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ALKALOIDS OF *ALSTONIA MUELLERIANA**

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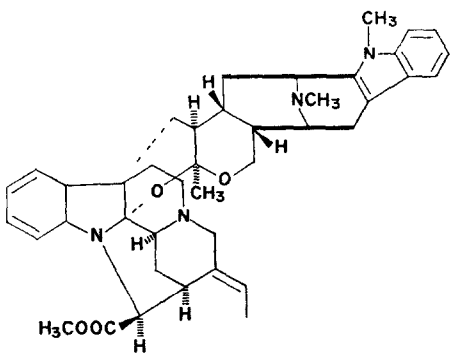
Abstract—Four indole alkaloids have been isolated and characterized from the tree bark of *Alstonia muelleriana* Domin One of these is the previously known dimeric indole alkaloid, villalstonine (I) A second probably dimeric indole alkaloid, alstonisidine (II), and two monomeric indole alkaloids, alstonisine (III) and alstonerine (IV) are also described

INTRODUCTION

IN CONTINUATION of our investigations of the alkaloidal constituents of various *Alstonia* species, we report the isolation and characterization of two new monomeric indole alkaloids, one new dimeric indole alkaloid, and the known dimeric indole alkaloid, villalstonine (I), from the tree bark of *Alstonia muelleriana* Domin (Apocynaceae).‡

We have been unable to find a report of any previous investigation of this species.



Villalstonine (I)

RESULTS

The finely ground bark was extracted successively with ligroin (70–90°) and 70% ethanol. From the concentrate of the ethanol extracts, the four alkaloids were separated by a com-

* Part XI in the series "Alstonia alkaloids" For Part X see R C ELDERFIELD and G MANALO, *J Philipine Pharm Ass* **50**, 91 (1964)

This paper is based on a Ph D dissertation submitted by Robert E Gilman to the University of Michigan, 1959

A preliminary summary of this work is given in R C ELDERFIELD, *Am Sci* **48**, 193 (1960)

† Eli Lilly & Co Fellow 1956–1957 National Institutes of Health Predoctoral Fellow 1957–1959.

‡ We wish to acknowledge the cooperation of Dr J R Price of the Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, in providing a supply of the bark We also acknowledge the cooperation of Parke-Davis & Company who ground the whole bark

bination of column chromatography and counter current distribution methods according to the scheme outlined in Fig. 1 and given in detail in the Experimental section.

The major alkaloid isolated was I (originally, in Gilman's Ph.D dissertation, called *B*) which has been isolated previously by Sharp[1] from a number of *Alstonia* species The identity

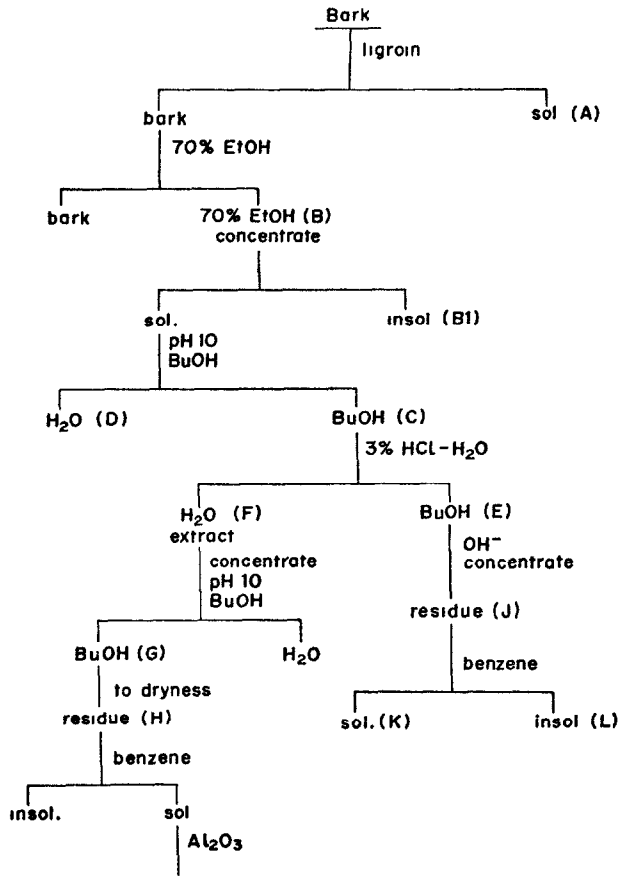


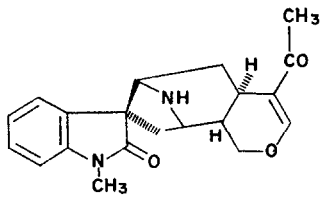
FIG. 1.

of *B* with I has subsequently been confirmed by Nordman and Kumra[2] by comparison of single crystal X-ray patterns with those of an authentic sample I has the empirical formula $C_{41}H_{48}N_4O_4$ and a dimeric indole structure has been assigned by Nordman and Kumra[2] from X-ray crystallographic analysis and simultaneously by Swiss workers[3] on conventional chemical evidence. Both groups agree on the absolute configuration of I on the assumption that the C-15 rule is valid

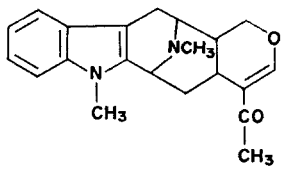
[1] T M SHARP, *J Chem Soc* 1227 (1934)
[2] C E NORDMAN and S K KUMRA, *J Am Chem Soc* **87**, 2059 (1965).
[3] M HESSE, J HURZELER, C W. GEMENDEN, B S JOSHI, W I. TAYLOR and H SCHMID, *Helv. Chim. Acta* **48**, 689 (1965)

Chatterjee *et al.*[4,5] have also obtained I from *A. macrophylla*. They also reported that I gave an *O*-acetyl derivative with acetic anhydride-pyridine.[4] Inasmuch as our alkaloid failed to give such a derivative and gave no absorption in the IR hydroxyl region some confusion as to the identity of the two existed This has now been resolved by the observation that I, when recrystallized from methanol, retains one methanol of crystallization very tenaciously whereas from acetone, the solvent-free base is obtained *

The second dimeric indole alkaloid was originally designated alkaloid *A* and we now propose the name alstonisidine (II) It possesses the empirical formula $C_{43}H_{52}N_4O_4$†, contains 4 methoxyl groups, and forms a dihydrochloride



Alstonisine (III)



Alstonerine (IV)

The structure of one of the monomeric indole alkaloids, originally designated alkaloid *C* and for which we now propose the name alstonisine (III), has been determined by X-ray analysis by Nordman and Nakatsu[6] who also revised the empirical formula to $C_{20}H_{22}N_2O_3$. The alkaloid is an oxindole derivative, forms a monohydrochloride, and contains no methoxyl groups.

Finally, the structure of the other monomeric indole alkaloid, originally designated alkaloid *D* and renamed alstonerine,[7] has been deduced on the basis of mass spectral and NMR data. It has the corrected empirical formula $C_{21}H_{24}N_2O_2$, contains no methoxyl groups and two NCH_3 groups.

All of the above alkaloids were chromatographically pure (paper and TLC) [8] In addition to those reported here, there are some 18 other alkaloids in the alcoholic extract of the bark as judged by paper chromatography Of these the known alstonine and tetrahydroalstonine were identified on the basis of R_f values Work is continuing on the isolation and characterization of those remaining

EXPERIMENTAL‡

Extraction of the tree bark of Alstonia muelleriana § A thick slurry of the finely ground bark in ligroin (70–90) was allowed to stand for 24 hr and pressed dry in a fruit press This extraction was repeated 5 times.

* Mrs Chatterjee has informed us that her acetyl derivative was indeed an artifact

† This was originally assigned the formula $C_{21}H_{24-26}N_2O_2$ At the time of its isolation no reliable molecular weight data could be obtained Dr. P W LeQuesne of these laboratories has now found on the basis of mass spectrographic data that the alkaloid probably is a double molecule

‡ All m p's are uncorrected IR spectra were taken as Mujol mulls Microanalyses were by Spang Microanalytical Laboratory, Ann Arbor, Mich or by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

§ Letters refer to fractions in Fig 1

[4] A Chatterjee, S K Talapatra and N Adityachaudhury, *Chem & Ind* 667 (1961)
[5] A Chatterjee and G Ganguli, *J Sci Res* (India) **23**, 178 (1964)
[6] C E. Nordman and K Nakatsu, *J Am Chem Soc* **85**, 353 (1963).
[7] J M Cook, P. W LeQuesne and R C Elderfield, *Chem Commun.* 1306 (1969)
[8] P. W. LeQuesne, Personal communication

Aside from β-sitosterol no other constituents were isolated from the ligroin extract (A) The air-dried bark was slurried 5 times (24 hr) with 70% EtOH and pressed dry after each extraction The combined EtOH extracts (B) were concentrated* to *ca* 20% the original volume The aqueous concentrate was decanted from the dark brown tarry residue (Bl) which was extracted thoroughly with water and discarded The combined aq extracts (B) were adjusted to pH 10 and extracted 4× with *n*-BuOH The aqueous layer (D) was discarded and the *n*-BuOH extracts (C) were extracted once with 3% HCl and twice with H_2O This series of extractions was repeated 7 times The extracted BuOH (E) was put aside for later examination The combined acid and H_2O extracts (F) were adjusted to pH 5 with dil NaOH and concentrated to *ca* 20% of the volume After bringing the pH to 10 with NaOH, it was extracted 4× with *n*-BuOH and discarded The combined BuOH extracts (G) were washed (H_2O) and taken to dryness (H)

Isolation of villalstonine (I) (a) *By chromatography* The extracted *n*-BuOH (E) was washed (dil NH_4OH and H_2O) and concentrated to dryness The dark syrupy residue (J) was extracted with hot benzene The benzene insoluble material (L) was discarded After removal of the benzene from the extracts 10 g of the residue (K) in 50 ml of benzene was chromatogrammed over 200 g of alumina † The column was eluted with benzene followed by benzene–$CHCl_3$ (1 1) giving three main fractions all of which gave I on recrystallization from acetone Analyses and physical constants (m p and $[\alpha]_D$) of I and its hydrochloride, oxalate and methiodide agreed with those previously published [1,3 4]

(b) *By continuous extraction with petroleum* The residue from the above dried benzene extract (K) was extracted (Soxhlet) with light petroleum (30–60°) for 36 hr Concentration of the extract and crystallization of the residue from acetone gave I

Isolation of alstonisidine (II), I, *and alstonisine* (III) The aqueous acid extracts (F) were concentrated, adjusted to pH 10 with 10% NaOH and extracted 5× with MeOH–Et_2O (1 10) Concentration of the combined extracts left a residue which was chromatogrammed in 10 g portions in benzene over alumina Elution was by benzene followed by benzene–$CHCl_3$ (5, 10, 20, 40% $CHCl_3$) and finally $CHCl_3$ A light blue fluorescent purple band came off first and gave I from acetone A light yellow fluorescent band followed which gave a solid refractory to crystallization It was dissolved in 30 ml MeOH and acidified to Congo red with HCl On refrigeration the hydrochloride of III separated as small crystals, m p 250–260° (dec *in vacuo*), $[\alpha]_D^{25}$ +155° (*c* = 1 0 in H_2O) The IR spectrum showed strong bands at 1690, 1680, 1620, 1610, 1590 and 1560 cm^{-1} (Calc for $C_{20}H_{22}N_2O_3$ HCl C, 64 05, H, 6 19, N, 7 47, Cl, 9 65 Found C, 63 27, H, 5 78, N, 7 25, Cl, 9 65%)

An aq solution of the hydrochloride was made basic with Na_2CO_3 and extracted with Et_2O giving III, m p 168–169°, $[\alpha]_D^{25}$ +200° (*c* = 1 0 in EtOH), after recrystallization from dil MeOH The IR spectrum showed bands at 1690s, doublets at 1615 and 1905, and 1645 m cm^{-1} The UV spectrum was characteristic of an oxindole with λ_{max} at 254 and λ_{min} at 231 nm (Calc for $C_{20}H_{22}N_2O_3$ C, 71 40, H, 5 99, N, 8 33, OCH_3, 0 00 Found C, 71 31, H, 5 96, N, 8 62, OCH_3, 0 00%)

Oxalate of III Colorless needles, m p 209–211° (dec *in vacuo*), $[\alpha]_D^{25}$ +119° (*c* = 1 0 in H_2O), from EtOH (Calc for $C_{20}H_{22}N_2O_3$ $C_2H_2O_4$ C, 61 64, H, 5 67, N, 6 54 Found C, 61 11, H, 5 78, N, 6 40%)

Hydrochloride of II Et_2O was added to the MeOH filtrate from the first crop of III hydrochloride obtained above to a slight turbidity Refrigeration of this solution for 4 days gave crystalline material which was not identical with III hydrochloride on the basis of IR Crystallization from MeOH gave the hydrochloride of II, m p 268° (dec *in vacuo*), $[\alpha]_D^{25}$ +137° (*c* = 0 6 in H_2O) The IR spectrum showed bands at 1725s and 1610m cm^{-1} (Calc for $C_{43}H_{52}N_4O_4$ 2HCl C, 67 69, H, 7 25, N, 7 35, Cl, 9 31 Found C, 67 27, H, 7 01, N, 7 24, Cl, 9 22%)

Alstonisidine (II) Liberation of the alkaloid from the hydrochloride gave II as small colorless rods, m p 322–325° (dec *in vacuo*), $[\alpha]_D^{25}$ −234° (*c* = 1 0 in EtOH), from dil MeOH The IR spectrum showed a strong CO band at 1730 cm^{-1} and the UV spectrum showed λ_{max} at 230, 286 and 294, λ_{min} at 265, and a shoulder at 252 nm (Calc for $C_{43}H_{52}N_4O_4$ C, 74 94, H, 7 60, N, 8 14, 4 OCH_3, 18 02 Found C, 75 07, H, 7 19, N, 8 50, 4 OCH_3, 18 9%)

Isolation of II, III *and Alstonerine* (IV) The dark tarry residue (H) was triturated with petroleum (30–60°) and filtered The insoluble residue was chromatogrammed in 30 g portions over alumina (300 g) in benzene in which about one half was soluble Elution was with benzene–$CHCl_3$ (9 1) and 15 one liter fractions were collected The first 4 l contained mostly I and IV on the basis of IR The next 2 l contained mainly II and III with traces of I and IV The next 7 l contained II and III A solution of the material (7 g) contained in the last 7 l in 25 ml of benzene deposited 380 mg of II on standing

Separation of II and III from the appropriate fractions was readily accomplished by a 9 plate modifica-

* This and all subsequent concentrations were done at aspirator vacuum under N_2 at 40–50°

† In this and subsequent chromatograms Merck alumina (pH of an aq slurry 10) was used Progress of the chromatogram was followed by observing the movement of fluorescent bands when the column was illuminated with UV light

tion of the Craig counter current distribution method*[9] using 9 125 ml separatory funnels and a system of benzene and citrate buffer (pH 3)

Separation of I *and* IV A solution of the residue from the first 4 l of the above chromatogram in benzene–petroleum (1 1) was chromatogrammed on 75 g of alumina and seventeen 50-ml fractons were collected Elution was with benzene–petroleum (1 1) through Fraction 15 Benzene and $CHCl_3$ were the eluents for Fractions 16 and 17 After concentration the fractions were examined by IR Fractions 1 and 2 were mostly I, Fractions 3–5 were a mixture of I and IV, Fractions 6–15 contained a much higher percentage of IV and the percentage of I increased again in Fraction 16 All attempts to crystallize the residues from Fractions 6–16 were unsuccessful and the combined residues (750 mg) were subjected to the Craig counter current distribution scheme[9] with benzene–pH 3 citrate buffer The fractions from the distribution which contained only IV gave pure IV (170 mg) as colorless crystals, m p 172–173°, $[\alpha]_D^{25}$ −195° (c = 0 6 in EtOH), from Et_2O The IR spectrum showed bands at 1620s and 1650s cm^{-1} The UV spectrum showed λ_{max} at 231 and 260, λ_{min} at 245, and shoulders at 285 and 293 nm (Calc for $C_{21}H_{24}N_2O_2$ C, 74 96, H, 7 20, N, 8 33, 2 NCH_3, 8 9 Found C, 74 70, H, 6 99, N, 8 82, 2 NCH_3, 8 5%)

* Details can be found in Gilman's Ph D dissertation, which is available from Xerox, University Microfilms Division, Ann Arbor, Mich. 48106

[9] L C Craig, C Golumbic, H Mighton and E O Titus, *J Biol Chem* **161,** 321 (1945)

Key Word Index—Alstonia muelleriana, Apocynaceae, indole alkaloids, villalstonine, alstonisidine, alstonisine, alstonerine