

## The Alkaloids of *Rauwolfia Serpentina*, Benth. Part I.

BY SALIMUZZAMAN SIDDIQUI AND RAFAT HUSSAIN SIDDIQUI.

In the course of a general chemical examination of the roots of *Rauwolfia serpentina*, we have already communicated (*J. Indian Chem. Soc.*, 1931, 8, 667), the isolation of five new alkaloids and characterised them in some detail. We were engaged since in the study of the three main alkaloids, chiefly *ajmaline*, but owing to lack of sufficient substance we could not so far get to decisive conclusions about their constitution. As however, a recent publication of Van Itallie and Steenhauer (*Arch. Pharm.*, 1932, 270, 313) on the subject has brought up some points of controversy, we feel constrained to publish the results already obtained and discuss them in the light of that publication.

The Dutch authors have been able to isolate only three alkaloids from the root of *R. S.*, which seem to correspond to *ajmaline*, *ajmalinine* and *serpentinine*. Their alkaloids B and C melt three degrees lower than *serpentinine* and *ajmalinine* respectively and the rotation of the alkaloid C ( $[\alpha]_D = -76.4^\circ$ ) is  $-20.6^\circ$  less than that of *ajmalinine* ( $[\alpha]_D = -97^\circ$ ), due probably to its contamination with the strongly positive *ajmaline*. Their failure to get *serpentine* and *ajmalicine* is easily accounted for by their using ammonia and ether as a means of extraction. *Serpentine* is not liberated by ammonia and both *serpentine* and *ajmalicine* are almost insoluble in ether. The authors have given *ajmaline* another name "*Rauwolfine*," on the basis of a formula, higher than that given by us for *ajmaline* by one carbon atom, in the face of almost identical colour reactions, optical activity and melting points: *Ajmaline*, m.p.  $158^\circ-60^\circ$ ,  $[\alpha]_D^{33} = +128^\circ$  (in 1 %  $\text{CHCl}_3$  solution). *Rauwolfine*, m.p. about  $160^\circ$ ,  $[\alpha]_D = +131.1^\circ$  (in 1 %  $\text{CHCl}_3$  solution).

The slightly lower rotation of *ajmaline* is easily explained by the fact that *ajmaline* containing  $3\frac{1}{2}$  molecules of water of crystallisation, i.e., 17.0% of active matter, was used by us for the determination

of optical activity, whereas the base used by the Dutch authors contained 1  $\text{CH}_3\text{OH}$  of crystallisation, i.e., only 8.7% of optically inactive matter.

The reason for the disparity in the formulae of ajmaline and rauwolfine lies in the fact, that we had assigned the formula  $\text{C}_{20}\text{H}_{26}\text{O}_2\text{N}_2$  to ajmaline on the basis of dehydration at  $100^\circ$  in *vacuo* while the Dutch authors combusted their base after heating it to constant weight at  $150^\circ$  to remove the methyl alcohol of crystallisation. As later observations showed, ajmaline does tenaciously retain  $\frac{1}{2}$  a molecule of water more, after losing three molecules of water at  $100^\circ$ , which it gives up only on heating upto about  $150^\circ$ . This view is further supported by the fact that ajmaline shows the presence of 2 active H after heating to constant weight at  $100^\circ$  and only 1 active H after complete dehydration at  $150^\circ$ . In the light of these observations the original formula of ajmaline  $\text{C}_{20}\text{H}_{26}\text{O}_2\text{N}_2$  resolves itself into  $\text{C}_{21}\text{H}_{26}\text{O}_2\text{N}_2$ ,  $\frac{1}{2}\text{H}_2\text{O}$ . [Found: (*loc. cit.* p. 673) C, 73.0; H, 8.12; N, 8.8.  $\text{C}_{21}\text{H}_{26}\text{O}_2\text{N}_2$ ,  $\frac{1}{2}\text{H}_2\text{O}$  requires C, 72.6; H, 7.8; N, 8.1 per cent.  $\text{C}_{20}\text{H}_{26}\text{O}_2\text{N}_2$ , requires C, 73.6; H, 8.0; N, 8.6 per cent.]. The platinum value of the chloroplatinate as given by us in the last communication (*loc. cit.*) though within the limits of error for the  $\text{C}_{20}$  formula agrees even more closely with the  $\text{C}_{21}$  formula. [Found: Pt, 17.9.  $(\text{C}_{21}\text{H}_{26}\text{O}_2\text{N}_2, \text{HCl})_2, \text{PtCl}_4$  requires Pt, 18.0 per cent.  $(\text{C}_{20}\text{H}_{26}\text{O}_2\text{N}_2, \text{HCl})_2, \text{PtCl}_4$  requires Pt, 18.4 per cent.]. The nitrogen value is a little too high for the  $\text{C}_{21}$  formula, but in the nature of the case the C and Pt values have to be given greater weight in deciding between the two formulae, differing by one carbon atom.

But while all the general characteristics of the base rauwolfine point to its identity with ajmaline we have to postpone a definite conclusion to this effect as the Dutch authors have made no mention of the presence of an active H in rauwolfine in the course of its group analysis and have, moreover, showed after the method of Herzig and Meyer the absence of  $\text{N-CH}_3$  in the base, whereas we believe to have established the presence of an  $\text{N-CH}_3$  and an active H in ajmaline, and showed the likelihood of ajmaline containing the group  $\text{:NHCH}_3$  in its molecule.

The secondary character of ajmaline we concluded from its yielding a non-basic crystalline nitroso derivative and a non-basic crystalline monobenzoate. A crystalline acetyl derivative could not be formed but we succeeded in getting a crystalline methyl derivative

of ajmaline out of a crystalline methiodide, which showed the presence of two  $\text{N-CH}_3$  groups whereas ajmaline itself was found to contain only one  $\text{N-CH}_3$ . As one of the two N atoms in ajmaline is non-basic, it appears that the methyl groups and the imino-hydrogen are both linked to one and the same nitrogen and the formula of ajmaline might be further represented as  $\text{C}_{20}\text{H}_{22}\text{O}_2\text{N}(\text{NHCH}_3)$ .

Ajmaline does not contain either a hydroxy or methoxy group and we have not been able so far to account for the two oxygen atoms contained in its molecule. On being heated upto about  $200^\circ$  ajmaline was changed into a brittle varnish-like reddish mass, out of which we could isolate a crystalline base melting at  $256^\circ$ , which we have provisionally called "pyroajmaline".

Serpentine, serpentinine, and ajmalicine also form nitroso derivatives and appear to be secondary bases. Considering the fact, that number of secondary bases in nature is very limited, their prevalence particularly in the roots of a plant is rather striking but as our investigation on the bark of *Holarrhena antidysenterica* (this volume) have brought to light a series of apparently secondary bases, the *Apocynaceae* family to which the two plants belong might be considered as particularly rich in secondary bases.

In the light of our observations in case of ajmaline the empirical formulae of ajmalinine and serpentine were also revised, but owing to the lack of sufficient amount of substance no decisive results could be so far obtained. It may, however, be noted here, that serpentine also appears to retain some more water of crystallisation after heating to constant weight at  $150^\circ$ , while ajmalicine does not. Neither of these bases were found to contain an  $\text{N-CH}_3$  group, but each of them showed the presence of one  $\text{OCH}_3$  group.

A further point of interest has been brought up through the isolation of 3 new bases from an African species of *Rauwolfia*, *Rauwolfia caffra*, by Koepfli (*J. Amer. Chem. Soc.*, 1931, 54, 2412). The chief alkaloid has been named rauwolfine ( $\text{C}_{20}\text{H}_{26}\text{O}_3\text{N}_2$ , d. p. 235-38°) and appears to be a quaternary base. Though the two plants are so allied, the base from *R. caffra* appear to be very different from those of *R. serpentina*.

#### EXPERIMENTAL.

*Determination of active H in ajmaline* (Zerewitinoff's method).—After complete dehydration at  $150^\circ$  [Found: H, 0.28.

$C_{21}H_{26}O_2N_2$  requires (for 1 active H) H, 0.29 per cent.]. After heating to constant weight at  $100^\circ$  in *vacuo* [Found: H, 0.59.  $C_{21}H_{26}O_2N_2$ ,  $\frac{1}{2}H_2O$  requires (for 2 H) H, 0.59 per cent.].

*Determination of N-CH<sub>3</sub> in ajmaline* (Herzig and Meyer's method) ajmaline showed the presence of 1N-CH<sub>3</sub>. [Found: CH<sub>3</sub>, 5.06.  $C_{21}H_{26}O_2N_2$  requires (for 1N-CH<sub>3</sub>) CH<sub>3</sub>, 4.43 per cent.].

*Nitrosoajmaline*.—To a cooled solution of ajmaline (1 mol., 0.38 g.) in 10 p. c. acetic acid, a well cooled solution of sodium nitrite (1.5 mol., 0.12 g.) was slowly added. A cream yellow granular precipitate was produced, which was filtered after keeping the reaction mixture well corked at room temperature overnight and well washed first with warm 5 p. c. acetic acid, then with water, to remove the unchanged base. On recrystallising from dilute alcohol nitrosoajmaline formed pale yellow needles, m. p.  $209^\circ$ . [Found: (after drying to constant weight at  $100^\circ$  in *vacuo*) C, 67.7; H, 7.11.  $C_{21}H_{25}O_3N_3$  requires C, 68.67; H, 6.81 per cent.  $C_{21}H_{25}O_3N_3$ ,  $\frac{1}{2}H_2O$  requires C, 67.02; H, 6.91 per cent.].

*Benzoylajmaline*.—To a solution of ajmaline (0.5 g.) in pyridine (4 c. c.) was added benzoyl chloride (0.75 c. c.) under good cooling. The thick oil which settled down overnight was washed with dilute acetic acid and taken up with alcohol and water. On cooling the solution, long, white, broad needles (0.3 g.) were obtained, which softened at  $134^\circ$  and melted at  $180^\circ$ . On recrystallisation from benzene after washing it with dilute acetic acid the benzoate began to darken from  $140^\circ$  onwards and melted at  $214-16^\circ$ . (Found: C, 74.6; H, 8.05.  $C_{21}H_{25}O_3N_2 \cdot COC_6H_5$  requires C, 76.02; H, 6.79 per cent.). Evidently this product was not pure enough to give a correct analysis but its distinctly non-basic character confirmed the presence of an imino H in ajmaline.

*Methylajmaline*.—To a solution of ajmaline (1 mol., 1 g.) in chloroform (3 c. c.) methyl iodide (1.5 mol., 0.7 g.) was added and the mixture left well corked at the room temperature ( $40^\circ$ ). After 2 days the separated crystalline product was filtered, washed with chloroform and dried (yield 0.95 p. c.). Methylajmaline hydroiodide thus obtained melted at  $230-31^\circ$ . Its N-CH<sub>3</sub> estimation showed the presence of two N-CH<sub>3</sub> groups. [Found: CH<sub>3</sub>, 6.2.  $C_{22}H_{28}O_2N_2$  HI requires (for 2 NCH<sub>3</sub>) CH<sub>3</sub>, 6.3 per cent.]. The chloroform solution on evaporation gave a residue (0.27 g.), which melted between  $230-50^\circ$ , but the amount being too small could not be investigated further.

The crystalline hydriodide was dissolved in acetic acid and the base precipitated from the solution with concentrated NaOH solution, because methylajmaline could not be precipitated by ammonia, and thus, rather contrary to expectations, appears to be stronger than the secondary base, ajmaline. The precipitate was shaken with chloroform, the chloroform solution dried over sodium sulphate and the clear pale yellow residue left on removing the solvent crystallised from moist ethyl acetate in star shaped clusters of needles, m.p.  $130^{\circ}\text{--}131^{\circ}$  (softening at  $127^{\circ}$ ). It is easily soluble in alcohol, less so in ethyl acetate, difficultly soluble in ether, insoluble in petroleum ether. It gives the same colour reactions as ajmaline. (Found: C, 74.90; H, 8.85.  $\text{C}_{22}\text{H}_{28}\text{O}_2\text{N}_2$  requires C, 75.0; H, 7.95 per cent.).

The salts of methylajmaline were prepared in the same manner as in case of ajmaline.

*Methylajmaline hydrochloride* forms a white powder, soluble in alcohol but difficultly so in cold water. It softens at  $125^{\circ}$ , froths up at  $132\text{--}134^{\circ}$  (giving off water of crystallisation) and melts at  $272^{\circ}$ .

*Methylajmaline picrate* forms a bright yellow powder, which is easily soluble in alcohol but difficultly so in cold water. It begins to froth at  $176^{\circ}$  and melts at  $186^{\circ}$ .

*Methylajmaline chloroplatinate* is an amber coloured powder, soluble in alcohol but insoluble in water. It shrinks with darkening at  $203^{\circ}$  and melts at  $215\text{--}220^{\circ}$  (decomp.).

*Effect of heat on ajmaline: pyroajmaline.*—On dehydration at  $100^{\circ}$  in *vacuo* ajmaline melts indefinitely from  $160\text{--}170^{\circ}$ . On heating to constant weight at  $150^{\circ}$  it further loses 2.3 p. c. of water ( $\text{C}_{21}\text{H}_{26}\text{O}_2\text{N}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$  requires  $\text{H}_2\text{O}$ , 2.88 p. c.) and begins to soften at  $170^{\circ}$  sticking slowly to the sides of the m. p. tube by  $185^{\circ}$ , the sticky mass melting down at about  $256^{\circ}$ . On heating ajmaline at  $200^{\circ}$  for about  $\frac{1}{2}$  hour a brittle reddish brown mass is produced which crystallises partly out of moist ethyl acetate in slender needles, m. p.  $256^{\circ}$  (shrinking at  $240^{\circ}$ ). They lose 6.9 p. c. water of crystallisation when heated to constant weight at  $150^{\circ}$  (showing  $1\frac{1}{2}$  mol of water of crystallisation, if the molecule of pyro-ajmaline should be the same, as that of ajmaline). It is difficultly soluble in chloroform.

*Pyroajmaline hydrochloride.*—The hydrochloride, prepared by adding ethereal hydrochloric acid in a chloroform-ether solution of the base is an amorphous powder which darkens at  $226^{\circ}$ , softens at  $230^{\circ}$  and melts at  $238\text{--}240^{\circ}$  (decomp.). It is easily soluble in alcohol and water.

*Determination of  $\text{OCH}_3$  in ajmalinine* (Zeisel's method) showed the presence of one  $\text{OCH}_3$  group. (Found:  $\text{OCH}_3$ , 8.8.  $\text{C}_{20}\text{H}_{23}\text{O}_4\text{N}$  requires  $\text{OCH}_3$ , 8.96 per cent.).

*Determination of  $\text{OCH}_3$  in serpentine* showed the presence of one  $\text{OCH}_3$  group. (Found:  $\text{OCH}_3$ , 9.27.  $\text{C}_{21}\text{H}_{23}\text{O}_4\text{N}$  requires  $\text{OCH}_3$ , 8.78 per cent.).

RESEARCH INSTITUTE,  
A. U. TIBBI COLLEGE,  
DELHI.

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