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

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From the roots of *Rauwolfia serpentina, Benth.*, obtained from the Bihar province of India, S. Siddiqui and R.H. Siddiqui[1] isolated in 1931* a series of crystalline bases, namely, ajmaline, ajmalinine, ajmalicine, serpentine, and serpentinine, taking advantage of the differences in their basic strength on the one hand, and the solubilities of their hydrochlorides on the other. Later, in 1939, working on the roots and root bark of the plant, obtained from the more temperate climate of the Dun Valley, S. Siddiqui[2] reported the isolation of two isomers of ajmaline, namely isoajmaline and neoajmaline, a base melting at 220°C. and, from the neutral fraction of the alcoholic extract, a white crystalline alkaloid melting at 234°C., in a yield of 0.1%. From this last mentioned base and its mother liquors it was subsequently possible through repeated fractional crystallisations from methanol and acetone to isolate a crystalline substance, which melted at 270-273°C., and yielded on mild hydrolysis an acid and a base. These findings, however, were not reported, awaiting further work on the problem which had to be held over during the War and its aftermath. In 1952, Schlittler et al.[3] reported the isolation of this base under the name, reserpine, from the so-called 'oleo-resin' fraction which actually corresponds to the 'neutral fraction' referred to above. These authors further claimed reserpine as the sole therapeutic agent in the dual role of its sedative action on the one hand, and blood pressure reducing property on the other. This claim had to be somewhat modified later on, particularly with the isolation of rescinnamine, which has similar pharmacological action.[4] The isolation of this and other minor bases subsequently reported over the years by various authors and listed in Table 1 has not however, materially affected the position claimed for reserpine in respect of its therapeutic action.

Against the background of earlier work briefly referred to above and also taking into account the conflicting pharmacological and clinical findings of various groups of workers in respect of the individual alkaloids and preparations of *Rauwolfia serpentina*, it was considered of interest to isolate and study the various alkaloidal factors as far as possible in the form they occur in the plant body. Moreover, as stocked samples of *Rauwolfia* roots were found to give comparatively low yields of crystalline bases, and profiting from the observation that the crystalline constituents isolated from fresh plant materials through dialysis with solvents can get completely destroyed on drying,[8] apparently due to enzymic and oxidative degradations, fresh undried roots of *Rauwolfia serpentina* were employed for this study.

Working on fresh roots harvested from an experimental plantation in East Pakistan, a procedure was evolved and briefly reported by Siddiqui for the isolation of a number of alkaloidal complexes,[9] on the basis of their varying solubilities in polar and non-polar solvents and expressly avoiding the use of acids, alkalies and other chemical reagents. This procedure which is described in detail in the experimental, consists in dialysing undried roots chopped into 2-3 cm. lengths with alcohol, removing the solvent from the combined dialysates, and resolving the semisolid residue into the following fairly well-defined alkaloidal factors, on the basis of their respective solubility in petroleum ether, ethyl acetate, amyl alcohol and water:

(1) A petroleum ether soluble, greenish viscous liquid, which has been named 'resajmaline' mainly consisting of fatty matter, serposterol and other unsaponifiables. It contains 2.3% reserpine, 0.5% rescinnamine, some of the other weaker bases, and traces of ajmaline (yield 0.5-0.8%).

(2) An ethyl acetate soluble, petroleum ether and water insoluble, alkaloidal complex, named 'ajmalexine' which forms a cream coloured powder consisting mainly of the alkaloids of the ajmaline group, and the weaker *Rauwolfia* bases including 5.5% of reserpine and 2.5% rescinnamine (yield 0.2–0.3%).

(3) An ethyl acetate insoluble, amyl alcohol soluble factor, designated as 'serpajmaline' which forms a light cream coloured powder and is readily soluble in water. It is chromatographically free from reserpine and other weaker bases of the group, and mainly consists of the alkaloids, ajmaline, serpentine and serpentinine, along with two unknown substances giving a greenish and an orange fluore-

* A year later, in 1932, van Itallie and Steenhauer[5] reported the isolation of a base from *Rauwolfia serpentina* which was obviously identical with ajmaline, but they named it as rauwolfine. This has been responsible for a double confusion in nomenclature, due to the added fact that the isolation of a base with that name was also reported in the same year by Koepffli as a constituent of *R. caffra.*

scence. Besides these three fairly well-defined fractions, which account for about 70% of the total assayed alkaloidal content, a comparatively small quantity of an ill-defined light brown product partially soluble in water and aqueous mixtures of amyl alcohol is also obtained in this working along with a reddish, syrupy, water soluble ballast containing about 5–10% of the residual alkaloids.

Apart from protecting the bases against aerial oxidation and enzymic degradation, a further advantage of working with freshly harvested material lies in the fact that the cell walls of the fresh roots virtually act as a semi-permeable membrane, retaining a substantial portion of the non-alkaloidal ballast, and the dialysates thus yield on removal of the solvent only 5% of a semisolid matter on air dried basis, as against 10–12% extractive from the alcoholic percolates of the air dried powdered roots, the non-alkaloidal ballast which greatly complicates the isolation work being thus reduced by about half.

In contrast to the rather long drawn out procedures which had to be adopted by Siddiqui and Siddiqui,[1] and later by Robinson et al.,[10] and other workers for the isolation of alkaloids from the alcoholic extracts of dried powdered roots, the complexes referred to above have served as convenient source materials for the various alkaloids, following a comparatively smooth and simple procedure described for their isolation in the experimental. Apart from ajmaline, neoajmaline, serpentine, serpentinine and reserpine, a base melting at

TABLE 1.—ALKALOIDS FROM *Rauwolfia serpentina*.[7]

Name	Empirical formulae	M. p. °C.	First isolated by
Ajmaline	$C_{20}H_{26}O_2N_2$	158 - 160	Siddiqui & Siddiqui (1931)
Ajmalinine	$C_{20}H_{26}O_3N_2$	180 - 181	,, ,, ,,
Ajmalicine		250 - 252	,, ,, ,,
o-Yohimbine	$C_{21}H_{24}O_3N_2$	258 - 259	Weisenborn et al. (1954)
Raubasine (Alkaloid II) ..	,,	247 - 248	Popelak et al. (1953)
Alkaloid F	,,	253 - 254	Neuss et al. (1954)
Tetrahydroserpentine ..	,,		Bader & Schwarz (1952)
Serpentine	$C_{21}H_{20}O_3N_2$	157 - 159	Siddiqui & Siddiqui (1931)
Serpentinine	$C_{21}H_{22}O_3N_2$ (?)	263 - 265	,, ,, ,,
Isoajmaline	$C_{20}H_{26}O_2N_2$	264 - 266	Siddiqui (1939)
Neoajmaline	$C_{20}H_{26}O_2N_2$	205 - 207	,, ,,
Rauwolfinine	$C_{19}H_{26}O_2N_2$	235 - 236	Chatterjee & Bose (1951)
Reserpine	$C_{33}H_{40}O_9N_2$	277 - 278	Mueller et al. (1952)
Raupine .. (Identical)	$C_{19}H_{22}O_2N_2$	325	Bodendorf & Eder (1953)
Serpagine ..		over 300	Stoll & Hofmann (1953)
Reserpinine ..		238 - 239	Schlittler et al. (1954)
Alkaloid A ..		243 - 244	Neuss et al. (1954)
Alkaloid C .. (Identical)	$C_{22}H_{26}O_4N_2$	240	Hofmann (1954)
Raubasinine (Alkaloid I) ..		228	Popelak et al. (1953)
Corynanthine (Rauhimbine) ..	$C_{21}H_{26}O_3N_2$	218 - 225	Hofmann (1954 & 1954)
Isorauhimbine	$C_{21}H_{26}O_3N_2$	225 - 228	Hofmann (1954)
Yohimbine	$C_{21}H_{26}O_3N_2$	234 - 236	Bader et al. (1954)
Rescinnamine	$C_{35}H_{42}O_9N_2$	226	Haack et al. (1954 & 1955)
		238	Klohs et al. (1954)
3-epi α-yohimbine ..	$C_{21}H_{26}O_3N_2$	125 - 128 / 181 - 183	Bader et al. (1954)
Serpine	$C_{21}H_{26}O_3N_2$	213	Chatterjee & Bose (1954)
Serpinine	$C_{20}H_{24}ON_2$ or $C_{21}H_{26}ON_2$	315 - 317	Bose (1955)
Thebaine	$C_{19}H_{21}O_3N$	195	Hofmann (1954)
Papaverine	$C_{20}H_{21}O_4N$	147	,, ,,
Reserpiline	$C_{23}H_{28}O_5N_2$	amorphous	Klohs et al. (1954)

300-310°C. and probably identical with serpinine[11] has also been isolated in the present working.

Several batches of *Rauwolfia serpentina* roots harvested from the plantation in East Pakistan at various age levels were worked out according to this procedure with the results tabulated in the experimental (Table 2). In the course of this work it has been noted that the maturity of the roots has a considerable bearing on the yields of the various complexes. In one of the batches harvested at the end of July, very little of the fraction corresponding to ajmalexine could be isolated. Moreover, the age of the roots was also found to influence the alkaloidal content of the serpajmaline complex. Alkaloidal content of this complex was found to be about 35% in comparatively young roots and nearly 50% in the roots of about 2 year old plants.

The seasonal variations seemed to be even more significant. The dialysates of the roots harvested during the summer months, May-July, on partitioning between water and ethyl acetate did not completely divide up between these two solvents, leaving some quantity of an insoluble middle layer, which seemed to increase with the age of the roots. The roots harvested in January (about 2 years old) did not give such a middle layer under parallel conditions of working. Moreover, only neoajmaline was obtained from serpajmaline in this working while all the batches received during the summer months gave the normal isomer, ajmaline. This finding recalls the earlier observation of Siddiqui[8] that neoajmaline is formed under more temperate climatic conditions, as the principal alkaloidal constituent of *Rauwolfia*.

The fact that the major part of the total alkaloids occurs in the form of 'complexes,' rather than as simple salts or in the free state, is fairly evident from the fact that ajmalexine is insoluble in water or dilute acetic acid, but on breaking up the complex with alkali, the liberated bases readily dissolve in dilute acids. Again, in contrast to all the *Rauwolfia* alkaloids and their simple salts, the bases contained in resajmaline are readily soluble in petroleum ether, and cannot be extracted from the ethereal solution with the help of dilute acetic or mineral acids, which should point to their occurrence in the form of a petroleum ether soluble complex. The solubility of serpajmaline in amyl alcohol also leads to the same conclusion.

On the basis of fairly exhaustive studies in the pharmacology of serpajmaline,[12] which is obtained in an yield of 1.3-1.6% on dry weight basis, it has been found that it is highly potent in its hypotensive activity, without the therapeutically complicating sedative and central depressant action characteristic of reserpine and other *Rauwolfia* preparations. It has been further noted that serpajmaline has a strong antifibrillant action on the isolated heart of the rabbit and the dog.[13] On the other hand, it has very low toxicity, dosages as high as 1000 mg./kg. body weight proving non-fatal in mice.

In order to determine the role of its non-alkaloidal constituents in this cardiac action of serpajmaline, its carbohydrate component was isolated free from basic impurities, employing mildest possible methods. A nearly colourless, treacly, nitrogen-free, water soluble product thereby obtained in an yield of about 15% on the weight of the complex, was found to have the same order of antifibrillant action as serpajmaline and ajmaline. None of its individual constituents, however, quantitatively account for the total activity of the serpajmaline complex, which would appear to be derived from the mutually potentiating, synergistic action of the various alkaloidal and non-alkaloidal components of the complex. Further work on problems arising out of the findings recorded in the present paper is in progress.

Experimental

The procedure employed for obtaining the various alkaloidal complexes was basically identical with that briefly described earlier by Siddiqui.[9] Sixteen kg. of fresh undried roots of *Rauwolfia serpentina* (corresponding in dry weight to 7.2 kg.) were harvested when 21 months old from an experimental farm in East Pakistan, flown to Karachi and immediately on receipt cut into 2-3 cm. lengths and soaked in ethanol. The extract was removed after every 48 hrs. About 20 litres of ethanol (95%) was needed each time to soak the chopped roots. Each extract after removal was first concentrated in a cyclone evaporator to about 5 litres and finally *in vacuo* to a treacly mass, not allowing the temperature to exceed 50°C. The weights of the liquidish residues obtained for the 5 percolates were 750, 600, 75, 68, and 61 g., respectively. A further extraction was carried out with 80% ethanol and the semisolid mass obtained was 135 g. The extraction was considered complete as a subsequent extract with 80% ethanol gave a very small amount of the semisolid residue.

The roots were initially assayed for their alkaloidal content and then after the withdrawal of each percolate:

Initial	2.04%	After ext. IV	0.15%
After ext. I	0.99%	,, V	0.12%
,, II	0.55%	,, VI	0.06%
,, III	0.49%	,, VII	0.03%

The still liquidish residue (about 2 litres) was stirred up with water (2 litres) and shaken with ethyl acetate when the extract divided up into ethereal and aqueous layers. The lower aqueous layer was exhaustively extracted with ethyl acetate (200 ml. each time, total 1 litre) until the ethyl acetate layer was only faintly yellow. The combined ethyl acetate extracts were washed with water (100 ml., 3 times) and the aqueous washings added to the main aqueous extract.

Isolation of Serpajmaline.—The aqueous extract was repeatedly shaken out with amyl alcohol (250 ml. each time, total 5 litres) till further extractions gave only negligible quantities of residue on removal of the soevent from an aliquot fraction. The combined amyl alcoholic extract was shaken out repeatedly with water (300 ml. each time, 5 times). The aqueous extracts on complete removal of the solvent *in vacuo* below 50°C. and subsequent drying yielded a light cream coloured powder (97 g.).

The amyl alcohol was then concentrated to about half the volume *in vacuo* and again exhausted with water. The aqueous extracts were shaken out with ethyl acetate, and on complete removal of water *in vacuo* yielded a further quantity of light cream coloured residue (18 g.), making for a total yield of 115 g. (1.59%).

Separation of Resajmaline and Ajmalexine.—The combined ethyl acetate extracts were freed of the solvent *in vacuo* and to the residue (80.8 g.) enough petroleum ether was cautiously added to adequately cover the residue without stirring, and was carefully decanted off after every 48 hrs. Further extractions were similarly carried out with petroleum ether until a colourless extract was obtained, leaving practically no residue on removal of the solvent.

Resajmaline.—The petroleum ether extract on complete removal of the solvent yielded a green thick oily product (55 g., 0.76%).

Ajmalexine.—The petroleum ether insoluble residue on drying *in vacuo* gave a light cream coloured powder (24 g., 0.33%) on the weight of the dry roots.

Several batches of fresh *Rauwolfia* roots obtained from the experimental farm were worked according to the process detailed above and gave the various complexes in yields indicated in Table 2.

Serpajmaline and its Constituent Bases.—Serpajmaline is a light cream coloured amorphous powder readily soluble in water, absolute alcohol and moist acetone, insoluble in benzene, ethyl acetate, ether and other non-miscible solvents. It begins to soften at 85°C., sticks to the sides at about 100°C., and froths up around 130°C. The aqueous solutions of serpajmaline show a pH of 5-6.

Attempts were initially made to separate the stronger, yellow bases from the comparatively weaker colourless bases of the ajmaline group by extracting with immiscible solvents after adjusting the pH of the aqueous solution to 7 with dilute ammonia. This procedure of isolation, however, did not work as the complex does not seem to break at this pH.

Serpajmaline (5 g.) was treated with cold dilute 10% ammonia (2 ml.) and the yellow granular precipitate was filtered and washed with ice-cold water under suction. The aqueous filtrate was extracted thrice with ethyl acetate (15 ml.). The precipitate (2 g.) was taken up in the ethyl acetate washings when practically the whole of it went into solution leaving a negligible quantity of a dark

TABLE 2.—YIELDS OF *Rauwolfia* ALKALOIDAL COMPLEXES FROM DIFFERENT BATCHES OF FRESH ROOTS.

Harvesting period	Age	Fresh roots kg.	Calculated dry weight kg.	Serpajmaline %	Ajmalexine %	Resajmaline %
End of January 1958 ..	15 months	18.0	9.0	1.55	0.22	0.833
Second week of July 1958 ..	10½ ,,	14.5	8.12	1.13	0.244	0.532
End of July 1958 ..	11 ,,	16.5	9.075	1.123	—	0.462
First week of October 1958..	13 ,,	17.0	7.65	1.2	0.29	0.60
End of May 1959 ..	21 ,,	16.0	7.20	1.6	0.33	0.76

brownish insoluble residue. The moist ethyl acetate extract was cooled in ice and saturated with carbon dioxide, finally with the addition of petroleum ether (about 10 ml.) whereby the stronger, yellow bases were removed as carbonate, leaving a faint yellow solution. After filtration and drying over anhydrous sodium sulphate the solvent was removed *in vacuo*, the residue (1 g.) extracted with benzene and the benzene soluble fraction (0.77 g.) after removal of the solvent was crystallised from methanol. The colourless crystallisate thus obtained was identified as ajmaline. The benzene insoluble fraction on crystallisation with methanol gave colourless long needles, subliming at 300-310°C., and probably identical with serpinine[11] (m.p. 315°C.). The yellow sticky precipitate of the carbonates was completely freed of the solvent *in vacuo*, dissolved in the least quantity of 10% acetic acid, charcoaled and treated with an ice-cold aqueous solution of potassium iodide. The resulting yellowish precipitate was filtered, taken up in methyl alcohol (8-10 ml.) and kept in the cold, when glistening yellow rods of serpentine hydriodide crystallised out (m.p.278°C., decomp.). The alcoholic filtrate was again treated with activated charcoal, slightly concentrated and carefully basified with dilute caustic soda, when serpentinine almost immediately came out in lemon yellow crystals which after washing with cold dilute methanol melted at 264°C., and showed no depression in m.p. on admixture with an authentic sample of serpentinine.

In a subsequent working, the basic fraction obtained on treatment of a cold aqueous solution of serpajmaline with ammonia was directly separated into a benzene soluble and an insoluble fraction avoiding the use of ethyl acetate. A little petroleum ether was added to the benzene solution when on saturation with carbon dioxide it became almost colourless. The residue on removal of the solvent was crystallised from methanol yielding ajmaline. The precipitated insoluble matter along with the benzene insoluble fraction containing the yellow bases could then be worked as described above for the isolation of serpentine and serpentinine.

Serpajmaline complex obtained from the roots harvested at the end of January yielded only neoajmaline melting at 205-207°C.

Ajmalexine.—It is a light cream coloured amorphous powder soluble in ethyl acetate, mostly soluble in benzene fairly so in ethanol and methanol and insoluble in petroleum ether. No quite satisfactory method of isolating the individual bases from this complex could be evolved owing to the fact that the complex connot be effectively broken without treatment with stronger alkali which results in the partial hydrolysis of the weaker bases like reserpine and rescinnamine. For the isolation of reserpine, ajmalexine (6 g.) was dissolved in hot ethyl acetate (25 ml.), and the solution after quickly drying over sodium sulphate was adsorbed over a column of silica gel (E. Merck, for partition chromatography), 25 cm. long and 2 cm. in diameter. The column was first eluted with ethyl acetate until the eluate became colourless and then exhausted with methyl alcohol. The ethyl acetate extract was freed of the solvent *in vacuo*, the residue taken up in hot benzene and cooled, and the small quantity of the greenish precipitate which deposited was filtered off and added to the methanol extract. The benzene soluble fraction on removal of the solvent *in vacuo* was dissolved in a small quantity of methanol and kept in the cold when colourless crystals (0.19 g.) melting at 268-271°C., were obtained and identified as reserpine. The methanolic eluate and the mother liquor of reserpine which gave positive HNO_3 colour reaction for ajmaline were not followed for the isolation of other alkaloidal constituents for the present.

Resajmaline.—The separation of individual alkaloidal components from this lipoid soluble complex also presented many manipulative difficulties. In one of the workings, resajmaline (50 g.), obtained from comparatively young roots harvested in February, was taken up in a mixture of ether, petroleum ether and methanol, and kept in the cold. The crystalline deposit thereby obtained was repeatedly crystallised from a mixture of acetone and methanol when it finally gave colourless silky needles m.p. 155-160°C., identified as serposterol. The dark green filtrate from the original crystallisate was extracted with a mixture of methanol and dilute 10% aqueous ammonia (50:50). The petroleum ether layer was freed of the solvent *in vacuo* and was not pursued further for the present. The ammoniacal extract was acidified with dilute acetic acid to pH 6 and the solvent removed *in vacuo*. The residue gave through ethyl acetate a crude crystallisate, the ether soluble fraction of which on repeated crystallisation from methanol yielded colourless needles (m.p. 205°C), identified as neoajmaline. The ether insoluble portion of the crude crystallisate gave from methanol, on repeated crystallisation, colourless needles m.p. 250-254°C., identified as ajmalicine, by taking mixed melting point with an authentic sample.

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