Tetrahedron Letters,Vol.29,No.35,pp 4381-4384,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

APHANORPHINE, A NOVEL TRICYCLIC ALKALOID FROM THE BLUE-GREEN ALGA *APHANIZOMENON FLOS-AQUAE*

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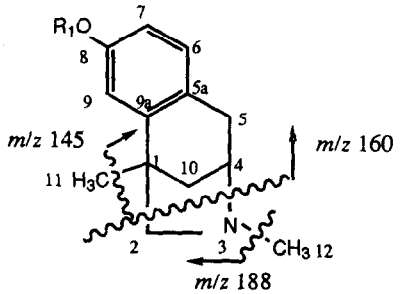
Abstract: An alkaloid with a benzazepine skeleton, aphanorphine (**1**) was isolated from the blue-green alga, *Aphanizomenon flos-aquae* together with its previously known constituents neosaxitoxin and saxitoxin.

A strain of the freshwater blue-green alga (cyanobacterium), *Aphanizomenon flos-aquae*, was reported to contain neurotoxins by Jackim and Gentile.[2] Further studies on these toxins by Alam[3] *et al.* confirmed the presence of saxitoxin and a mixture of three other toxins as toxic constituents. Neosaxitoxin, which was isolated from the dinoflagellate *Gonyaulax tamarensis* and characterized by Shimizu and coworkers,[4] was also reported from *A. flos-aquae* strains NH-1 and -5[5,6,7]. Another basic compound (named aphanorphine), which shows a blue-fluoresent spot on TLC upon 1% H_2O_2 spraying followed by heating, was isolated during the biosynthetic studies of neosaxitoxin in this alga.[6,8] In this paper we wish to report the isolation and structure elucidation of aphanorphine.

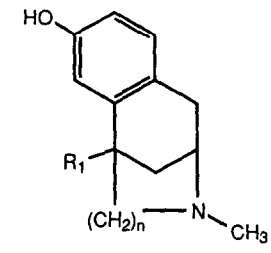
Cultured cells of *A. flos-aquae*[6,9] were extracted with aqueous AcOH, concentrated, and the extract was washed with CH_2Cl_2 to remove lipophillic substances. The aqueous layer was concentrated, and dialyzed against 0.08N AcOH. The aqueous solution was concentrated and chromatographed on BioGel P-2, and the toxin fraction was repeatedly chromatographed on Bio Rex-70 weak ion-exchange resin. Aphanorphine, which is non-toxic to mice at 25 mg/kg (IP), was eluted after saxitoxin and neosaxitoxin bands (0.04% yield from dry weight). Further purificatin was carried out on Chelex-100 to remove chelated metal ion impurities. The free base was obtained by basification of aphanorphine hydrochloride solution with Na_2CO_3 and extraction with CH_2Cl_2.

Aphanorphine (**1**), was crystallized from acetone to prisms, mp. 223-229°C, $[\alpha]_D^{25}$ -43.7° (*c* 0.47, HCl salt in H_2O). The EI high resolution mass spectrum of aphanorphine hydrochloride gave a molecular ion at *m/z* 203.1312 which matched the molecular formula of $C_{13}H_{17}NO$ (calcd *m/z* 203.1311). The EI low resolution mass spectrum showed major fragments: *m/z* 188 (M^+-Me), 160



1. R_1 = H *m/z* 203

2. R_2 = Ac



3a. R_1 = H, n=1

3b. R_1 = H, n=2

3c. R_1 = CH_3 , n=3

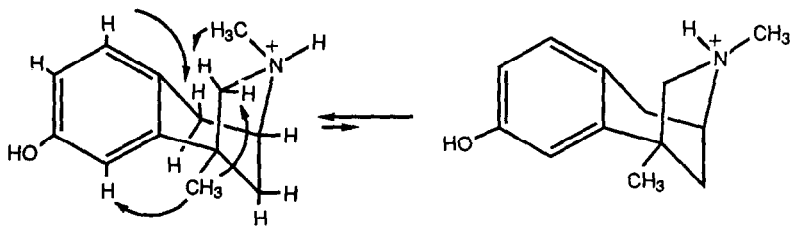


Figure 1. Selected observed NOEs and the two equlibrium forms of **1**

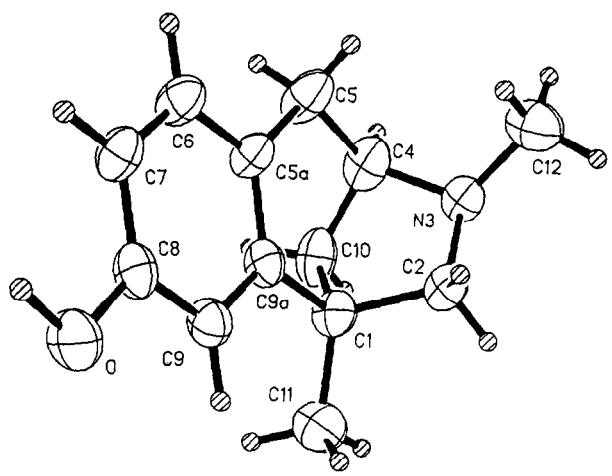


Figure 2. A computer generated perspective drawing of the final X-ray model of **1**

(M^+-C_2H_5N) and 145 (M^+-C_3H_8N). The IR (KBr) absorption bands ν_{max} 3413 and 1613 cm^{-1} were indicative of the presence of an OH group and an aromatic ring system. Acetylation of **1** with Ac_2O/pyridine gave a monoacetate **2**; $C_{15}H_{19}NO_2$, (calcd. *m/z* 245.1417; observd. *m/z* 245.1411); ^1H NMR (in D_2O) δ 2.26 (3H, phenol acetate). The presence of a phenolic moiety in **1** was further confirmed by its UV spectrum; λ_{max}(HCl salt in H_2O) 225 nm(ε4700) and 278(ε1600). The ^1H NMR spectrum of **1** (HCl salt in D_2O) showed the presence of a deshielded tertiary methyl group at δ 1.40 (3H, s); an N-Me group at δ 2.84 (3H,s); three methylene groups at δ1.98 (1H, dd, *J*=1.1,12.7 Hz), and δ 2.27 (1H, dd, *J*=6.18,12.7 Hz); δ 3.05 (2H, br s); δ 3.56 (1H, dd, *J*=1.1,11.4 Hz) and δ 2.89 (1H, d, *J*=11.4 Hz); and a methine proton at δ 4.05; three aromatic protons at δ 6.56 (1H, dd, *J*=2.5,8.3 Hz), δ 6.69 (1H, d, *J*=2.5 Hz) and δ 6.95 (1H, d, *J*=8.3 Hz). The assignment was also supported by ^{13}C NMR and DEPT spectra (HCl salt in D_2O) which showed aromatic carbons at δ 110.4 (C-7), 114.8 (C-9), 121.6 (C-5a), 130.6 (C-6), 143.4 (C-9a), 154.2 (C-8) confirming a trisubstituted aromatic ring system; a quaternery carbon at δ42.9 (C-1); a methyl at δ 18.5 and an N-Me at δ 42.8; two methylenes at δ 34.1 (C-10) and 38.3 (C-5) and a hetero atom bearing methylene at δ 70.6 (C-2), and a hetero atom bearing methine at δ 67.4 (C-4).

The carbon connectivity was established by decoupling studies; irradiation at δ 4.05 gave a doublet at δ 2.27 (*J*=12.7 Hz) and sharpened the broad methylene singlet at δ 3.05; irradiation of δ 3.05 changed the multiplet at δ 4.05 to a doublet (*J*=6 Hz), and sharpened the signal at δ 6.95 indicating that methylene at δ 3.05 was long range coupled with the aromatic proton at δ 6.95; irradiation of the signal at δ 2.27 collapsed the signal at δ 1.98 and δ 4.05 to broad singlets. Furthermore the proton at δ 3.56 showed W coupling (*J*=1.1 Hz) with the proton at δ 1.98. The aromatic ring was 1,2,4-trisubstituted as shown in the coupling pattern. From these data, we arrived at the structure **1** for aphanorphine. The difference NOE spectrum of **1** showed NOEs as given in figure 1., which are also in good agreement with the structure **1**.

The ^1H and ^{13}C NMR spectra of **1** in strongly acidic solutions showed two sets of signals (*ca* 6:1), which collapsed to a set of averaged signals in higher pH (~ pH 4).[10] A distinct difference was seen in the chemical shifts of N-Me groups; the signal of the dominant compound was more deshielded (δ 2.84) than that of the other isomer (δ2.65). The above observation suggests that in low pH, **1** exists in two "fixed" conformations, which is probably due to the difference of N-Me orientation (Figure 1.). To determine its orientation and also to confirm the structure unequivocally, a single crystal X-ray diffraction studies of **1** was carried out.

Crystals of aphanorphine formed in space group C2 with **a**=15.630(5), **b**=8.290(2), **c**=11.450(4), and β=131.06(2)° and one molecule of composition $C_{13}H_{17}NO$ forming the asymmetric unit (Z=4). All unique diffraction maxima with $2\theta \leq 114^\circ$ were collected using θ-2θ scans and graphite monochromated CuKα radiation (1.5418 Å). A total of 820 unique reflections were collected, and 816 were judged observed ($|F_o| \geq 3\sigma(F_o)$). A phasing model was found using the SHELXTL series of programs. Full-

matrix least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.041 for the observed data.[11] A computer generated perspective drawing of the final X-ray model is given in Figure 2. The amino nitrogen, N-3, is pyramidalized to orient the methyl group, C-12, towards the aromatic ring and away from the methylene group C-10. There is an intermolecular hydrogen bond in the crystal from N-3···H O [1/2+x, -1/2+y, z].

These results confirm that 8-hydroxy-1,3-dimethyl-2,3,4,5-tetrahydro-1,4-methano-3-benzazepine is the structure of **1**. The N-Me of the dominant solution structure is also facing towards the aromatic ring (*endo*) as evidenced by the observed ring current effect on its chemical shift. Interestingly the desmethyl derivative of **1** and other similar ring systems have been synthersized as morphine analogs (**3a-c**) and found to be moderately analgesic.[12,13] We are currently studying the absolute stereochemistry of **1** and its biological activity.

Acknowledgements: NIH (Grant CA24487) and New York State Sea grant are gratefully acknowledged for the financial support rendered for this work.

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(Received in USA 25 May 1988)