

Studies on Philippine Species of *Rauwolfia*

1. Isolation of Reserpine and Rescinnamine from *Rauwolfia amsoniaefolia* A. DC.

There are four *Rauwolfia* species reported as native to the Philippines: *Rauwolfia amsoniaefolia* A. DC., *Rauwolfia samarensis* Merr., *Rauwolfia membranacea* Merr., and *Rauwolfia loheri* Merr. Of these only the first two are available for investigation.

Rauwolfia amsoniaefolia A. DC., known in Tagalog as sibakong, is found in the thickets and secondary forests of Cagayan, Ilocos Norte, Batangas, Camarines provinces, Mindoro, Masbate, and Mindanae.

Very little is known about the alkaloidal content of this native plant species. There was a report on the preliminary phytochemical investigation on the leaves, roots, and bark of *R. amsoniaefolia* by GOMEZ and CARANDANG¹. An isolation of a creamish, amorphous alkaloid, amsoniaefoline, was reported by GOMEZ, DA SILVA, and GARCIA².

Experimental. Determination of the total alkaloid content of the different parts of the plant by the acetic acid

Preliminary analysis of the crude drug by paper chromatography indicated the presence of the following alkaloids: reserpine, rescinnamine, deserpidine, aricin, yohimbine, methyl reserpate, possibly ajmalicine, and an unidentified alkaloid from the stem bark. The roots showed the presence of the same alkaloids except deserpidine. However, smaller amounts of reserpine and rescinnamine were indicated for the roots.

The material used for the isolation of the constituent alkaloids was the stem bark of mature plants collected in Mindoro. The air-dried, finely ground material was percolated to exhaustion with methanol. The percolate was concentrated under reduced pressure to a volume such that 1 ml of the concentrated extract was equivalent to 1 g of the drug. The concentrated methanolic extract, after dilution with an equal volume of water, was shaken several times with chloroform. The chloroform extract was then chromatographed on alumina. The various fractions of eluate were evaporated to dryness and crystallized from methanol. Four crops of crystals were obtained (Fig. 1-4) which gave the following melting points: crystal 1, 246 to 249°C; crystal 2, 246–249°C; crystal 3, 225–227°C; crystal 4, 234–236°C.

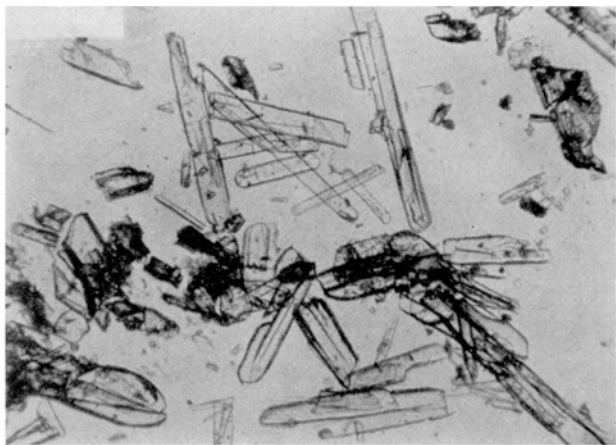


Fig. 1

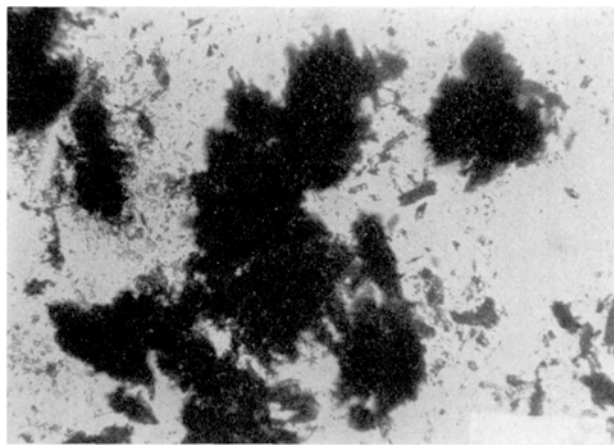


Fig. 3

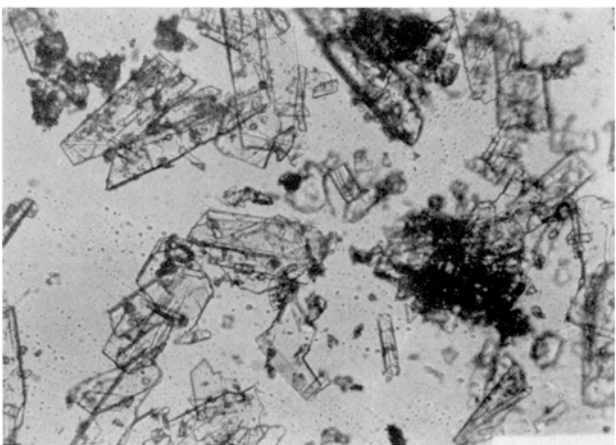


Fig. 2



Fig. 4

method³ gave the following results:

Stem bark	2.06%	Bark of root	4.16%
Whole root	2.44%	Leaves	0.96%
Wood of root	1.39%		

¹ L. GOMEZ and L. V. CARANDANG, J. Phil. Pharm. Assoc. 43, 102 (1956).

² L. GOMEZ, P. DA SILVA, and P. GARCIA, J. phil. pharm. Assoc. 44, 127 (1957).

³ F. NEUWALD and W. LOGES, Arch. Pharm. 289, 226 (1956).

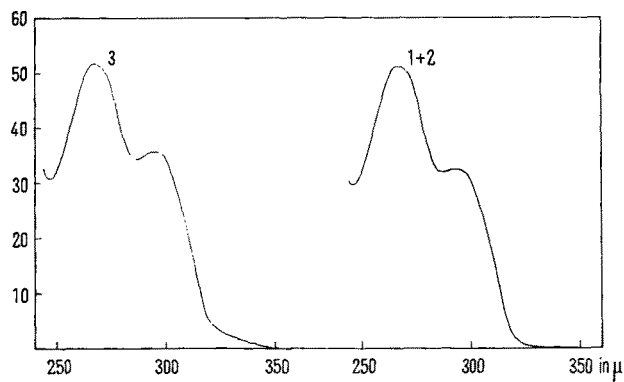


Fig. 5

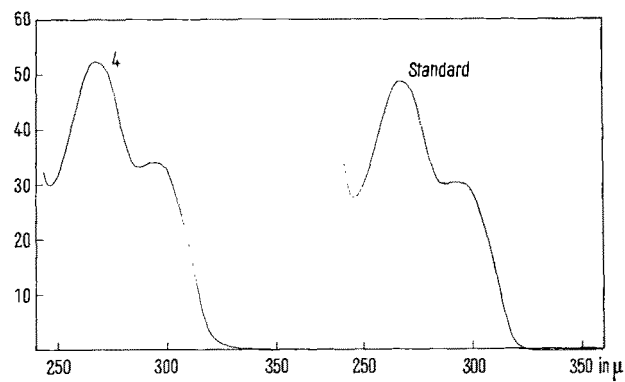


Fig. 6

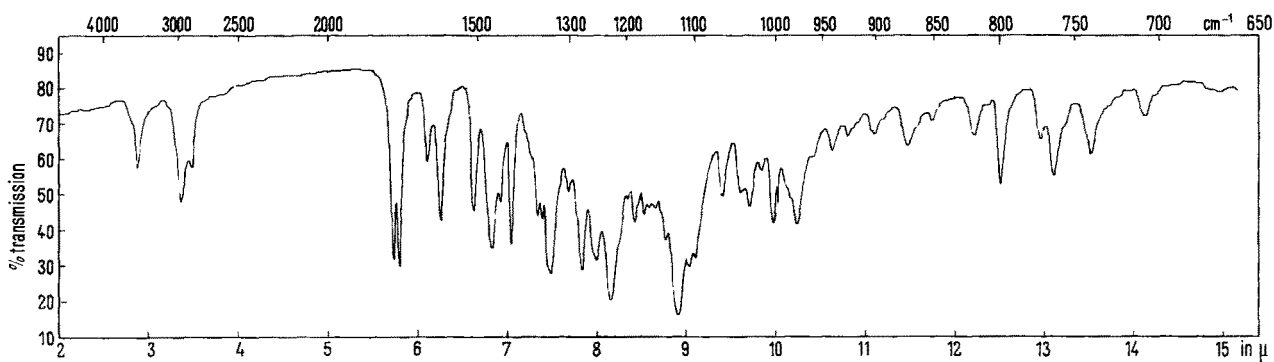


Fig. 7

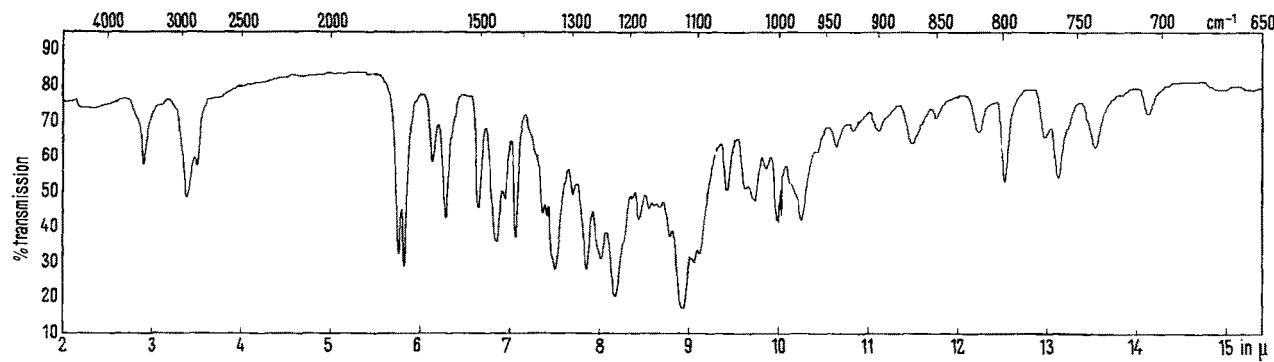


Fig. 8

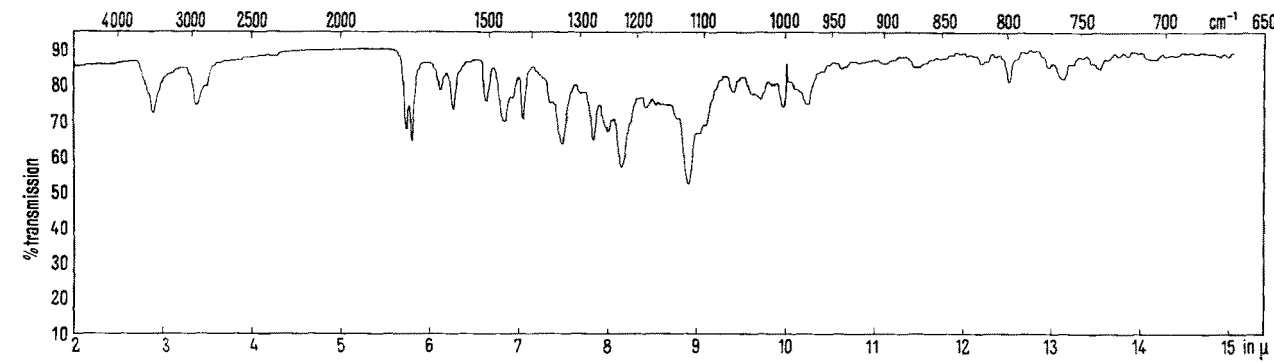


Fig. 9

The same colour reactions characteristic of reserpine and rescinnamine were given by the four crystals: with Froehde's reagent, yellow-green to blue; with concentrated sulfuric acid, slightly greenish yellow and with concentrated nitric acid, brick red turning to greenish yellow solution.

Elementary analysis yielded the following results:

Crystal 1: C 64.76, H 6.71, N 4.81

Crystal 2: C 65.30, H 6.78, N 4.08

Crystal 3: C 65.88, H 6.83, N 4.61

Crystals 1 and 2 were identified by comparison on a paper chromatogram with pure reserpine and by their ultraviolet (Fig. 5, 6) and infrared absorption spectra (Fig. 7, 8, 9). Crystals 3 and 4 were identified by paper chromatography to be mixtures of reserpine and rescinnamine. Quantitative analysis showed crystals 1 and 2 to be 99% reserpine, crystal 3 is 90% reserpine and 10% rescinnamine and crystal 4 contains 95% reserpine and 5% rescinnamine.

The elementary analyses were made by Misses LYDIA JASON and GLORY LLEANDER, NSDB, and the UV- and IR-spectra by J. CAROL and staff, FDA, Washington.

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College of Pharmacy, University of the Philippines and National Science Development Board, Manila, August 17, 1959.

Zusammenfassung

Aus *Rauwolfia amsoniaefolia* A. DC. wurden ziemlich reines Reserpin und ein Gemisch von Reserpin und Rescinnamin isoliert und charakterisiert.

Note on the Specificity of Mercuric Bromophenol Blue for the Cytochemical Detection of Proteins

Quite recently BAKER¹ has expressed some doubts on the specificity of bromophenol blue (BPB) as a protein stain. He seems to be of the opinion that whatever is stained by bromophenol blue does not necessarily contain proteins, and hence this fact does not qualify this stain to act as a reliable agent for the detection of proteins.

The purpose of this communication is to emphasize that, in addition to what BAKER¹ has observed, whatever is not stained with this dye is not always devoid of proteins.

The credit of bringing this powerful acid dye to the forefront as a specific stain for various proteins goes to MAZIA, BREWER, and ALFERT², who modified the older techniques employed by DURRUM³, KUNKEL, and TISELIUS⁴ and GESCHWIND and LI⁵ and standardized a new technique.

I have studied in detail the mucinogenesis in the goblet cells of different vertebrates [viz., *Mystus seenghala* (fish), *Rana tigrina* (amphibian), *Hemidactylus* sp. (reptile), and the house- and white rats (mammals)]. The mucus which these cells secrete has been established histochemically as acid mucopolysaccharides (see also PEARSE⁶ and PALAY⁷) as is evident from the following reactions (PAS +, Best's carmine –, Alcian blue⁸ +, mucicarmine +, SBB –, intensely metachromatic after Feyrter's 'enclosure' method⁹, and toluidine blue⁶ etc. etc.). Further, mucopolysaccharides and acid mucopolysaccharides invariably contain proteins (+ after coupled tetrazonium⁹) as one of their constituents (PEARSE⁶ and LILLIE¹⁰).

During the present investigations, mercuric bromophenol blue staining has been employed both on paraffin sections from the various fixatives such as Regaud, Lewitsky, Lewitsky saline¹¹, Champy, Helly, Zenker-formol, Zenker and Carnoy (both with and without chloroform) and gelatin sections from formaldehyde calcium¹², Lewitsky saline¹¹, and pyridine extracted (prior fixation in weak Bouin) material¹³. In none of the above preparations does the mucus in the thecae of the goblet cells give blue coloration with mercuric bromophenol blue technique of MAZIA, BREWER, and ALFERT².

So in the present state of knowledge, as BAKER¹ has also pointed out, it is safe to regard bromophenol blue merely as a 'powerful acid dye, capable of making direct links with basic groups in tissue-constituents' and perhaps also with certain acidic groups, through mercury.

In short, whatever is stained with mercuric bromophenol blue does not always contain proteins and whatever is not stained is not necessarily devoid of proteins.

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Résumé

Le bromophénol mercurique bleu n'est pas un colorant spécifique des protéines. Tout ce qui est teint par lui ne contient pas toujours des protéines et tout ce qui n'est pas teint n'est pas nécessairement dépourvu de protéines.

¹ J. R. BAKER, Quart. J. micr. Sci. 99, 459 (1958).

² D. MAZIA, P. A. BREWER, and M. ALFERT, Biol. Bull. 104, 57 (1953).

³ E. L. DURRUM, J. Amer. chem. Soc. 72, 2943 (1950).

⁴ H. G. KUNKEL and A. TISELIUS, J. gen. Physiol. 35, 89 (1951).

⁵ I. R. GESCHWIND and C. H. LI, J. Amer. Chem. Soc. 74, 834 (1953).

⁶ A. G. E. PEARSE, Histochemistry (Churchill Ltd., London 1954).

⁷ S. PALAY, Frontiers in Cytology (New Haven, Yale University Press 1958), p. 305.

⁸ H. F. STEEDMAN, Quart. J. micr. Sci. 91, 477 (1950).

⁹ J. F. DANIELLE, Symp. Soc. exp. Biol. 1, 101 (1947).

¹⁰ R. D. LILLIE, Histopathologic Technic and Practical Histochemistry (The Blakiston Company Inc., New York 1954).

¹¹ J. R. BAKER, Quart. J. micr. Sci. 97, 621 (1956).

¹² J. R. BAKER, Quart. J. micr. Sci. 85, 1 (1944).

¹³ J. R. BAKER, Quart. J. micr. Sci. 87, 441 (1946).

¹⁴ The department has now shifted to Chandigarh.

Enzymatische Hydrierungen im Ring A von Steroiden mittels Streptomyzeten¹⁻³

Die Fähigkeit, Steroide im Ring A enzymatisch zu hydrieren, ist unter den Mikroorganismen ziemlich verbreitet⁴. Die umgekehrte Reaktion hingegen, die Hydrierung von Δ^4 -3-Keto-steroiden zu den entsprechenden im

¹ 10. Mitt. über mikrobiologische Reaktionen; 9. Mitt. siehe J. URECH, E. VISCHER und A. WETTSTEIN, Helv. chim. Acta 43, 1077 (1960).

² 170. Mitt. über Steroide; 169. Mitt. siehe R. NEHER und A. WETTSTEIN, Helv. chim. Acta 43, 1171 (1960).

³ Auszugsweise vorgetragen am VII. Internationalen Kongress für Mikrobiologie, Stockholm 1958.

⁴ Vgl. zum Beispiel E. VISCHER und A. WETTSTEIN, Adv. Enzymol. 20, 237 (1958).