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THE SUBSIDIARY ALKALOIDS OF *RAUWOLFIA VOMITORIA* AFZUELIA

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Apart from the isolation of ajmaline, reserpine and tetraphyllicine, the occurrence of N-methyl ajmaline, ajmalidine, ajmalinine and neoajmaline has been established for the first time in *Rauwolfia vomitoria*.

The isolation, characterization and structural elucidation of two new alkaloids from *Rauwolfia vomitoria* has been communicated earlier [1,2]. The present paper deals with the isolation of four known bases namely N-methyl ajmaline, ajmalidine, ajmalinine and neoajmaline, which had not hitherto been reported in this species of *Rauwolfia*. The isolation of these bases was carried out by employing mild techniques with the avoidance of column chromatographic methods which failed to yield the six bases reported in the present series of papers [1,2]. As recorded in the experimental, it was principally based on the varying basic strength and differences in the solubilities of the free bases and their salts in polar and nonpolar solvents and solvent mixtures. It may be noted, however, that in the isolation of ajmalidine it was found helpful to remove the lingering traces of ajmaline through column chromatography. The identity of these alkaloids was established on the basis of their physical characteristics and UR, IR, mass and NMR spectral data.

The controversial status of neoajmaline as an isomer of ajmaline, has been dealt with earlier by Siddiqui [3,4], Robinson [5] and Ahmad [6]. The IR spectra of the two bases are quite similar but nevertheless vary in the fingerprint region. Further support to this view has been provided by the high resolution mass spectrum of neoajmaline, which showed all the fragments of ajmaline varying in relative intensities. The similarity in mass spectra of neoajmaline and ajmaline, however, rules out the possibility of epimerization at C-2 as the latter gives completely different fragmentation pattern [7]. The difference in stereochemistry at C-17, C-20 and C-21 reportedly does not change the fragmentation pattern and neoajmaline evidently differs from ajmaline in having epimerization at either one or more of these asymmetric centres. The absolute configuration of

neoajmaline could not be established due to lack of sufficient quantity.

EXPERIMENTAL

Darkish viscous methanolic extract of *Rauwolfia vomitoria* (2 kg) was partitioned between ethyl acetate and water. The aqueous layer was treated with a little dil. ammonia to bring up its pH from 4.5 to 6 and a dilute solution of common salt added on to it which threw out a darkish resinous material which was cottoned off. The turbid cottonate was charcoaled and to the bright red clear filtrate was added sodium chloride till faint turbidity and kept overnight in the ice chest when it yielded the major crop of ajmaline hydrochloride as amber-coloured rhombohedral crystals which were filtered. The filtrate was saturated with common salt and the mixed hydrochloride of the bases thereby obtained was subjected to three cornered fractional crystallization from HCl (alc). In this operation the head fractions yielded a further crop of ajmaline hydrochloride. The base recovered from the tail fractions, was divided up into benzene-soluble and insoluble fractions out of which the latter crystallized out from methanol as colourless rectangular plates which melted at 320–21° (yield 600 mg) and analyzed for $C_{20}H_{24}N_2O$. (Found: C 77.89, H 7.80, N 9.11%, and M^+ peak at 308. $C_{20}H_{24}N_2O$ requires: C 77.92, H 7.79, N 9.09%, and mol. wt. 308). It could be identified as tetraphyllicine through superimposable UV, IR and mass spectra as well as through the formation of crystalline methiodide, m.p. 283–5° (lit. [8] 285°) and perchlorate m.p. 305° (lit. [8] 305°).

The benzene-soluble fraction was chromatographed over alumina. The elution was carried out with benzene, benzene-ether (1:1), ether, ether-chloroform, chloroform

and benzene-methanol. The ether-chloroform and chloroform fractions yielded crystalline residues which on recrystallization from methanol, gave a colourless base as beautiful prisms which melted at 240–41° and analyzed for $C_{20}H_{24}N_2O_2$. (Found: C 73.89, H 7.42, N 8.65%, and M^+ peak at 324. Calcd for $C_{20}H_{24}N_2O_2$: C 74.07, H 7.39, N 8.64%, and mol. wt. 324). It gave various colour reactions of ajmaline [3] but contained two hydrogen atoms less. The hydroxyl group determination showed the presence of one OH group while determination of N- CH_3 by Herzig and Meyer method showed the presence of 1 N- CH_3 .

The UV spectrum in ethanol was similar to ajmaline with maxima at 247–48 nm (ϵ 9152) and 294–95 nm (ϵ 3018). The IR spectrum showed common features to ajmaline with an additional carbonyl band at 1740 cm^{-1} . It showed close similarity to the standard IR spectrum of vomalidine [9] (12-methoxy-17 keto ajmaline) which indicated the presence of carbonyl group at position 17 in the ajmaline skeleton which was also confirmed by the position of band at 1740 cm^{-1} , which is indicative of a ketonic group in five-membered ring. The base was, therefore, identified as 17-keto ajmaline (ajmalidine) and the inference was further substantiated through analytical data and comparison of high resolution mass, UV and IR spectra with the published spectral data of ajmaline and ajmalidine [7, 10–15].

The aqueous fraction left over after the recovery of sparingly soluble hydrochloride forming bases, was treated with KI. The methanolic solution of the hydroiodide precipitate gave a brownish pasty mass on addition of water. On recycling this operation of solvation of the pasty mass with methanol and precipitation with the addition of water, two distinct fractions were obtained one of which was insoluble in dilute methanol and the other fairly soluble. The base from the latter was taken up in dilute acetic acid and treated with NaCl to eliminate the residual ajmaline hydrochloride which was decanted off. The turbid decantate was charcoaled and to the bright yellow clear solution was added KI. The colourless semicrystalline hydroiodide thus obtained, crystallized from methanol as colourless rectangular plates, m.p. 230°. The base liberated from the hydroiodide crystallized out from moist methanol in star shaped colourless needles, m.p. 130–31° [a]_D²⁴ + 124° (absolute). It gave various colour reactions of ajmaline [3] and analyzed for $C_{21}H_{28}N_2O_2$ (Found: C 74.19, H 8.30, N 8.26%, and molecular ion peak at 340. Calcd for $C_{21}H_{28}N_2O_2$: C 74.11, H 8.25, N 8.23%, and mol wt 340). The presence of 2 N- CH_3 and an alcoholic group were respectively shown by Herzig and Meyer method and acetylation of the base. The UV spectrum indicated the characteristic features of dihydroindoles (λ_{\max} in methanol at 245

nm, 288–89 nm and λ_{\min} at 267 nm). The IR spectrum in KBr was similar to ajmaline with additional carbonyl stretching at 1710 cm^{-1} which together with C-H stretching at 2770 cm^{-1} , provided evidence for an aldehydic group. The presence of ajmaline skeleton was also evident from the mass spectrum. The peaks at m/e 144, 157, 158, 160, 182, and 200 are common to ajmaline and arise from N-methyl indolic system with C-7 to C-17 bond and hydroxyl group at C-17 [7, 10–13]. The analytical and spectral data coincided with N-methyl ajmaline and it was confirmed by proton NMR spectrum in deuterated chloroform. In contrast to the carbinolamine structure of ajmaline, the base gave peaks for the aldehyde group (δ 9.038, 1H, doublet), aromatic protons (δ 6.34 to 7.668, 4H, multiplet), N₁-methyl (δ 2.766, 3H, singlet), N₄-methyl (δ 2.45, 3H, singlet), C-18 ethyl group (δ 0.938, 3H, triplet; δ 1.4, 2H, multiplet) and alcoholic group (δ 4.15, 1H, singlet). The identity of the base as N-methyl ajmaline was further substantiated by the preparation of hydrochloride, m.p. 270° (lit. [3] 272°), picrate m.p. 187° (lit. [3] 186–87°) and chloroplatinate m.p. 214–16° (lit. [3] 215–20°C). It may be noted here that the isolation of N-methyl ajmaline constitutes only the second case of the occurrence of a natural derivative of ajmaline following the earlier isolation of 17-acetyl ajmaline from *Rauwolfia vomitoria* by Bombardelli and Ezio *et al.* in 1967 [8].

Examination of Ethyl Acetate-soluble Fraction. The ethyl acetate-soluble fraction which formed a brownish viscous mass on removal of the solvent, was digested out with petroleum-ether to remove the fatty matter. The methanolic solution of the insoluble residue gave on cooling a crystallize which on recrystallization melted at 276–77° and could be identified as reserpine. The residue left on removal of the solvent from the mother liquor of reserpine was purified through ethyl acetate, ether and petroleum-ether which eliminated a negligible quantity of a blackish resinous mass. The light yellow solution thereby obtained was extracted out with 5% acetic acid, the acidic fraction basified with dilute ammonia, and the liberated bases shaken out with ethyl acetate. The residue from the ethyl acetate fraction was taken up in HCl (alc) when on cooling a crystalline hydrochloride was obtained. On addition of dil ammonia to the aqueous solution of the hydrochloride, and crystallizing the liberated base from methanol, rectangular plates melting at 205–7° were obtained (yield 200 mg). It analyzed for $C_{20}H_{26}N_2O_2$. (Found: C 73.60, H 7.99, N 8.52%, and M^+ peak 326. $C_{20}H_{26}N_2O_2$ requires: C 73.60, H 8.00, N 8.6%, and mol.wt. 326). The analytical data corresponded to neoajmaline [4] and the identity was further substantiated through superimposable UV, IR and mass spectra as well as mixed m.p. with an

authentic sample of the base.

The base liberated from the residue left on removal of the solvent from the mother liquors of neoajmaline hydrochloride, was taken up in ethyl acetate and purified through ether and petroleum-ether which eliminated some darkish resinous matter. The residue from the purified fraction crystallized out from moist methanol in colourless silky hexagonal plates which melted at 180–81°, $[\alpha]_D^{35} -97^\circ$ (yield 350 mg). It analyzed for $C_{20}H_{26}N_2O_3$. (Found: C 70.20, H 7.91, N 8.10%. $C_{20}H_{26}N_2O_3$ requires: C 70.18, H 7.60, N 8.19%). The UV spectrum in alcohol was characteristic of indoles and showed maxima at 273 and 300 nm respectively. The IR spectrum in KBr showed NH/OH stretching at 3420 cm^{-1} , carbonyl stretching of methyl ester (1700 cm^{-1}), aromatic C=C vibration (1620 cm^{-1}), and C–OH stretching of OH in 5- or 6-membered ring (1070 cm^{-1}). The spectral data and colour reactions [3] of the base identified it as ajmalinine and the identity was further substantiated through the formation of hydrochloride m.p. 242° (lit [3] 243–45°), picrate m.p. 201–3° (lit. [3] 200–205°) and methiodide m.p. 232° (lit. [3] 233–34°C).

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