

PAKISTAN JOURNAL
OF
SCIENTIFIC AND INDUSTRIAL RESEARCH

Vol. 4, No. 1

January 1961

CONSTITUENTS OF RAUWOLFIA VOMITORIA FROM EAST PAKISTAN

SALIMUZZAMAN SIDDIQUI AND M. MANZUR-I-KHUDA

*Drugs and Pharmaceuticals Research Division, Central Laboratories, Pakistan Council
of Scientific and Industrial Research, Karachi*

(Received December 22, 1960)

R. vomitoria is native to French Guinea and is histologically similar to the American species *Rauwolfia heterophylla* Roem. and Schult.

Paris¹ reported the isolation of the ajmaline series of bases,² ajmaline, *iso*-ajmaline, small quantities of ajmalinine and ajmalicine along with traces of a yellow base, from the roots of the plant grown in French West Africa. From the roots of the same plant Schlittler and co-workers³ isolated alstonine.^{4,5,6} Poisson and co-workers⁷ reported the isolation of reserpine⁷ and two new alkaloids namely raumitorine and seredine.⁸ In 1955, Haack and co-workers isolated rauvomitine⁹ and Kidd reported the isolation of rescinnamine¹⁰ from the same plant. *Iso*-reserpiline and serpagine were isolated from it in 1955.¹¹

In view of the interesting results obtained with the alkaloidal complex serpajmaline as a hypotensive agent,^{12,13} the present work was undertaken for isolating the active principles of the plant in the form of their naturally occurring complexes and investigating their chemical constituents. The extracts of the fresh undried drug material were worked up as outlined in the experimental to give three alkaloidal complexes, of which the water soluble complex I corresponded to serpajmaline isolated from *Rauwolfia serpentina*, and had marked hypotensive action on dogs. The action of the drug was prolonged and the material from root bark was found to be more potent than that from the whole root. Like serpajmaline it also had antifibrilliant action on the isolated heart of rabbit. This complex was found to contain alstonine and ajmaline as the major alkaloidal constituents, along with traces of serpentinin and an undefined amphoteric base. With

modified working conditions it also gave a hydroiodide which showed green fluorescence.

Complex II was isolated as a pale yellow powder, insoluble in petroleum ether, benzene, ether and water. It gave ajmaline and reserpine together with a crystalline material which could be identified as serposterol.

Complex III was obtained as a petroleum ether soluble fraction which gave ajmaline, reserpine and serposterol on chromatography over alumina. Four sugars, maltose, sucrose, glucose and mannose were identified from the aqueous ballast on paper chromatography.

Experimental

The material for this work was obtained through the courtesy of Dr. S. Hedayetullah, from an experimental plantation in Dacca.

Isolation of the Alkaloidal Complexes.—Freshly harvested roots (moisture content 55%; total alkaloids 1.7% on moisture free basis) were chopped into approximately 1 cm. long pieces (1/2 kg.) and repeatedly extracted as such with ethanol (95%, 2 1/2 l. × 1; 80%, 2 1/2 l. × 1; 70%, 2 1/2 l. × 2). The combined extracts were concentrated to semisolid residue (18 g.) below 40°C. in vacuo.

Fresh root bark (1 kg., moisture content 70%; total alkaloids 1.6 % on moisture free basis) was also similarly extracted with ethanol (2 l. × 5) to give a dark green semisolid residue (25.0 g.) after removing the solvent. This (25.0 g.) was triturated with water and ethyl acetate. The

a queous layer was shaken out with water and extracted with amyl alcohol ($3\times$). The combined amyl alcohol extracts were then repeatedly shaken out with water. The aqueous extract on removal of the solvent in vacuo gave a cream yellow powder designated as complex I (5 g.). The ethyl acetate layer was dried over Na_2SO_4 and freed of the solvent in vacuo and the semisolid residue exhaustively extracted with petroleum ether. The petroleum ether extracts gave on removal of the solvent a fatty residue designated as complex III. The petroleum ether insoluble residue formed a cream coloured powder designated as complex II.

Constituents of Complex I.—Five g. of this complex was dissolved in a small amount of water and the aqueous solution (pH 4–5) was treated with dilute ammonia (1%) to pH 7.5 and repeatedly extracted with ethyl acetate ($7\times$). The ethyl acetate solution, dried over Na_2SO_4 , gave on removal of the solvent a glassy mass which, after charcoaling in methanol, slowly crystallised from dilute methanol to give ajmaline, m.p. 152°C ., twice crystallised from the same solvent it gave m.p. 158°C . (Found: C, 71.85; H, 7.95; O, 11.7. $\text{C}_{20}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 71.5; H, 8.05; O, 11.9). Mixed m.p. with ajmaline undepressed (m.p. 158°C .) $[\alpha]^{22}_{\text{D}} + 125^\circ$ (95% ethyl alcohol). Literature value $[\alpha]^{33}_{\text{D}} + 128^\circ$.

The aqueous layer from the above working was concentrated in vacuo and made acid with 10% acetic acid and treated with potassium iodide. The semi-crystalline precipitate thereby obtained was washed with a little water and crystallised from methanol to give alstonine hydroiodide, m.p. $282\text{--}4^\circ\text{C}$. decomp. (0.96 g.), twice crystallised from the same solvent it gave glistening colourless plates m.p. 286°C . decomp. (Found C, 52.95; H, 4.6; N, 5.6; O, 10.45; I, 26.8. $\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2 \cdot \text{HI}$ requires C, 52.95; H, 4.45; N, 5.85; O, 10.1; I, 26.7). It had $[\sigma]^{27}_{\text{D}} + 162^\circ$ in absolute ethanol. Literature m.p. of alstonine hydroiodide, 292°C . decomp. (corrected). The free base was obtained by adding dilute sodium carbonate solution to an aqueous solution of the hydroiodide. The base which is known to have a very unstable character had to be handled with great care in the process of isolation. It crystallised as light orangish yellow needles which decomposed above 150°C . without melting or sintering on slow heating. By decomposing the hydroiodide solution with dilute caustic soda no crystals could be obtained; adding it dropwise until the pH reached 8 resulted in deposition of violet needles. Alstonine decomposed slowly even when kept in a desiccator in vacuo (Found C, 69.45; H, 5.85; N, 8.0; $\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 68.85; H, 6.05; N, 7.65). Salts prepared from

the free base failed to crystallise.

The aqueous filtrate from alstonine hydroiodide was made strongly alkaline with dilute caustic soda. A yellow precipitate thrown out was collected, washed with water and crystallised (charcoaled) from dilute ethanol to give serpentinine, m.p. 276°C . undepressed on admixture with an authentic specimen.

In a modified working based on liberation of the total base with alkali and separation of the bases through the hydroiodides, a methanol insoluble crystalline iodide was obtained which melted at 257°C . and could not be studied further due to insufficient quantity of the material.

Alstonine Hydrochloride.—Alstonine hydroiodide was triturated with freshly precipitated silver chloride (washed free from nitrate) and filtered warm. The residue was washed twice with hot methanol and warm water. The filtrate was evaporated in vacuo, the residue dissolved in methanol and charcoaled. The methanolic solution was diluted with ether when it deposited long prismatic needles, m.p. $282\text{--}4^\circ\text{C}$. decomp. $[\alpha]^{21}_{\text{D}} + 140.5^\circ$ in distilled water. (Found C, 64.45; H, 5.4; N, 7.25; Cl, 8.9. $\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2 \cdot \text{HCl} \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 64.05; H, 5.65; N, 7.15; Cl, 9.00). Literature m.p. 286°C . decomp. (corrected). Literature value $[\alpha]^{22}_{\text{D}} + 139.9^\circ$ in water.

Complex II.—The cream yellow product was triturated with 2% caustic soda solution and extracted with ethyl acetate. The sticky residue was then triturated with 5% acetic acid solution and extracted with ethyl acetate. This alternate treatment with alkali and then with acid was repeated till all the material distributed itself between ethyl acetate and the aqueous layer which was kept alkaline. The bulked ethyl acetate layer was dried over Na_2SO_4 and freed of the solvent in vacuo. It was transferred with benzene to a column of alumina and chromatographed with the same solvent followed by mixtures of benzene, ether, chloroform and methanol. The concentrated benzene and chloroform eluates readily crystallised in rhombs, which melted at 273°C . and showed no depression in m.p. when mixed with an authentic specimen of reserpine. The 25% benzene and chlor. form eluate also crystallised from dilute methanol to give ajmaline, m.p. 158°C . (mixed m.p. with an authentic specimen showed no depression). The rest of the chromatographic fractions did not crystallise.

Complex III.—The semisolid complex obtained from the root material was transferred on to a

column of alumina with petroleum ether (b.p. 60-80°C.) and chromatographed with the same solvent followed by mixtures of benzene, ether, chloroform and methanol. The 1:1 ether/benzene eluate crystallised to give a material of m.p. 152°C., mixed m.p. with serposterol (m.p. 150-52°C.), 151-2°C.

The 1:2 chloroform/benzene eluate gave a substance of m.p. 245°C., recrystallised ex. ethanol m.p. 275°C. mixed m.p. with reserpine m.p. 275°C. (Found C, 65.95; H, 6.7; N, 4.75; O, 21.9. $C_{33}H_{40}O_6N_2$ requires C, 65.15; H, 6.65; N, 4.6; O, 23.65) $[\alpha]_D^{22} + 116^\circ$, Literature value $[\alpha]_D^{23} - 117^\circ$; the end fractions collected with the same solvent as eluate gave crystals m.p. 158°C. (mixed m.p. with ajmaline 158°C.).

Paper Chromatography of Aqueous Ballast.—A mixture of butanol (10 ml.), glacial acetic acid (10 ml.) and water was shaken thoroughly and the organic layer allowed to separate. Spots of the aqueous ballast and different sugars (mannose, glucose, gulose, sucrose, rhamnose, fructose and maltose) were chromatographed on a filter paper sheet for 3 days. It was allowed to dry and then sprayed with 1% silver nitrate solution, allowed to dry again and sprayed with 1% caustic soda solution. The resulting four brown spots of different sugars from both the root and root bark material were found to correspond to the spots of mannose, glucose, sucrose and maltose.

Summary

Complexes corresponding to serpajmaline, ajmalexine and resajmaline as isolated from *Rauwolfia serpentina* have now been obtained from *Rauwolfia vomitoria* grown in an experimental plantation in East Pakistan. The complex corres-

ponding to serpajmaline was found to be a strong hypotensive agent in dogs and also had anti-fibrillant action on isolated heart of rabbit. The chemical constituents of these complexes have been examined and the one corresponding to 'serpajmaline' was found to contain alstonine as the major alkaloidal constituent, together with ajmaline and serpentinine. The other two complexes gave reserpine, ajmaline and serposterol. The extracts were found to contain maltose, sucrose, glucose and mannose as the sugar constituents.

Acknowledgements.—The analyses were done by A. Bernhardt, Mulheim, W. Germany. The authors wish to thank Dr. M. I. Khan, Pharmacological Section, P. C. S. I. R. for experiments on animals and Mr. A. A. Qureshi for some experimental help.

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