

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

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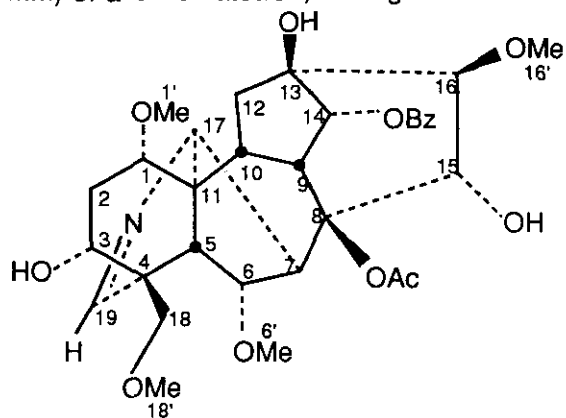
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Abstract – From a commercial source of aconitine, "Aconitine Potent Merck", isolated from *Aconitum napellus* L., Lot No. 30169, a new minor norditerpenoid alkaloid, "merckonine" has been isolated. Structure (1) for the new alkaloid was assigned on 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.

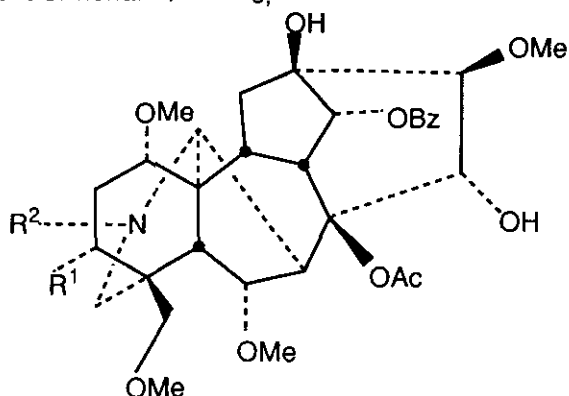
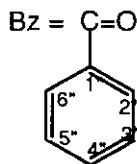
Use of the toxic norditerpenoid alkaloid aconitine in pharmacological testing to induce arrhythmias in test animals has been known for a long time.¹ The commercial aconitine isolated mainly from the root tubers of *Aconitum napellus* L., was marketed by Merck and Company in the early 19th century.² There 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 chromatography⁶ 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 frac-



1 Merckonine



2 $\text{R}^1 = \text{OH}$, $\text{R}^2 = \text{Et}$

3 $\text{R}^1 = \text{H}$, $\text{R}^2 = \text{Et}$

4 $\text{R}^1 = \text{OH}$, $\text{R}^2 = \text{Me}$

5 $\text{R}^1 = \text{OH}$, $\text{R}^2 = \text{H}$

tions (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).

FABHRMS m/z 616.2758 $[\text{M} + 1]^+$ indicated the molecular formula $\text{C}_{32}\text{H}_{41}\text{NO}_{11}$ (MW=615.2680) for the amorphous homogeneous compound, $[\alpha]_{\text{D}} -29.86^\circ$ ($c=0.309$, CHCl_3); IR ν_{max} 3495, 3485 ($-\text{OH}$), 1715, 1685 ($-\text{C}=\text{O}$), 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 benzoyl ester 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-14 β , 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-14 β appears as a doublet at δ 3.73.¹² The H-14 β 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 Chemical 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 $\delta \sim 13$ -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 chemical shifts was similar to those reported for *N*-deethylaconitine (**5**).¹³ (see comparison in the Table). Compound (**5**), $\text{C}_{32}\text{H}_{43}\text{NO}_{11}$, 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 $-\text{N}=\text{C}-19\text{H}$ 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 $-\text{N}=\text{C}-19\text{H}$ 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 conditions¹⁵, furnished a product which had identical TLC, 1H and ^{13}C NMR spectra as compared with those of merckonine (1).

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