MERCKONINE, A NEW ACONITINE-TYPE NORDITERPENOID ALKALOID WITH A –*N*=C–19H FUNCTIONALITY

Haridutt K. Desai, L. Phillip Silverman, and S. William Pelletier*

Institute for Natural Products Research and Department of Chemistry, The University of Georgia, Athens, Georgia 30602-2556, U. S. A.

Abstract – From a commercial source of aconitine, "Aconitine Potent Merck", isolated from *Aconitum napellus* L., Lot No. 30169, a new minor norditerpenoid alkaloid, "merckonine" has been isolated. Structure (**1**) for the new alkaloid was assigned on the basis of its physical and spectroscopic data and synthesis from aconitine. The known alkaloids 3-deoxyaconitine, aconitine and mesaconitine were also isolated in pure form from this commercial source.

Use of the toxic norditerpenoid alkaloid aconitine in pharmacological testing to induce arrhythmias in test animals has been known for a long time.[1] The commercial aconitine isolated mainly from the root tubers of *Aconitum napellus* L, was marketed by Merck and Company in the early 19th century.[2] There were two types of products available in the market: aconitine amorphous, which was a mixture of amorphous alkaloids (left after crystallization of aconitine).[3] The second product, "Aconitine Potent Merck", a crystalline product, was marketed by Merck and Company. Inc., Rahway, U.S.A.[4] From a bottle of this aconitine bearing lot number 30169 we isolated about 95% of aconitine (**2**), along with 3-deoxyaconitine (**3**), mesaconitine (**4**) and a mixture of some very minor polar (on Al_2O_3) alkaloids.[5] We have examined this mixture several times.[6,7] From time to time we needed pure samples of aconitine to prepare its derivatives for pharmacological testing[8,9] and to study its reactions.[10] The isolation of pure aconitine was carried out by vacuum liquid chromatography[6] and purification by centrifugally accelerated radial thin layer chromatography on an Al_2O_3 rotor of a Chromatotron.[7] During each isolation of pure aconitine we had accumulated fractions that were rich in the polar (on Al_2O_3) alkaloids present in this Merck crystalline aconitine.

We report here the isolation of a new norditerpenoid alkaloid designated as merckonine (1) from this accumulation of mixed polar alkaloids.

The polar alkaloid mixture was subjected to a fractionation on an Al_2O_3 (basic, EM 1104) rotor (1 mm) of a Chromatotron, eluting with a solvent gradient of hexane, $CHCl_3$, and EtOH. Several fractions (20 mL each) were collected, evaporated *in vacuo*, and examined by TLC. Most of the fractions were found to be mixtures. Fraction 4 eluted with $CHCl_3$ gave a mixture of at least three compounds which were separated by preparative TLC. One of these three compounds gave a homogeneous amorphous alkaloid (4 mg) and on the basis of its spectroscopic data (IR, NMR, HRMS) it was characterized and identified as the new alkaloid merckonine (**1**).

1 Merckonine

Bz = C=O

2 R^1 = OH, R^2 = Et
3 R^1 = H, R^2 = Et
4 R^1 = OH, R^2 = Me
5 R^1 = OH, R^2 = H

FABHRMS m/z 616.2758 $[M + 1]^+$ indicated the molecular formula $C_{32}H_{41}NO_{11}$ (MW=615.2680) for the amorphous homogeneous compound, $[\alpha]_D$ -29.86° (c=0.309, $CHCl_3$); IR ν_{max} 3495, 3485 (–OH), 1715, 1685 (–C=O), 1280, 1220, 1100, 1035, 755, 715 cm^{-1}. The ^1H and ^{13}C NMR spectra revealed the following: a one-proton singlet at δ 8.10 and a methine carbon at δ 163.1 indicating the presence of a proton in an unsaturated system besides the presence of five protons at δ 8.03 (2H, d, J=7 Hz), 7.60 (1H, t, J=7.4 Hz) and 7.47 (2H, t, J=7.1 Hz) supported by the carbon signals at δ 166.0 (s), 129.6 (s), 129.6 (d, 2C), 128.6 (d, 2C), 133.5 (d, 1C) indicating the presence of a benzoylester group. The presence of four methoxyl groups was indicated by the proton signals at δ 3.78, 3.31, 3.25, 3.13 (each 3Hs) and the methyl carbons at δ 55.6, 57.4, 59.2 and 61.1. The presence of the signals at δ 61.1 (q) and δ 89.8 (d) assignable to the methoxy at C-16 and C-16, respectively, indicated the presence of an α–OH group at C-15 as in aconitine.[11] A one-proton doublet at δ 4.88 (J=5 Hz) assigned to H-14β, indicated the presence of an β-OH group on C-13 and not at C-9. When β-OH is present on C-9 as in sepaconitine, H-14β appears as a doublet at δ 3.73.[12] The H-14β doublet at δ 4.88 also indicates that the benzoyl group is present on C-14 and not on C-8 since the three-proton singlet at δ 1.34 along with the carbon signals at δ 172.3 (s) and 21.4 (q) indicate the presence of an acetate group at C-8. The presence of an α–OH group at C-3 is indicated by a carbon signal at 42.1 (s) assignable to C-4.

Table. ^{13}C NMR Chemcial shift* assignments for **1** and its comparison with **2**[11] and **5**[13]

Carbon	2	1	5	Carbon	2	1	5
1	82.4	82.6d	81.6	17	60.9	57.8d	55.7
2	33.9	33.5t	34.9	18	76.2	74.9t	77.0
3	71.9	69.9d	71.0	19	47.0	163.1d	40.7
4	43.2	42.1s	43.8	20	48.9	—	—
5	46.9	50.6d	51.4	21	13.4	—	—
6	83.4	79.5d	83.4	1'	55.9	55.6q	55.7
7	44.8	42.8d	43.6	6'	58.0	57.4q	57.6
8	92.1	90.2s	91.6	16'	61.1	61.1q	61.0
9	44.2	48.2d	47.3	18'	59.1	59.2q	59.1
10	40.8	40.4d	41.0	8-C=O	172.3	172.3s	171.9
11	50.0	49.4s	49.4	CH_3	21.4	21.4q	21.3
12	35.9	34.2t	34.9	BzC=O	165.9	166.0s	165.9
13	74.0	74.5s	74.1	1"	129.8	129.6s	129.0
14	78.9	78.8d	78.9	2", 6"	129.6	129.6d	129.6
15	78.8	78.4d	78.9	3", 5"	128.6	128.6d	128.6
16	90.1	89.8d	89.9	4"	133.2	133.5d	133.1

*Chemical shifts in ppm downfield from TMS.

The ^1H NMR spectrum showed the absence of an *N*- Et group in the molecule which was supported by the absence of the carbon signal at δ ~13-14. The ^{13}C NMR spectrum exhibited 32 lines for the 32 carbons of the molecule. The multiplicity of these carbons as revealed by the DEPT experiments, showed the presence of seven quaternary, seventeen methine, three methylene and five methyl carbons. The pattern of the chemcial shifts was similar to those reported for *N*- deethylaconitine (**5**).[13] (see comparison in the Table). Compound (**5**), $C_{32}H_{43}NO_{11}$, has seven quaternary, sixteen methine, four methylene and five methyl carbons. Thus the difference in carbon multiplicities between compounds (**1**) and (**5**) is in the methine and methylene carbons. Compound (**1**) has one methylene carbon less and one methine carbon more than those in **5**. The presence of a methine carbon at δ 163.1 indicates that there is a *–N=* C-19H group present in the molecule which is also supported by the presence of one proton singlet at δ 8.10 in the ^1H NMR spectrum. In the case of the alkaloid barbelline, which has a *–N=* C-19H group, C-19H appears as one-proton singlet at δ 7.44 and C-19 appears at δ 169.3 as a methine.[14] That the double bond is not between *N* and C-17 can be ruled out as there would have been one more quaternary carbon present in the

molecule. All of the above data support structure (**1**) for merckonine. This is the first report of an aconitine-type alkaloid having a *–N=* C-19H functionality among some more than ~300 norditerpenoid alkaloids reported in the literature.

Structure (**1**) for merckonine was confirmed by its synthesis from aconitine (**2**). $KMnO_4$ oxidation of **2**, under neutral conditions[15], furnished a product which had identical TLC, ^1H and ^{13}C NMR spectra as compared with those of merckonine (**1**).

ACKNOWLEDGMENTS

Financial support by Grant HL 32562 from National Institutes of Health is gratefully acknowledged. We thank Dr. Dennis R. Phillips for the mass spectrum.

REFERENCES AND NOTES

1. J. T. Cash and W. R. Dunstan, *Phil. Trans.,* 1898, **190B**, 239.
2. J. T. Cash and W. R. Dunstan, *Proc. R. Soc. London,* 1901, **68**, 384.
3. W. Freudenberg and E. F. Rogers, *J. Amer. Chem. Soc.,* 1937, **59**, 2572.
4. W. Freudenberg and E. F. Rogers, fourth communication on aconite alkaloids, presented at the Rochester meeting of the American Chemical Society, Sept. 1937.
5. S. W. Pelletier, B. S. Joshi, and H. K. Desai, "Techniques for Isolation of Alkaloids" in *Advances in Medicinal Plant Research,* ed. by A. J. Vlietinck and R. A. Dommisse, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1985, 153.
6. S. W. Pelletier, H. P. Chokshi, and H. K. Desai, *J. Nat. Prod.,* 1986, **49**, 892.
7. H. K. Desai, B. S. Joshi, A. M. Pann, and S. W. Pelletier, *J. Chromatogr.,* 1985, **322**, 723.
8. Y. Bai, H. K. Desai, and S. W. Pelletier, *J. Nat. Prod.,* 1994, **57**, 963.
9. Y. Bai, H. K. Desai, and S. W. Pelletier, *J. Nat. Prod.,* 1995, **58**, 929.
10. H. K. Desai, H. P. Chokshi, and S. W. Pelletier, *J. Nat. Prod.,* 1989, **52**, 1296.
11. B. S. Joshi, J. K. Wunderlich, and S. W. Pelletier, *Can. J. Chem.,* 1987, **65**, 99.
12. V. A. Tel'nov, M. S. Yunusov, N. D. Abdullaey, and M. G. Zhamierashvilti, *Khim. Prir. Soedin,* 1988, 556.
13. P. Kulanthaivel and S. W. Pelletier, *Tetrahedron,* 1986, **44**, 4313.
14. B. S. Joshi, E. A. El-Kashouri, H. K. Desai, E. M. Holt, J. D. Olsen, and S. W. Pelletier, *Tetrahedron Lett.,* 1988, **29**, 2397.
15. G. Liming, Z. Shangzhen, H. Xiaojiang, W. Xiaoxiong, and S. Xuwei, *Indian J. Chem.,* 1997, **36B**, 682.

Received, 17th February, 1998