Phytochemistry, 1966, Vol. 5, pp. 977 to 983. Pergamon Press Ltd. Printed in England

AN EXAMINATION OF *RAUWOLFIA VERTICILLATA* OF HONG KONG—II*

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(*Received* 19 *January* 1966)

Abstract—A complete examination of the bark, roots, leaves and wood of *Rauwolfia verticillata* of Hong Kong led to the isolation of twenty-two compounds, several of which are new.

IN A complete examination of the bark, root, leaves and wood of *Rauwolfia verticillata* of Hong Kong twenty-two different compounds have been shown to be present:

(*a*) *Bark*: β-amyrenyl acetate, Quercus alcohol A1, β-sitosterol, Triterpenoid RB-17, Triterpenoid RB-7, δ-yohimbine, reserpine, Triterpenoid RB-16, Alkaloid RB-19, yohimbine, serpentine, ajmaline, Alkaloid RB-20, Alkaloid RB-35. (*b*) *Root*: Quercus alcohol A1, β-sitosterol, δ-yohimbine, Alkaloid RR-5, Triterpenoid RB-7, Quercus ketone K1, reserpine, Compound RR-31, δ-yohimbine, Alkaloid RR-22, Triterpenoid RR-28, ajmaline, yohimbine. (*c*) *Leaves*: β-amyrenyl acetate, Quercus alcohol A1, β-sitosterol, Alkaloid RB-19, Alkaloid RB-20, serpentine, the flavonoid glycoside robinin. (*d*) *Wood:* β-sitosterol, Steroid RW-11, Alkaloid RB-19, yohimbine, Alkaloid RW-47, Alkaloid RB-20, ajmaline.

Alkaloids RB-19, RB-20, RR-5, and RW-47 on comparison with samples in the collection of Lilly & Co, Indianapolis, by Dr. N. Neuss, whom we thank, appear to be new. Triterpenoids RB-17, RB-7, RB-16 and RR-28, have not as yet been identified and may be new. New work on robinin reported by Chiang *et al.*[1] in the Mainland species will be described in Part III and the mixtures of quaternary bases isolated as the Reineckates (RB-18), and as precipitates with Mayer's Reagent (RB-38) in Part IV. Further work is contemplated on Steroid RW-11 which appears to be new among those from plant and fungal origin.

The new Alkaloids RB-19 and RB-20 occur in the bark, leaves, and wood but apparently not in the root, which, however, contains Alkaloid RR-5 in very low yield. Alkaloid RW-47 occurs only in the wood. Commonly-known alkaloids were present in various plant parts. The Triterpenoids RB-17, RB-7, RB-16, and RR-28 are under investigation.

Except for Steroid RW-11 which appears to be an α,β-unsaturated ketone and not to have been described among those of plant and fungi origin, and a higher fatty alcohol and ketone first reported in Hong Kong Quercus species[2] the other compounds isolated are common.

R. verticillata has been investigated before by one of us (H. R. A.).[3] In 1958 Yamaguchi and Shoji[4] found 0·736 per cent total alkaloids (on dry wt.) in *R. chinensis* (*R. verticillata*)

* δ-Yohimbine from the bark of *Rauwolfia verticillata*, H. R. ARTHUR, *Chem. & Ind.* (*London*) **85** (1956) is regarded as Part I of this series.

[1] T. C. CHIANG, L. HUANG, S. F. CHEN and T. N. SHANG, *Yao Hsueh Hsueh Pao* **10**, 614 (1963).
[2] H. R. ARTHUR, K. F. CHENG, M. P. LAU and K. J. LIE, *Phytochem.* **4**, 969 (1965).
[3] H. R. ARTHUR, *Chem. & Ind.* (*London*) 85 (1956).
[4] K. YAMAGUCHI and H. SHOJI, *Eisei Shikenjo Hokoku* **76**, 99 (1958).

of which 1·55 per cent was reserpine. They also detected the presence of ajmaline and ajmalicine (δ-yohimbine). In the same year Liu, Lo and Shi[5] isolated an alkaloid, samatine, from the roots. This resembled rauwolfine to some extent. The authors concluded that samatine probably possessed a carbon skeleton similar to that of δ-yohimbine.

EXPERIMENTAL

Microanalyses were carried out by the Microanalytical Laboratories of the Universities of Melbourne and Singapore. Specific rotations were measured in chloroform, except where otherwise stated; i.r. spectra on a P-E model 137 Infracord spectrophotometer. Melting points of the alkaloids were taken on a Kofler block. The alumina used was B.D.H. preparative grade and was acid-washed (5 ml 20% acetic acid per 100 g alumina). Light petroleum refers to the fraction, b.p. 60–80°. All known samples were compared in m.p., mixed m.p. and i.r. spectra and sometimes by paper chromatography with authentic samples and were found identical.

Extraction of R. verticillata. (*a*) *Bark*

Air-dried bark (6·8 kg) was milled then extracted with light petroleum at room temperature for 6 days then continuously with methanol (Soxhlet) for 3 days. (i) The petroleum extract was concentrated to 1 l. then applied to a chromatographic column (300 g of adsorbent). Elution with light petroleum gave β-amyrenyl acetate which crystallized from light petroleum as colourless plates $[\alpha]_D$ +82·7 (*c* = 0·76) (Found: C, 81·5; H, 11·0. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%); Quercus alcohol A1 eluted with 10% C_6H_6 in petroleum crystallized from light petroleum in the form of "cotton wool" (Found: C, 82·9; H, 14·3%); β-sitosterol eluted with C_6H_6 crystallized from EtOH in long colourless needles. (Found: C, 83·3; H, 12·4. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·2%.) *Triterpenoid* RB-17 eluted with 30% MeOH in $CHCl_3$ crystallized from MeOH as a colourless micro-crystalline compound, m.p. 288–290°, $[\alpha]_D$ +128 (*c* = 0·39) (Found: C, 76·1; H, 10·6. Calc. for $C_{30}H_{50}O_4$: C, 75·9; H, 10·6%). (ii) The methanol extract was concentrated under reduced pressure until all solvent was removed. The residue was mixed with an equal volume of 5% acetic acid. The turbid suspension was extracted with *n*-hexane and the remaining acid solution was cooled to 5° and adjusted to pH 10 with solid sodium carbonate. The brown amorphous precipitate which formed was collected and the filtrate was extracted with chloroform until negative when tested with Mayer's reagent. The precipitate was combined with the concentrated chloroform extract and the resulting mixture (X), and was treated as shown in Fig. 1. The alkaline solution was acidified with conc. HCl to give solution G (and in the case of the leaves *only*, precipitate H).

n-*Hexane extract* (from methanolic extract; see above). This was concentrated to dryness. The residue (20 g) was taken up in light petroleum and, after removal of tar by filtration, the solution was applied to a column of adsorbent (250 g). Elution with 10% benzene in petroleum yielded β-sitosterol (3·0 g), m.p. 135–139°, as obtained in (*a*)i. Elution with 10% $CHCl_3$ in C_6H_6 yielded *Triterpenoid* RB-17 (0·1 g), m.p. 288–90°, as obtained in (*a*)i. Elution with 10% MeOH in $CHCl_3$ gave the *Triterpenoid* RB-16, which on crystallization from MeOH deposited as long colourless needles (0·2 g), m.p. 286–290°, $[\alpha]_D$ +128° (*c* = 0·23 in 1:1 $CHCl_3$–MeOH) (Found: C, 72·6; H, 10·6. Calc. for $C_{30}H_{50}O_5$: C, 73·4; H, 10·3%). Elution with $CHCl_3$ gave the *Triterpenoid* RB-7 (0·1 g), m.p. 283–286°, $[\alpha]_D$ +46·9 (*c* = 0·47)

[5] C. C. LIU, J. Y. LO and L. L. SHI, *K'o Hsueh T'ung Pao* **2**, 52 (1958).

obtained as colourless needles after crystallization from methanol. (Found: C, 78·0; H, 11·2. Calc. for $C_{30}H_{50}O_3$: C, 78·5; H, 11·0%.)

Residue A (Fig. 1). This residue (20·1 g) was triturated with ether (which removed tars) and the green residue (10·0 g) was dissolved in hot C_6H_6 (200 ml) and applied to a column of acid-washed alumina (250 g). Elution with benzene gave δ-yohimbine (1·0 g) which crystallized from MeOH as needles, m.p. 254–255°, $[\alpha]_D = -63{\cdot}4°$ ($c = 1{\cdot}53$). (Found: C, 71·4; H, 7·0; N, 9·1. Calc. for $C_{21}H_{24}O_3N_2$: C, 71·6; H, 6·9; N, 8·0%.) (See Table 1.) Elution with 10% $CHCl_3$ in C_6H_6 yielded reserpine (0·3 g) which crystallized from MeOH as needles, m.p. 255–256°, $[\alpha]_D$ −120·5° ($c = 1{\cdot}42$). (Found: C, 65·0; H, 6·7; N, 5·0. Calc. for

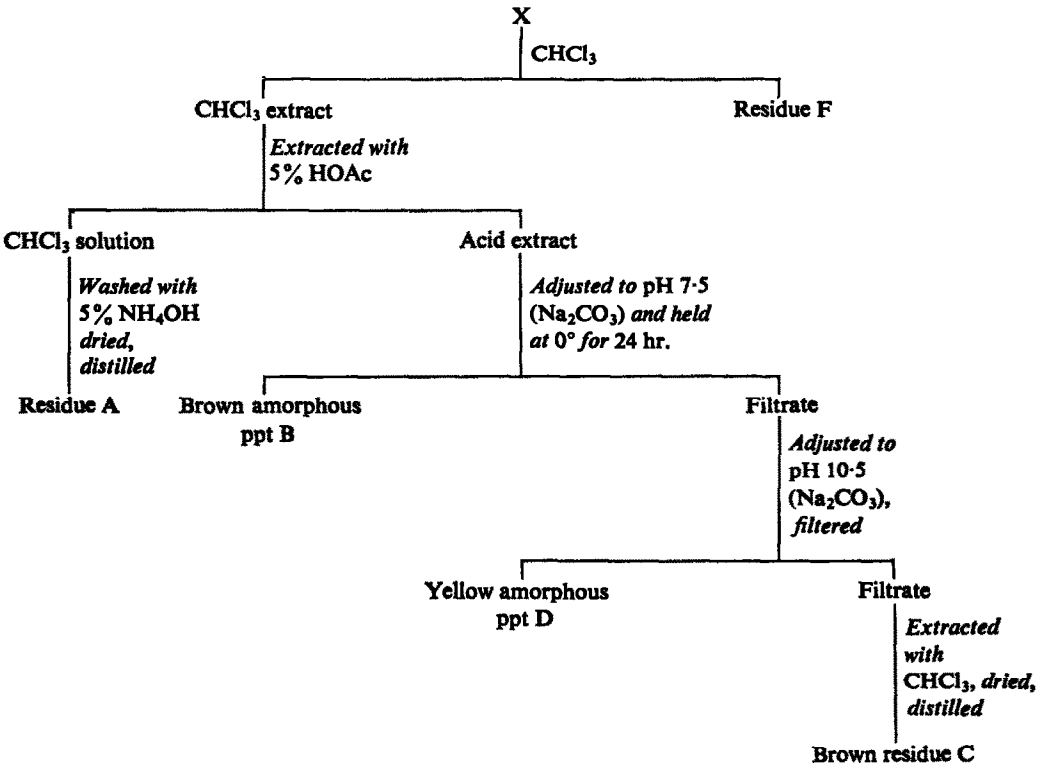

FIG. 1.

$C_{33}H_{40}O_9N_2$: C, 65·1; H, 6·6; N, 4·6%.) Elution with 50% $CHCl_3$ in C_6H_6 yielded more reserpine (0·1 g). Elution with 30% MeOH in $CHCl_3$ yielded the Triterpenoid alcohol RB-16 (0·1 g) which crystallized from EtOH as fine needles, m.p. 285–289°, identical with that from the *n*-hexane extract.

Fraction B (Fig. 1). This brown precipitate (50 g) was exhaustively extracted with hot benzene. The residue from the concentrated extract was dissolved in C_6H_6:$CHCl_3$ (1:1) (500 ml) and applied to a column of adsorbent (200 g). Elution with C_6H_6 gave δ-yohimbine (0·3 g) which on crystallization from MeOH had m.p. 254–255°. Elution with 20% $CHCl_3$ in C_6H_6 yielded *Alkaloid* RB-19 (0·2 g), which on crystallization from $CHCl_3$–C_6H_6 had m.p. 285–288°, $[\alpha]_D$ +175·7 ($c = 0{\cdot}23$). (Found: C, 77·9; H, 7·3; N, 10·1. Calc. for $C_{26}H_{29}ON_3$:

C, 78·2; H, 7·3; N, 10·5%.) Yohimbine was also shown to be present in fraction B (see Table 1).

Fraction C (Fig. 1). A little of this yellow residue (5·0 g) was subjected to paper chromatography and the presence of serpentine, yohimbine and ajmaline was proven by comparison with authentic samples. (See Table 1.) Column chromatography of fraction C was carried out as for fraction B. Elution with 70% $CHCl_3$ in C_6H_6 yielded *Alkaloid* RB-20 (0·2 g), m.p. 185–188°, $[\alpha]_D$ +34·5 (c=0·59) obtained as nearly colourless fine needles on crystallization from $CHCl_3$–MeOH. (Found: C, 64·5; H, 6·8; N, 8·0. Calc. for $C_{21}H_{28}O_5N_2$: C, 64·9; H, 7·2; N, 7·2%). Elution with $CHCl_3$ yielded a product which on crystallization from C_6H_6 gave the yellow *Alkaloid* RB-35 (0·5 g), m.p. 145–149°. (Found: C, 66·3; H, 7·6; N, 7·5. Calc. for $C_{23}H_{32}O_5N_2$: C, 66·3; H, 7·7; N, 6·7%.)

Fraction D (Fig. 1). A portion (10·0 g) of this yellow residue (50 g) was column chromatographed (100 g of adsorbent) as stated for fraction B. Elution with C_6H_6 gave Alkaloid RB-19 (0·5 g), m.p. 285–288°, identical with that from fraction B. Elution with $CHCl_3$

TABLE 1. R_f VALUES FOR ALKALOIDS

	Systems					
	a		*b*		*c*	
Alkaloid	Found	Lit.[6]	Found*	Lit.	Found	Lit.
δ-Yohimbine	0·17 (0·17)	0·18	0·18 (0·2)	0·67	0·91 (0·85)	0·9
Serpentine	0·17 (0·17)	0·13	0·0 (0·0)	0·0	0·0 (0·0)	0·0
Yohimbine	0·42 (0·47)	0·45	0·15 (0·15)	0·03	0·036 (0·031)	0·40
Ajmaline	0·65 (0·65)	0·65	0·0 (0·0)	0·0	0·10 (0·10)	0·15

System (*a*)—H_2O (developer) in H_2O/HOAc atmosphere: Whatman No. 1. System (*b*)—C_6H_6–C_6H_{12} (1:1) on formamide impregnated[7] Whatman No. 1. System (*c*)—C_6H_6–$CHCl_3$ (1·1) on formamide impregnated Whatman No. 1.

Values in brackets refer to "found" R_f values of authentic specimens. The values given for various fractions are typical of all.

* Descending technique.

yielded a product which on crystallization from $CHCl_3$–MeOH gave Alkaloid RB-20 (0·1 g), m.p. 185–188°, as obtained from fraction C. Yohimbine was shown to be present by paper chromatography (see Table 1).

Solution G (from methanolic extract). To this an acid solution of ammonium Reineckate was added until precipitation was complete. The dried precipitate, RB-18 (20·1 g) was pink in colour. To the filtrate Mayer's solution was added. The dried precipitate RB-38 (5·0 g) was white. RB-18 and RB-38 are under investigation.

(*b*) *Root*

Air-dried root (17·9 kg) was extracted as for the bark.

(i) The petroleum extract was chromatographed as for the bark. The compounds obtained with various eluents are shown in Table 2.

[6] F. A. HOCHSTEIN, K. MURAI and W. H. BOEGEMANN, *J. Am. Chem. Soc.* **77**, 3551 (1955).

[7] A. ZAFFARONI, R. B. BURTON and L. H. KENTMANN, *Science* **111**, 6 (1950). O. SCHLINDLER and T. REICHSTEIN, *Helv. Chim. Acta* **34**, 108 (1951).

Alkaloid RR-5 separated in almost colourless needles (0·005 g) from methanol and had m.p. 209–212° and was shown to differ from known alkaloids.

(ii) The methanol extract was treated as for bark (see Fig. 1).

n-*Hexane extract.* The residue (5·0 g) from this was chromatographed as stated for the bark. Elution with 50% C_6H_6 in petroleum yielded β-sitosterol (4·0 g), m.p. 136–138°.

Residue A. This dark brown residue (25·0 g) was triturated with ether. The dry ether-insoluble residue was dissolved in warm absolute ethanol (tar neglected) and the solution was kept at 0° overnight. The yellow amorphous precipitate A-1 (5·0 g) which formed was collected. The ethanol filtrate was combined with the ether extract; concentration of the mixture gave a yellow precipitate A-2 (6·0 g).

Precipitate A-1 was treated as for Fraction B (bark). The residue from the C_6H_6 extract was chromatographed (adsorbent, 170 g). Elution with C_6H_6 yielded solids which were neglected. Elution with 30% $CHCl_3$ in C_6H_6 gave reserpine (0·1 g), m.p. 264–265°. Elution with 1% MeOH in $CHCl_3$ yielded a solid which on attempted crystallization from C_6H_6 gave *Compound* RR-31 (0·5 g), m.p. 98–100°, as a yellow amorphous powder which is under investigation.

TABLE 2. COMPOUNDS FROM THE PETROLEUM EXTRACT OF ROOT

Eluent	Yield (g)	m.p. (°C)	Identification
5% C_6H_6 in petroleum	0·05	84	Quercus alcohol A1[2]
C_6H_6	3·0	136–138	β-Sitosterol
C_6H_6	0·20	253–255	δ-Yohimbine
30% $CHCl_3$ in C_6H_6	0·005	209–212	*Alkaloid RR*-5
30% MeOH in $CHCl_3$	0·50	283–286	Unidentified Triterpene RB-7
50% MeOH in $CHCl_3$	0·10	75	Quercus ketone K1[2]

* Identified as shown in (*a*)(i) and (*a*)(ii).

Precipitate A-2 was treated as for A-1 (adsorbent, 200 g). Elution with benzene gave δ-yohimbine (2·0 g), m.p. 253–255°, then a solid which on crystallization from MeOH yielded *Alkaloid* RR-22 (0·1 g) as fine colourless needles, m.p. 132–137°, followed by a further amount (0·2 g) by elution with 5% $CHCl_3$ in C_6H_6. Further elution with 5% and with 10% $CHCl_3$ in C_6H_6 gave similar mixtures of δ-yohimbine (2·8 g), m.p. 253–255°, and reserpine (2·6 g), m.p. 264–265°. Elution with 30% MeOH in $CHCl_3$ yielded *Triterpenoid* RR-28 (0·5 g) which crystallized in colourless needles, m.p. 264–267°, from MeOH. It had $[\alpha]_D$ +9·1 (c=1·02 in pyridine) and gave a red→purple→brown colour in the Liebermann–Burchard test. (Found: C, 71·8; H, 10·7. Calc. for $C_{30}H_{50}O_4$. $2CH_3OH$: C, 71·4; H, 10·8%.)

Fraction B. This brown residue (20·0 g) was treated as for that from the bark (adsorbent used, 170 g). Elution with C_6H_6 yielded mixtures of δ-yohimbine (2·0 g), m.p. 250–251°, and reserpine (0·5 g), m.p. 260–263°. Further elution with C_6H_6 gave a colourless solid which on crystallization from $CHCl_3$–C_6H_6 yielded ajmaline (0·005 g), m.p. 155–157°.

Fraction C. This black residue (2·1 g) was chromatographed as for fraction B (adsorbent, 15·0 g). Elution with C_6H_6 gave yohimbine (0·1 g), m.p. 239–240°. (See also Table 1.)

Fraction D. This residue (4·0 g) yielded no identifiable compound.

(*c*) *Leaves*

Air dried leaves (1·8 kg) were extracted as stated for the bark.

(i) The petroleum extract was treated and then chromatographed on 300 g adsorbent as stated for the bark. Elution with petroleum gave β-amyrenyl acetate (0·2 g), m.p. 237–240°. Elution with 10% C_6H_6 in petroleum gave Quercus alcohol A1 (0·1 g), m.p. 82°. Elution with 50% C_6H_6 in petroleum gave β-sitosterol (1·0 g), m.p. 136–138°.

(ii) The methanol extract was treated as for that of the bark (see Fig. 1).

n-*Hexane extract*. Yielded no crystalline compounds.

Residue A. The green residue (2·0 g) yielded no crystalline compounds.

Fraction B. Half (10·0 g) of this brown fraction was treated then chromatographed as stated for the bark. Elution with C_6H_6 gave Alkaloid RB-19 (0·001 g), m.p. 286–288°. Elution with 70% $CHCl_3$ in C_6H_6 gave Alkaloid RB-20 (0·5 g), m.p. 185–188°.

Fraction C. As much as possible of this red residue (10·1 g) was taken into C_6H_6:$CHCl_3$ and the solution was chromatographed (adsorbent, 100 g). Elution with 70% $CHCl_3$ in C_6H_6 gave Alkaloid RB-20 (0·5 g), m.p. 185–188°. Paper chromatography of fraction C showed the presence of serpentine (see Table 1).

Fraction D. Half of this yellow residue (20·1 g) was treated as for fraction C (above). Elution with 50% $CHCl_3$ in C_6H_6 gave Alkaloid RB-20 (0·5 g), m.p. 186–187°.

Precipitate H. Crystallization of H from methanol yielded fine yellow needles (3·0 g) of robinin, m.p. 197–199°. (Found: C, 53·8; H, 5·9; OMe, 0·0. Calc. for $C_{33}H_{40}O_{19}$: C, 53·5; H, 5·4%.)

(*d*) *Wood*

The dry milled wood (10·9 kg) was extracted as stated for the bark.

(i) The petroleum extract was treated then chromatographed (250 g adsorbent). Elution with C_6H_6:petroleum gave very low yields of compounds which were not identified. However, the last compound eluted with petroleum, when crystallized from EtOH, deposited elongated plates (0·1 g) of *Steroid* RW-11, m.p. 96–98°. (Found: C, 84·4; H, 11·8. Calc. for $C_{29}H_{48}O$: C, 84·4; H, 11·7. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·2%.) This had λ_{max} 245 (EtOH), ϵ, 12,400 based on M = 400 which suggests that the compound is a conjugated steroid ketone some confirmation of which was obtained in the i.r. spectrum which showed ν_{max} (CCl_4) 1670 cm^{-1}. In the Liebermann–Burchard reaction Steroid RW-11 gave a yellow colour changing through orange to red and with tetranitromethane a pale yellow colour. Elution with benzene gave β-sitosterol (3·1 g).

(ii) The methanol extract was treated as for that of the bark (see Fig. 1).

n-*Hexane extract*. This yielded no crystalline substances.

Fraction A. This fraction (3·5 g) was treated as stated for the bark. No crystalline compounds were obtained.

Fraction B. 20·0 g of this fraction (100 g) was treated as for Fraction B of the leaves (chromatography 100 g adsorbent). Elution with 30% C_6H_6 in $CHCl_3$ gave Alkaloid RB-19 (0·01 g), m.p. 285–288°. Yohimbine was shown to be present in fraction B by paper chromatography (see Table 1).

Fraction C. The brown residue (3·0 g) was dissolved in 30% $CHCl_3$-C_6H_6 and the solution was chromatographed (adsorbent, 100 g). Elution with C_6H_6 gave a product which on crystallization from benzene yielded colourless plates (1·0 g) of *Alkaloid* RW-47, m.p. 130–132°, $[\alpha]_D$ +27·3 (c = 0·83). (Found: C, 72·2; H, 7·5; N, 9·3%, M, 149 (mass spec.). Calc. for $C_9H_{11}ON$: C, 72·4; H, 7·4; N, 9·3%, M, 149.) Elution with 30% $CHCl_3$ in

C_6H_6 gave Alkaloid RB-20 (0·5 g), m.p. 185–188°. Fraction C also contained ajmaline as shown by paper chromatography (see Table 1).

Fraction D. 20 g of this brown residue (100 g) was treated as for Fraction B then chromatographed (adsorbent 100 g). Elution with 30% $CHCl_3$ in C_6H_6 gave Alkaloid RB-19 (0·05 g), m.p. 285–288°. Elution with 70% $CHCl_3$ in C_6H_6 gave Alkaloid RB-20 (0·20 g), m.p. 185–188°.

Acknowledgements—The authors thank Mr. H. C. Tang, Government Herbarium, Hong Kong, for identification of plant material and the Tropical Products Institute, Ministry of Overseas Development, and the Research Grants Committee of the University of Hong Kong for grants-in-aid.