STUDIES IN THE ALKALOIDS OF RAUWOLFIA SERPENTINA, BENTH. AND THE MODE OF THEIR OCCURRENCE

Isolation of a new Hypotensive Factor and other Alkaloidal Complexes from the Roots

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IN the last communication on the alkaloids of Rauwolfia Serpentina, Benth.,[1] the importance of handling fresh drug material was stressed, as carelessly collected and stocked samples of roots gave very low yields of crystalline bases. Around the same time, it was noted by the author that while dialysis* of fresh uncrushed sprouts from the germinated Bengal gram (Cicer Arietenum, Linn.) with alcohol gave three crystalline products,[2] two of which later proved to be free isoflavones[3] the third being identified as asparagine,[4] none of these constituents could be isolated from air-dried sprouts, due apparently to enzymic and oxidative degradation.

Profiting from these observations on a resumption of studies in the alkaloidal constituents of Rauwolfia serpentina after a long unavoidable break during the war and its aftermath, fresh undried roots harvested from an experimental plantation in East Pakistan were chopped into 2 to 3 centimeter lengths and dialysed five times with alcohol. The dialysate which accounted for about 80% of the assayed alkaloidal content of the roots yielded on removal of the solvent 5% of semi-solid matter on air-dried basis, as against 10 to 12% extractive from the alcoholic percolates of the dried powdered roots, the non-alkaloidal ballast, which greatly complicates isolation work, being thus reduced by about half in this process. The product thus obtained has been resolved into four alkaloidal factors, noted below along with their paper chromatographic evaluation. In view of their probable therapeutic importance as a whole, the first three of these factors have been provisionally assigned names in accord with the nomenclature of the ajmaline series of bases, for the convenience of future reference.

1. Petroleum ether soluble 'oleo-alkaloid' fraction—resajmaline—greenish, viscous oily liquid containing the fatty matter, serposterol, and unsaturated higher alcohols along with around 2.3% reserpine[5] and 0.5% rescinnamine,[6] along with some other weaker bases and only traces of ajmaline. It is soluble in ethyl acetate, ether and petroleum ether, fairly so in alcohol and methanol.

2. Ethyl acetate-benzene soluble akaloidal complex—ajmalexine—forming a cream coloured powder with a concentration of the weaker Rauwolfia bases including 5.5% of reserpine, 2.5% of rescinnamine and some hitherto unknown substances with green and yellowish orange fluorescence. It is soluble in ethyl acetate, mostly soluble in benzene, fairly so in alcohol and methanol, and insoluble in petroleum ether.

3. Serpajmaline fraction, light cream coloured powder soluble in water and also in some partially miscible solvents, mainly containing the stronger bases serpentine, serpentinine, ajmaline and two unknown substances.

4. A cream coloured powder, soluble in water as well as in a mixture of alcohol and non-miscible solvents, containing the stronger bases and two unknown substances with a greenish and a yellowish orange fluorescence.

A reddish, syrupy, water soluble fraction forming the non-alkaloidal ballast was eliminated in the process of separation, which is based on the varying solubilities of the fractions in water and various polar and non-polar organic solvents, without the use of any chemical reagents. Two minor quantities of intermediate alkaloidal fractions were also separated off in the process which will be dealt with later.

While exhaustive studies in the therapeutically significant constituents of these factors and the mode of their occurrence are in progress, it may on the basis of the present pharmacological studies be reported that fraction No. 3 (serpaj-

* The expression 'dialysis' is considered appropriate as the unruptured cell membrances serve as a dialysing medium.

maline), which is chromatographically free from reserpine and other weaker bases and accounts for about 40% of the total alkaloids, is much more potent in its hypotensive activity than pure reserpine, without the complicating feature of the latter's sedative and central depressant action.

From the description of the various factors isolated from the alcoholic dialysate of the uncrushed roots, it will be further seen that none of them correspond to what could be termed as an 'oleo resin', an expression introduced by some of the later workers in this field, and that reserpine and rescinnamine are concentrated in the lipoid soluble factors, ajmalexine and resajmaline, the latter of which consists of fatty matter, serposterol, and higher alcohols[1] with an alkaloidal complex which in contrast to the free bases is readily soluble in petroleum ether. It will also be observed that rescinnamine and reserpine are present in fraction No. 2 (ajmalexine) in a ratio of 2:5 and even in the petroleum ether soluble fraction, which is neglected in the Ciba process for the isolation of reserpine,[7] the two bases occur in a ratio of 1:5, whereas rescinnamine seems to have been isolated in only very minute quantities from the Rauwolfia roots.

With reference to the isolation of reserpine, it may be recalled that in the last communication of the present author,[1] it was reported that the 'neutral fraction' of the alcoholic extracts of the whole roots and the bark gave a white crystalline alkaloid melting at 234°C., which appeared to have an amphoteric character. At that stage no name was assigned to this weak base on account of its doubtful uniformity, but working subsequently with larger quantities of the base and its mother liquors it was possible early in 1940 to obtain from them an alkaloid with a melting point varying with the rate of heating between 262-264°C. and 270-272°C., as against the melting points 262-263°C. and 272°C., later reported for reserpine. Recently, a product melting at 238-240°C. was isolated from the corresponding neutral fraction obtained from the alcoholic extracts of commercially available Rauwolfia roots, which on alternate repeated fractional crystallisation from methanol and acetone yielded two bases which could be identified with reserpine and ajmalicine.

It will be observed that the findings recorded in this communication are in sharp contrast to the results of extensive investigations reported in recent years on the therapeutic activity of various alkaloids and alkaloidal factors obtained from the extracts of dried powdered roots and root bark.[8] Definite conclusion in this regard will have to await further studies which are under way, but already at the present stage of work it would appear that many of the references in respect of the nature of the alkaloidal constituents of the drug and their therapeutic activity, particularly the claim for reserpine as the major therapeutic agent, may eventually have to be drastically revised. The procedure adopted in the present work for the isolation of therapeutically active constituents in their naturally occurring complex form would, moreover, seem to offer a new approach to studies in medicinal plant materials.

Experimental

Isolation of Serpajmaline

16 kg. of fresh undried roots of Rauwolfia serpentina, Benth., corresponding in air-dry weight to 7.650 kg., were chopped into 2 to 3 cm. lengths and soaked with ethanol in a percolator for 48 hours after which the first percolate was drained out. After 5 similar operations a sample of the percolated roots assayed for only 0.3% alkaloids as against 1.3% in the original roots on air-dry weight basis in each case. The combined percolates were completely freed of the solvent in vacuo below 60°C. The resulting semi-solid residue (400 g.) was partitioned between one litre of water and 500 cc. of ethyl acetate whereby the whole of it was divided up between these two phases without leaving any insoluble matter. The lower aqueous layer was repeatedly extracted with ethyl acetate (4.5 litres) till the ethyl acetate layer was found to extract only a negligible quantity of the material and had a slightly yellowish colour. The aqueous layer was then repeatedly extracted with amyl alcohol (6 litres) till further extractions were noted to yield only negligible quantities of residue on removal of the solvent from an aliquot fraction. The combined amyl alcoholic extract was then shaken out repeatedly with water (3 litres). The aqueous extracts on removal of the solvent in vacuo below 60°C. yielded a spongy residue which could be resolved into a light cream coloured powder (95 g.). The amyl alcoholic solution left after exhaustion with water was freed of the solvent and the residue was subjected to the operations carried out on the semi-solid residue left on removal of the solvent from the alcoholic percolates, whereby a second lot of serpajmaline was obtained (20 g.) making for a total yield of 115 g. (1.5%).

Serpajmaline thus obtained softened at 85°C., stuck to the sides at about 100°C. and frothed up at 130°C. Its 1% aqueous solution had a pH value around 6.

Separation of Resajmaline and Ajmalexine

The combined ethyl acetate extracts were freed of the solvent in vacuo and digested with petroleum ether till the petroleum ether did not extract any further quantity of the resulting light cream coloured powder. The petroleum ether extracts gave, on removal of the solvent, resajmaline in a yield of 53 g. (0.7%) , while the light cream coloured powder, ajmalexine, formed 31 g. (0.4%).

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References

1. S. Siddiqui, J. Indian Chem. Soc., **16,** 421 (1939).
2. S. Siddiqui, J. Sci. and Ind. Research (India), **4,** 68 (1945).
3. S. Siddiqui and J.L. Bose, J. Sci. and Ind. Research (India), **4,** 231 (1945).
4. M. Damodaran and C. Singh, Proc. Indian Acad. Sci., **33B,** 159 (1951).
5. J.M. Muller, E. Schlittler and H. J. Bein, Experentia, **8,** 338 (1952).
6. M.W. Klohs, M.D. Draper and F. Keller, J. Am. Chem. Soc., **76,** 2843 (1954).
7. Ciba Ltd., Pakistan Patent 105288 (July 28, 1953).
8. R.F. Woodson, H.W. Youngken, E. Schlittler and S.A. Schneider, *Rauwolfia (Botany, Pharmacognosy, Chemistry and Pharmacology)*, Little, Brown and Company, Boston.