.

13. A. J. Jones and M. H. Benn, *Can. J. Chem.*, 1973, **51**, 486.

14. B. S. Joshi, E. A. El-Kashoury, H. K. Desai, E. M. Holt, J. D. Olsen, and S. W. Pelletier, *Tetrahedron Lett.*, 1988, **29**, 2397.

15. J. S. Jin, *Zhongcbaoyao*, 1986, **17**, 49.

16. K. Wada, T. Yamamoto, H. Bando, and N. Kawahara, *Phytochemistry*, 1992, **31**, 2135.

17. S. Chen and L. Qiu, *Acta Bot. Yunnanica*, 1989, **11**, 267.

18. According to the already isolated nor- and bisnorditerpenoid alkaloids lamarckinine and liconosine A could derive biogenetically from the corresponding norditerpenoid alkaloid with a C(19)=N- azomethine group and subsequent loss of the methyl group at C-4.

Received, 28th September, 1992