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SHORT COMMUNICATION

MINOR ALKALOIDS OF *BLEEKERIA VITIENSIS**

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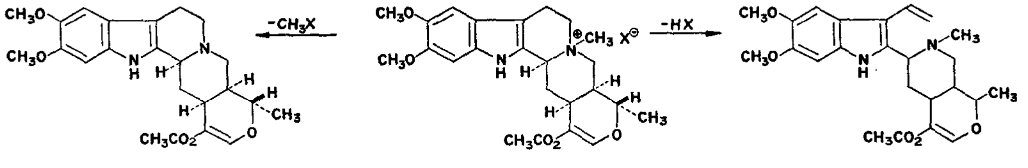
Key Word Index—*Bleekeria vitiensis*; Apocynaceae; indole alkaloids; 9-methoxyellipticine; holeinine; bleekerine; sterols.

Abstract—The chemical constituents of the stem-bark and wood of *Bleekeria vitiensis* are compared and the structure of a new alkaloid, bleekerine, is described.

In a previous paper[1] we showed that the combined stem-bark and wood of the Fijian plant *Bleekeria vitiensis* (Apocynaceae) A. C. Smith is the best natural source of the alkaloid 9-methoxyellipticine (0·083% based on dry plant material) yet reported. This compound has useful antitumour activity and, in order to confirm our results, we have examined a second specimen of the plant but this time the stem-bark and the wood were examined separately. The results are as follows: 9-methoxyellipticine is present in the bark and in the wood, although much more abundant in the former (0·165% vs. 0·006%). Ellipticine, isoreserpiline and isoreserpiline-ψ-indoxyl are also present in both parts of the plant and these alkaloids are accompanied by small amounts of sitosterol, stigmasterol and campesterol.

In addition, the bark yields the alkaloid holeinine (I), present as the mixed chloride–bromide. This alkaloid, previously isolated by Scheuer[2] from the related plant *Ochrosia sandwicensis*, was not detected in the first specimen of *Bleekeria vitiensis*.[1] On pyrolysis, holeinine forms isoreserpiline (II);[2] we have observed that this reaction is replicated in the mass spectrometer, giving rise to the spectra of isoreserpiline and the appropriate methyl halide. Moreover, a Hofmann-type elimination also occurs, affording the molecular species (III) or its equivalent, plus hydrogen chloride and hydrogen bromide. The presence of a molecular ion m/e 440·2310 ($C_{25}H_{32}N_2O_5$) is observed in the mass spectrum of holeinine and it is significant that the same ion is also present in the mass spectrum of a sample of



(II) (I) (III)

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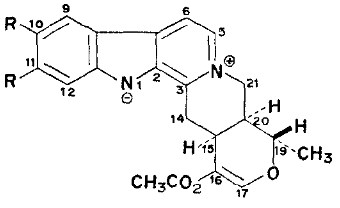
* Part II in the series "Extractives of the Ochrosiinae". For Part I see Ref. 1.

[1] K. N. Kilminster, M. Sainsbury and B. Webb, *Phytochem.* **11**, 389 (1972).

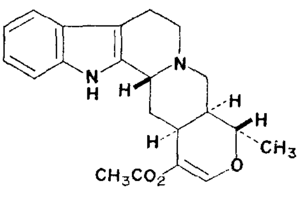
[2] P. J. Scheuer and J. T. Hamamoto Metzger, *J. Org. Chem.* **26**, 3069 (1961).

holeinine obtained from *O. sandwicensis*. It is possible that the origin of this molecular ion is the, as yet unreported, alkaloid salt N(a)-methylholeinine, but because of the extremely low concentration in the plant it has not been possible to confirm this.

Another alkaloid, bleekerine $C_{23}H_{24}N_2O_5$ m.p. 276–277° (EtOH) $[\alpha]^{22\cdot5}_{546\,nm} + 612$ (MeOH), is found in the stem-bark of *Bleekeria vitiensis*; this molecule has not been described previously. The UV spectrum λ^{EtOH}_{max} (ϵ) 208 (14 000), 242 (14 900), 276 (9850), 336 (12 150), and 394 (5600) nm suggests a highly conjugated structure and bands at 1693, 1618, 1225, 1210 and 1185 cm^{-1} in the IR spectrum (KBr) demonstrate the presence of a β-alkoxyacrylic ester unit, similar to that of isoreserpiline.[3] Bleekerine does not contain a > NH function, although a band at 1630 cm^{-1} is indicative of a $>C{=}N^+{=}$group. The molecular ion *m/e* 408 is the base peak of the mass spectrum which, apart from an $(M\text{-}15)^+$ ion (79·5%), shows little fragmentation and confirms the conjugated nature of the molecule. This mass spectrum is closely similar to that of alstonine (IV, R=H), *m/e* 348 (100%) (M^+), 333 (41·8%) $(M\text{-}15)^+$, and since on reduction, with $NaBH_4$ in methanol, bleekerine yields isoreserpiline, the structure of this new alkaloid must correspond to 10,11-dimethoxy-alstonine (IV, R=OCH_3). The NMR spectrum (CF_3CO_2H), which substantiates this structural allocation, is summarized in Table 1.



(IV)



(V)

TABLE 1. SUMMARY OF THE NMR OF BLEEKERINE

δ	Multiplicity	No. of protons	Assignment
1·56	d (J = 6·0 Hz)	3	$=CH\text{-}CH_3$
2·4–4·6	m	6	aliphatic protons
3·93	s	3	$-CO_2CH_3$ and $2\,x\text{-}OCH_3$
4·12	s	6	
4·92	double d (J = 14·0, 7·3 Hz)	1	$C_{21}\text{-}H$ (α)*
7·30	s	1	$C\text{-}H_9$, $C\text{-}H_{12}$, $C\text{-}H_{17}$
7·70	s	1	
7·95	s	1	
8·20	s	2	C_5H, $C_6\text{-}H$

* The signal due to $C_{21}\text{-}H$ (β) is superimposed upon that of $C_{19}\text{-}H$ at $\delta \sim 4{\cdot}6$.

Oxidation of isoreserpiline with lead tetra-acetate, under similar conditions[4] to those whereby akuammigine (V) is converted into alstonine (IV, R = H), affords bleekerine in very low yield. Crude bleekerine contains traces of a further quaternary alkaloid salt which, in the mass spectrometer, gives rise to a molecular ion *m/e* 422·1842 ($C_{24}H_{26}N_2O_5$) together

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[3] See for example B. GILBERT, *The Alkaloids* (edited by R.H.F. MANSKE), Vol. VIII, p. 335, Academic Press, New York (1965).

[4] E. WINTERFELDT, H. RADUNZ and T. KORTH, *Chem. Ber.* **101**, 318 (1968).

with ions due to methyl chloride and hydrogen chloride. Possibly this molecule is N(a)-methylbleekerine.

EXPERIMENTAL

M.p's are uncorrected. Al_2O_3 refers to Merck neutral grade 1 (column) or Merck GF_{254} Type E (TLC).

Wood. Pulverized dry wood (500 g) was extracted exhaustively with boiling EtOH (5 l.). Removal of the solvent gave a dark coloured residue (0·113 g). This material was chromatographed repeatedly on preparative Al_2O_3 TLC plates developing with 1 % MeOH–$CHCl_3$. In this way 9-methoxyellipticine (30 mg, 0·006 %), m.p. and m.m.p. 283–285° (lit.,[6] 280–285°) R_f 0·7 Al_2O_3, 6 % MeOH–$CHCl_3$, was obtained. From the first TLC separation a band which ran with the solvent front was collected and eluted to give a crude mixture of sterols (5 mg). MS examination of this mixture revealed molecular ions at *m/e* 414·3844 ($C_{29}H_{50}O$) 412·3705 ($C_{29}H_{48}O$) and 400·3705 ($C_{28}H_{48}O$), which correspond to sitosterol, stigmasterol and campesterol respectively. GLC: 2·5 % OV1 on chromasorb W, AW–DMCS 200°, R_ts (with respect to sitosterol) 1·0, 0·87, 0·74. These values are closely similar to those reported by Rowe[5] and the assignments are confirmed by reference to authentic samples. Trace amounts of ellipticine, isoreserpiline and isoreserpiline-ψ-indoxyl were also detected during the purification of 9-methoxyellipticine from the wood extract.

Bark. Finely divided dry stem-bark (500 g) was exhaustively extracted with ethanol (7 l.). After removal of the solvent, the residue (2·0 g) was chromatographed on Al_2O_3 eluting with petrol (60–80°)–$CHCl_3$ and $CHCl_3$–MeOH solvent systems. Three fractions *A*, *B* and *C* were collected: *A* 50 % $CHCl_3$–petrol. The residue from this fraction was rechromatographed on Al_2O_3 to give, (i) a crude mixture of sterols (45 mg) in which stigmasterol, sitosterol and campesterol were shown to be present, and (ii) the alkaloid isoreserpiline contaminated with isoreserpiline-ψ-indoxyl. *B* 75–100 % $CHCl_3$–petrol. On removal of the solvent, a brown semi-crystalline mass was obtained. This, when treated with MeOH, afforded pale yellow prisms (45·6 mg, 0·009 %) of the alkaloid bleekerine m.p. 276–277° (EtOH), R_f Al_2O_3 (EtOH) 0·46 (bright blue fluorescence under UV), MS *m/e* 408·1690. $C_{23}H_{24}N_2O_5$ req. 408·1685 (Found: C, 67·8; H, 6·0; N, 7·1. $C_{23}H_{24}N_2O_5$ reqd. C, 67·6; H, 5·9; N, 6·9 %). The MeOH mother-liquor was evaporated to yield a gum which crystallized when triturated with Et_2O, to give holeinine as the mixed chloride–bromide. This material was dissolved in water and treated with a few drops of $HClO_4$ affording holeinine perchlorate which was recrystallized from H_2O. Yield 82 mg (0·016 %), m.p. 224–226° (lit.[2], 228–231°.) Comparison (IR and m.m.p.) with a sample prepared from an authentic sample of holeinine showed that the two specimens are identical. (Found C, 54·6; H, 6·0; N, 5·3. Calc. for $C_{24}H_{31}O_9N_2Cl$: C, 54·6; H, 6·0; N, 5·3 %). The Et_2O layer, from which holeinine separated, was concentrated to yield isoreserpiline (25 mg) together with a further quantity of the sterol mixture (10 mg). *C* 5–100 % MeOH/$CHCl_3$. Evaporation of this fraction gave a crystalline residue which recrystallized from EtOAc to give 9-methoxyellipticine (0·824 g, 0·165 %) m.p. 280–282°. The mother-liquor on concentration and trituration with benzene yielded ellipticine (13 mg) contaminated with 9-methoxyellipticine. This mixture was separated and ellipticine characterized as previously described[1] (yield 9·5 mg, 0·002 %). Total yield of isoreserpiline in bark 50 mg, 0·01 %; total weight of mixed sterols 55 mg.

Reduction of bleekerine with $NaBH_4$. Bleekerine (10 mg) in MeOH was treated with $NaBH_4$ (20 mg) and the reaction mixture was heated under reflux until no further change was observed in the UV spectrum. Removal of the solvent gave a solid residue which was treated with H_2O (5 ml) and extracted with $CHCl_3$. Evaporation of the dry solvent phase yielded a gum which was purified by preparative TLC (Al_2O_3) eluting with 50 % $CHCl_3$–petrol. Removal of a band R_f 0·6–0·8, followed by extraction with $CHCl_3$ and repeated chromatography afforded isoreserpiline (1·8 mg), identical with an authentic specimen (TLC, IR, MS).

Oxidation of isoreserpiline with lead tetra-acetate. A solution of isoreserpiline (20 mg) in HOAc (2 ml) maintained at 60° was treated dropwise with $Pb(OAc)_4$ (30 mg) in HOAc (10 ml). The addition was stopped when the UV spectrum of the product no longer showed the presence of the indole chromophore. Removal of HOAc under reduced pressure gave a dark red oil, this was dissolved in CH_2Cl_2 and shaken with 2 N Na_2CO_3 solution. The dried solvent layer was reduced in volume and applied to preparative TLC plates coated with Al_2O_3. The plates were developed with $CHCl_3$–MeOH (1 : 1) and viewed in UV light. A band R_f 0·6–0·85 was removed from the plates and worked up to yield a mixture of isoreserpiline and isoreserpiline-ψ-indoxyl, whereas a band at R_f 0·5–0·35 showing bright blue fluoresence was extracted to give a semi-solid product. This was rechromatographed, affording 0·8 mg of pure material identical (MS, GLC and m.m.p.) with bleekerine from *Bleekeria vitiensis*.

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[5] J. W. Rowe, *Phytochem.* **4,** 1 (1965).

[6] S. Goodwin, A. F. Smith and E. C. Horning, *J. Am. Chem. Soc.* **81,** 1903 (1959).