THE OCCURRENCE OF RESERPINE IN *ALSTONIA CONSTRICTA* F. MUELL.*

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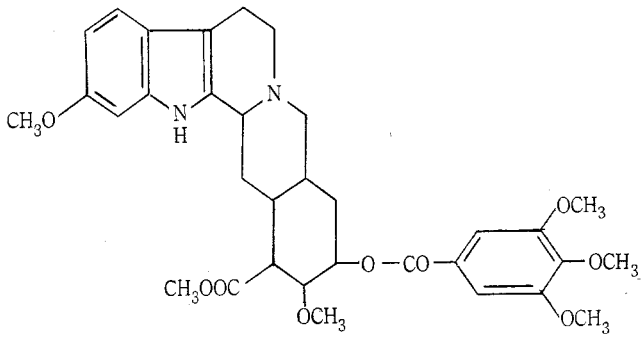
In recent years great interest has been aroused in the use of the alkaloid reserpine (I) in the treatment of hypertension and various mental disorders. The roots of *Rauwolfia serpentina* Benth., a member of the family Apocynaceae, from which the alkaloid was originally isolated by Mueller, Schlittler, and Bein (1952), have been used for centuries in India as a sedative, and have been the main commercial source. Further export of this material from India has been banned (Editorial 1955) and although the demand has been partially met by other eastern countries such as Burma, Siam, and Java, and by the use of other reserpine-containing species such as *R. vomitoria* Afz. (Poisson *et al.* 1954) and *R. heterophylla* Roem. et Schult. (Djerassi *et al.* 1953), an intensive search for new sources is being conducted by the pharmaceutical firms concerned in exploitation.



(I)

We wish to report the presence of reserpine in the root bark of *Alstonia constricta* F. Muell. ("bitter bark"), a small tree confined to north-eastern Australia and occurring in the Queensland rain-forests. No attempt at complete isolation of the alkaloids has been made, but reserpine has been isolated in approximately 0·05 per cent. yield and identified by comparison with an authentic specimen and by hydrolysis to reserpic acid and 3,4,5-trimethoxybenzoic acid. The results of assays based on the amount of the latter acid produced on hydrolysis of the crude tertiary bases are shown in Table 1. These values cover all alkaloids which produce a non-volatile acid on hydrolysis, but since substantially pure 3,4,5-trimethoxybenzoic acid was produced in each case, rescinnamine (Klohs, Draper, and Keller 1954) was probably absent.

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Deserpidine (Schlittler *et al.* 1955) may be present, but so far only reserpine has been isolated (apart, of course, from known *Alstonia* alkaloids such as alstonine and its congeners). A detailed investigation of the alkaloids of *A. constricta* is in progress and further results will appear in a subsequent paper.

Preliminary tests carried out by the Division of Animal Health and Production, C.S.I.R.O., with the alkaloid from *A. constricta* and authentic reserpine showed general similarity in pharmacological action.

TABLE 1

RESERPINE CONTENT OF ALSTONIA CONSTRICTA

Part of Plant	Drying Temperature (°C)	Reserpine (%)
Leaves	60–115*	Trace
Stem bark	60	0·03
Stem wood	60–115	Trace
Mature root bark	60	0·23
Root wood	60	Trace
Small roots	60–115	0·28
Small roots	60	0·26

* Half sample only, remainder dried at 60 °C.

Experimental

All m.p.'s are corrected. Microanalyses were carried out by the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Assay Procedure.*—The milled plant material (250–350 g) was extracted with methanol in a Soxhlet for 30 hr, the extract concentrated to remove the methanol, and the residue treated with $NaHCO_3$ solution and ether. Any resinous material separating at this stage was dissolved in a little methanol and retreated in the same way, this process being repeated thrice or until no more resin formation occurred. All the aqueous solutions were extracted four times with ether and the combined ether extracts washed with 2% acetic acid (3×100 c.c.) then 2% HCl (2×100 c.c.). The combined acid extracts were then basified (NH_3) and exhaustively extracted with ether ; any resin formed was redissolved in 2% acetic acid and retreated as already described. The crude tertiary bases obtained on evaporation were hydrolysed by refluxing for 1 hr with 5% methanolic KOH (*c.* 40 c.c./g), the solvent replaced by an equal volume of water and unhydrolysed bases removed with ether. Acidification and extraction with ether afforded the crude acids which were purified by extraction into $NaHCO_3$ solution and recovery into ether. Evaporation afforded the pure acids. The percentage reserpine content was calculated from the expression:

$$\frac{287 \times \text{mass of acid}}{\text{mass of plant material}}.$$

(*b*) *Isolation of Reserpine.*—Mature root bark (264 g) was treated as described above up to the stage where the crude bases were obtained in acid solution. Basification with NH_3 gave a resin which would not dissolve in ether. A solution of this material in a little methanol gradually deposited a crystalline precipitate which after several recrystallizations from methanol had m.p. 266–267 °C (Found : C, 65·3 ; H, 6·9 ; N, 4·7 ; MeO, 29·8%. Calc. for $C_{33}H_{40}O_9N_2$: C, 65·1 ; H, 6·6 ; N, 4·6 ; 6×MeO, 30·6%). Yield 0·07 g. A further quantity was obtained on longer standing. The material did not depress the m.p. of authentic reserpine and the infra-red spectra measured in a " Nujol " mull were identical. The alkaloid had $[\alpha]_D^{20}$ —116° (*c*, 1·0 in chloroform)

and in an evacuated tube melted at 277–278 °C with subsequent decomposition. Dorfmann, Furlenmeier, and Hueber (1954) quote $[\alpha]_D^{24}$ —118° and m.p. 277–277·5 °C under the same conditions.

Hydrolysis of the alkaloid from *A. constricta* as described in the assay procedure afforded 3,4,5-trimethoxybenzoic acid (m.p. and mixed m.p. with an authentic sample 169–170 °C), and reserpic acid, m.p. 236–237 °C.

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References

DJERASSI, C., GORMAN, M., NUSSBAUM, A. L., and REYNOSO, J. (1953).—*J. Amer. Chem. Soc.* **75**: 5446.

DORFMANN, L., FURLENMEIER, A., and HUEBER, C. F. (1954).—*Helv. Chim. Acta* **37**: 59.

EDITORIAL (1955).—*Industr. Engng. Chem. (News)* **33**: 1074.

KLOHS, M. W., DRAPER, M. D., and KELLER, F. (1954).—*J. Amer. Chem. Soc.* **76**: 2843.

MUELLER, J. M., SCHLITTLER, E., and BEIN, H. J. (1952).—*Experientia* **8**: 338.

POISSON, J., LE HU, R., GOUTAREL, R., and JANOT, M-M. (1954).—*C.R. Acad. Sci. Paris* **238**: 1607.

SCHLITTLER, E., ULSHAFER, P. R., PANDOW, MARY L., HUNT, REGINA M., and DORFMANN, L. (1955).—*Experientia* **11**: 64.