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N-DEETHYLACONITINE FROM *ACONITUM NAPELLUS* SSP. *VULGARE*

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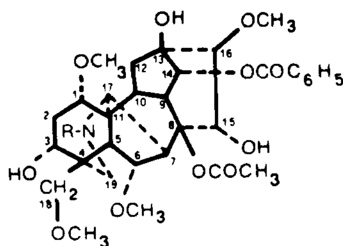
Some *Aconitum* species (Ranunculaceae) are well known to possess medicinal properties and are used clinically in eastern Asia (1-8). In our phytochemical study of the genus *Aconitum* of Northern Italy (9-11), we have started an extensive chemosystematic investigation on members of the taxonomically complex *A. napellus* group.

Aconitum napellus ssp. *vulgare* Rouy & Fouc. is a widely distributed plant in alpine zones, especially in the Western and Central Alps (12). The tlc alkaloid pattern of a population of this taxon shows a Dragendorff-positive spot with intensity comparable to that of aconitine and not attributable to any alkaloid described for the *Aconitum* genus. In the present paper we report the isolation and identification of the unknown alkaloid from this plant.

Extraction and subsequent chromatography of the raw plant material, as described in the Experimental section, yielded a new alkaloid [**1**], besides aconitine [**2**] and mesaconitine [**3**]. The structure of **1** was determined from ir, ms, and ^{1}H-nmr data. The ir spectrum of **1** was very similar to that of aconitine but exhibited a band at 3500 cm^{-1} for N-H stretching. The fd mass spectrum of **1** (Figure 1) showed the molecular ion as the base peak at *m/z* 617 and less abundant fragments at *m/z* 585 (M-CH_3OH), 557 (M-CH_3COOH), and 453 (MH-CH_3COOH-C_6H_5CO). This fragmentation pattern, very similar to that of aconitine [**2**] and mesaconitine [**3**] (Table 1), strongly suggested a similar structure for the new alkaloid.

The ^{1}H-nmr spectrum of **1** showed great similarity with the spectral data reported for aconitine [**2**] (13): the four methoxy substituents and the acetyl group exhibited the same chemical shifts as in aconitine [**2**], while the methylene hydrogens at C-18 were a singlet instead of showing diastereotopic character as in **2** (13), although the chemical shift was invariant (3.56 δ). Compared with aconitine, the absence of the ethyl substituent at the amino nitrogen and the shift to lower field ($\Delta\delta = +0.17$ ppm) of H-3 were noteworthy. These data suggested a structure of **1** identical with aconitine but having a secondary amino group bridging C-17 and C-19 instead of a tertiary amino nitrogen. To further prove the proposed structure, **1** was



1 R=H *N*-deethylaconitine
2 R=Et aconitine
3 R=Me mesaconitine

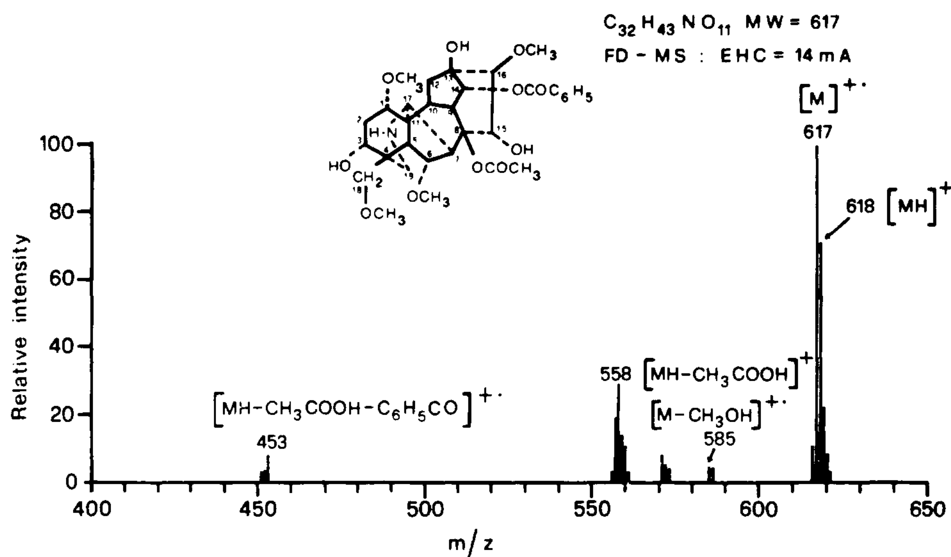


FIGURE 1. FD-Mass spectrum of compound **1**

treated with CH_3I to exhaustively methylate the secondary nitrogen (14): the product so obtained was identical by nmr with the product of the methylation of mesaconitine but different from mesaconitine itself, pointing to the formation of the quaternary dimethyl iodide salt of **1** and the methyl iodide salt of **3** under the reaction conditions. Mass spectra of methylated **1**, methylated **3** and **3** were identical, due to the facile loss of the alkyl iodide in the mass spectrometer source (Table 1) (15). Oxidation of aconitine with $KMnO_4$ gave compound **1** (14), as confirmed by tlc, hplc, and ms.

All the above chemical and spectroscopic data supported the assigned structure of the alkaloid *N*-deethylaconitine. This alkaloid has not previously been found in nature, but it has been prepared by oxidation of aconitine (14).

EXPERIMENTAL

PLANT MATERIAL.—*A. napellus* ssp. *vulgare* samples were collected in their natural habitat in Piani di Bobbio, Como, Italy, at 1750 m above sea level; a voucher specimen has been deposited in the Herbarium of the Dipartimento di Biologia of the Università di Milano, Italy. The roots were harvested during the late summers of 1984 and 1985.

CHEMICALS.—H_2O was distilled in glass and filtered on 0.45 μm Millipore filter. The extraction was performed with analytical reagent-grade $CHCl_3$ (Merck-Darmstadt-FRG). The hplc separation was performed with LiChrosolv-reagent-grade (Merck-Darmstadt-FRG). Ir absorption spectra refer to solutions in $CHCl_3$ and nmr spectra to solutions in $CDCl_3$. Aconitine and mesaconitine were obtained from Sarsynthex (Mérignac-Cedex-France).

TABLE 1. FD-MS of Aconitine [**2**], Mesaconitine [**3**], Methylated [**1**] and Methylated [**3**]

Ion/Compound	[**2**]	[**3**]	Methylated [**1**]	Methylated [**3**]
$[M H]^+$	646(42)	632(42)	632(72)	632(91)
$[M]^{+\cdot}$	645(100)	631(100)	631(100)	631(100)
$[M-CH_3OH]^{+\cdot}$	—	—	599(9)	—
$[M-CH_3COOH]^{+\cdot}$	585(14)	571(9)	571(14)	571(2)
$[MH-CH_3COOH-C_6H_5-CO]^+$	481(1)	—	—	467(2)

[a]*m/z* (rel. int.).

APPARATUS.—Ir Beckman IR 20 A; nmr Varian XL 200; ms Varian 311A mass spectrometer equipped with a combined FI/FD/EI ion source; the total potential difference between the field emitter anode and the cathode was 9 KV, the source temperature was 120°, and the data reported in Table 1 were obtained in the emitter heating current (EHC) range 12-14 mA. Liquid chromatography was performed on a liquid chromatograph consisting of a Waters Assoc. Model 6000 A solvent pump and a U6K universal injector, a Perkin-Elmer LC 75 UV variable wavelength detector and a Perkin-Elmer Sigma 10 computing integrator. The column was a DIOL 250×10 mm I.D. (10 μm particle size) Merck.

EXTRACTION AND FRACTIONATION.—Fresh roots (450 g) thinly minced in a blender (60 mesh) were exhaustively extracted at room temperature with 0.1 N HCl; the ratio w/v was 1:10. The aqueous phase was extracted with $CHCl_3$. The $CHCl_3$ extract was dried (anhydrous Na_2SO_4), concentrated under reduced pressure at 40°, and subjected to Extrelut (Merck) column separation. The column was eluted with *n*-hexane, Et_2O, MeCN, and MeOH. The fractions eluted with MeCN were chromatographed on a Si gel column (70-230 mesh) and eluted with $CHCl_3$-MeOH (9:1, v/v)+0.01% NH_4OH; the order of elutions of substances was aconitine, mesaconitine, and *N*-deethylaconitine. The fractions rich in compound **1** were pooled and the solvent removed to give the crude alkaloid (80 mg). The residue was chromatographed by semipreparative hplc, using $CHCl_3$-MeOH (98:2, v/v) as eluent, a flow rate of 3 ml/min, and detection at $\lambda=235$ nm. The order of elution was aconitine, mesaconitine, and *N*-deethylaconitine. Compound **1** (20 mg) was the main product isolated.

IDENTIFICATION.—Compound **1**: ir ν max 3500 (NH), 3050 (phenyl ring), 2980 (CH), 2820 (CH), 1740 (CO), 1600 (phenyl ring), 710 cm^{-1} (CH); ms: see Table 1; ^1H nmr (200 MHz, $CDCl_3$) 7.4-8.0 (m, 5H, C-14-OCO-*Ph*), 4.86 (d, *J*=5 Hz, 1H, *H-14*), 4.47 (d, *J*=5 Hz, 1H, *H-15*), 4.07 (dd, *J*=1,8 Hz, 1H, *H*-6), 3.94 (t, *J*=6 Hz, 1H, *H-3*), 3.56 (s, 2H, CH_2-*18*); 3.74, 3.31, 3.28, 3.15 (four s, 12H, C-1-OCH_3, C-6-OCH_3, C-16-OCH_3, C-18-OCH_3), 1.34 (s, 3H, C-8-OCOCH_3). Methylated **1** and **3** ms: see Table 1.

OXIDATION OF ACONITINE [**2**].—Oxidation of aconitine by the usual procedure (14) gave a compound identical with **1** (tlc, hplc, ms).

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