

STRUCTURE AND C-13 NMR SPECTRUM OF CADABINE

VIQAR UDDIN AHMAD and ANWER BASHA

Postgraduate Institute of Chemistry, University of Karachi, Karachi 32

ATTA-UR-RAHMAN

University Chemical Laboratories, Cambridge, England

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Abstract. Cadabine a compound isolated from the leaves of *Cadaba fruticosa* has been identified as stachydrine and its C-13 NMR studied.

Cadaba fruticosa (L). Druce (syn. *C. farinosa* Hook. f. et Thom. *C. indica* Lamk.) is a straggling shrub found in areas around Karachi. Different parts of this plant are used for the treatment of a variety of ailments in the indigenous system of medicine. The leaves and roots of the plant are attributed to possess purgative, antihelminthic, antispasmodic, deobstruent, amenagogue and aperient properties.¹ A poultice prepared from the leaves is used as an ointment for sores, and a decoction from them is used in uterine disturbances.

In 1971, Ahmad and Basha isolated hygroscopic crystalline needles, m.p. 98–100° from the leaves of the plant.² On the basis of its physical and chemical properties and the analyses of its picrate and auric chloride salts, it was suggested that the substance was a betaine of the formula $C_8H_{13}NO_3$, termed cadabine.

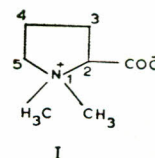
Since the earlier microanalyses had been carried out on the picrate and auric chloride salts of cadabine, it was thought necessary to check the composition by preparing its hydrochloride for further analyses. Cadabine picrate was dissolved in 20% hydrochloric acid and shaken with benzene. Evaporation of the aqueous layer afforded colourless needles of the corresponding hydrochloride, m.p. 222–25°. The hydrochloride salt analysed for $C_7H_{14}NO_2Cl \cdot H_2O$, indicating the correct molecular formula of the betaine to be $C_7H_{13}NO_2$ rather than the earlier assigned $C_8H_{13}NO_3$.

Attempted mass spectrometry of the betaine and its picrate salt failed to afford any parent peaks or readily distinguishable fragmentation patterns because of the low volatility of the zwitterionic materials. An aqueous solution of the betaine did not exhibit any absorptions in the UV region. The IR spectrum exhibited peaks at 1625 cm^{-1} (COO^-) and 3450 cm^{-1} ($-OH$).

Proton NMR spectroscopy of a solution of the betaine picrate in CF_3COOD afforded a fairly complex spectrum. A one-proton multiplet was located at 4.56 δ . Since no olefinic proton appeared to be present in the molecule, this low field signal was assigned to the methine proton adjacent to the quaternary nitrogen and the carboxyl group. A two-proton multiplet at 3.78 δ was assigned to the methylene protons also adjacent to the quaternary nitrogen. This multiplet was seen to collapse to a

broad singlet on spin-spin decoupling on irradiation at 2.5 δ . Two three-proton singlets were located at 3.37 and 3.54 δ , ascribed to the two methyl groups attached to the quaternary nitrogen atom. The remaining four aliphatic protons were present in the region 2.3–3.0 δ as broad multiplets.

The above NMR spectrum of the protonated betaine in CF_3COOD was similar to that earlier reported³ for stachydrine (I) in D_2O .



Since no C-13 NMR spectra of any betaines have previously been reported, it was considered interesting to record and interpret such a spectrum for the betaine in hand for confirmation of structure (I). The C-13 NMR spectrum was, therefore, recorded at 25.2 MHz in D_2O using dioxan as internal standard. Signal enhancement with Fourier Transform device afforded six sharp singlets in the fully decoupled spectrum at 75.3, 68.2, 52.7, 46.8, 24.8 and 19.2 p.p.m. The downfield signal at 75.3 p.p.m. was assigned to C-2 as it was tertiary and had two electron-withdrawing groups (N^+ and $C=O$) attached to it. The signal at 68.2 p.p.m. was assigned to C-5, this secondary carbon also being shifted significantly downfield due to the adjacent quaternary nitrogen atom. The two peaks at 52.7 and 46.8 p.p.m. were due to the two *N*-methyl groups, the downfield one being the one located in the anisotropic area of the carboxyl group. The signals at 24.8 and 19.2 p.p.m. were ascribed to C-3 and C-4 respectively.

In confirmation of the above structure, stachydrine was prepared by methylation of proline with methyl iodide and silver oxide, and converted into its hydrochloride. The two hydrochlorides were found to be identical, showing the same melting points, with no depression in mixed melting points, superimposable IR spectra, and possessing identical R_f values on paper chromatogram.

Experimental

M.p. are uncorrected. Proton and C-13 NMR spectra were recorded with a Varian 100 MHz instrument.

Fresh undried leaves of *Cadaba fruticosa* (1.6 kg containing 80% water) were collected from the shrubs growing locally. The dark greenish residue obtained on the removal of the solvent from the alcoholic percolates of the leaves was partitioned between water and ethylacetate. The ethylacetate portion which contained sterols, resinous matter was set aside for the isolation of nonalkaloidal constituents.

The aqueous extracts gave a crystalline orange yellow precipitate with Dragendorffs reagent but no precipitate with ammonia or alkali.

The aqueous layer was concentrated in a dish to a thick syrup and taken up in 80% alcohol whereby an inorganic crystallisate remained undissolved and was filtered off. The crystallisate gave positive tests for Na^+ , K^+ , Cl^- and NO_3^- . Solvent from the filtrate was removed *in vacuo* and the syrupy residue was passed through a column of Amberlite 120 resin (H^+ form). The unabsorbed acidic and neutral fractions were washed thoroughly with water and the column was subsequently eluted with 1N aqueous ammonia. The eluate was evaporated on the water-bath, yielding a brown syrupy residue which gave a positive Dragendorffs test.

The residue was treated with a solution of picric acid in chloroform and ground for 15 min whereby the yellow material afforded crude crystals. The crude crystalline mass was taken up in acetone,

some undissolved impurities remaining behind. Concentration of the acetone solution resulted in the precipitation of inorganic materials leaving the purer betaine picrate in the mother liquor. This process was repeated several times till the pure betaine picrate remained in the mother liquor, which on further concentration afforded pure stachydrine picrate. The picrate was dissolved in 20% HCl and shaken with benzene. On evaporation, the aqueous layer afforded stachydrine hydrochloride as hygroscopic needles. This was taken up in methanol and on adding ethyl acetate, needle-like crystals were obtained, m.p. 222–25° (yield 2.1 g., 0.6% on dry weight basis) $[\alpha]_D^{25} -33$ (0.007% EtOH). (Found: C, 42.54; H, 7.07; N, 6.69; Cl, 18.43%. $\text{C}_7\text{H}_{14}\text{NO}_2 \cdot \text{H}_2\text{O}$ requires: C, 42.5; H, 8.1; N, 7.0; Cl, 18.0%.) ν_{max} (nujol) 1710 cm^{-1} (—COOH), 3350 cm^{-1} (—OH); NMR (CF_3COOD): 4.56 δ (1H,m, C-2H); 3.78 δ (2H,m, C-5H); 3.37 δ (3H,s, N—CH₃); 3.54 δ (3H,s, N—CH₃); 2.3–3 δ (broad multiplets, 4H of C-3 and C-4); C-13 NMR (D_2O): 75.3 p.p.m. (C-2), 68.2 p.p.m. (C-5), 52.7 and 46.8 p.p.m. (two N—CH₃), 24.8 p.p.m. (C-3), 19.2 p.p.m. (C-4).

References

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