Phytochemistry, 1974, Vol. 13, pp. 2309 to 2312. Pergamon Press. Printed in England.

LOCHVINERINE: A NEW INDOLE ALKALOID OF *VINCA MAJOR*

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(*Received* 22 *February* 1974)

Key Word Index—*Vinca major*: Apocynaceae; periwinkle; alkaloids; reserpinine; vincamajoreine; majoridine; 10-methoxyvellosimine; lochvinerine.

Abstract—Five indole alkaloids have been isolated from the aerial parts of *Vinca major*. Four of these have been identified as reserpinine (pubescine), vincamajoreine, majoridine and 10-methoxyvellosimine. The fifth base, a new indole alkaloid *lochvinerine*, has been assigned the depicted structure.

INTRODUCTION

IN CONTINUATION of our work[1,2] on indole alkaloids we undertook the chemical investigation of the aerial parts of *Vinca major* L. var. *major*,[3] collected from Long Islands in the Andaman Islands situated in the Indian Ocean. The crude alkaloidal extract produces, by i.v. injection, a marked fall in the blood pressure of dogs.[4] Twenty-five indole alkaloids have been previously reported from this source.[5–7] In course of the present work, five indolic bases, including a new alkaloid designated lochvinerine, were isolated, the complete characterization of which is described in the present communication.

RESULTS

One of the five isolated bases was shown to be identical with reserpinine (pubescine) (**1**)[8,9] from spectral studies (UV, IR, NMR and MS) and by comparison with an authentic sample (m.m.p., co-TLC and superimposable IR spectra). Two others were identified as vincamajoreine (**2**)[10] and majoridine (*O*-acetylvincamajoreine) (**3**)[11,12] from spectral studies (UV, IR and MS) and direct comparison with authentic samples (co-TLC and m.m.p.).

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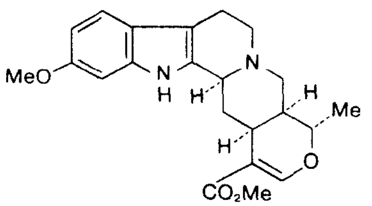
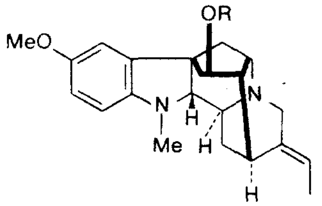
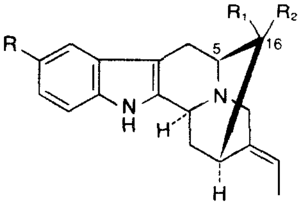


(1)



(2) R = H
(3) R = COMe

The fourth alkaloid, $C_{20}H_{22}N_2O_2$ (M^+ 322·1686; calc. for $C_{20}H_{22}N_2O_2$: 322·16813), m.p. 217–220° (dec.) showed colour reactions characteristic of tetrahydro-β-carboline alkaloids (Fröhde's reagent—deep green; Hopkins-Cole reagent—deep blue). Comparison of the spectral data (UV, IR and MS) of this compound with those published for 10-methoxyvellosimine (**4**),[10,13] previously isolated from this plant, suggested their identity. A detailed NMR study of the alkaloid lent support to this view. Signals in the 100 MHz NMR spectrum of this alkaloid in d_6-DMSO were observed at δ 9·57 (1H, *s*, C_{16}-CHO), 8·09 (1H, *s*, indole-NH-), 5·28 (1H, *q*, *J* 6·5 Hz, =C$\underline{H}$–Me), 3·76 (3H, *s*,-OMe) and 1·56 (3H, *d*, *J* 6·5 Hz, =CH–C$\underline{H}_3$). The characteristic ABX splitting pattern (δ 7·22, 1H, *d*, *Jo* 8·5 Hz, C_{12}-H; δ 6·86, 1H, *d*, *Jm* 2·5 Hz, C_9-H; δ 6·68, 1H, *d* of *d*, *Jo* 8·5 Hz, *Jm* 2·5 Hz, C_{11}-H) of the aromatic protons observed in its NMR spectrum demonstrated that the alkaloid was a 5-methoxyindole derivative. The appearance of the formyl proton at the normal value of δ 9·57 resembling the chemical shift (δ 9·58 in liq. SO_2) of the formyl proton of vellosimine (**5**)[14] demonstrated that the –CHO group at C-16 must be situated away from the aromatic nucleus. This observation confirmed the identity of the isolated base with 10-methoxyvellosimine (**4**). However, non-availability of an authentic sample of the latter precluded direct comparison. Reduction of 10-methoxyvellosimine with sodium borohydride afforded a dihydroderivative, $C_{20}H_{24}N_2O_2$ (M^+ 324), m.p. 199–200° in which the hydroxymethyl group must be away from the aromatic nucleus and which should thus be identical to lochnerine (**6**)[16] (Alkaloid-C[15], C-Alkaloid-T[17]), isolated previously from *Lochnera* (*Vinca*) *rosea* (L.) Reichb. var. *alba* Hubbd.



(4) R = OMe, R_1 = H, R_2 = CHO
(5) R = H, R_1 = H, R_2 = CHO
(6) R = OMe, R_1 = H, R_2 = CH_2OH
(8) R = OMe, R_1 = CH_2OH, R_2 = H

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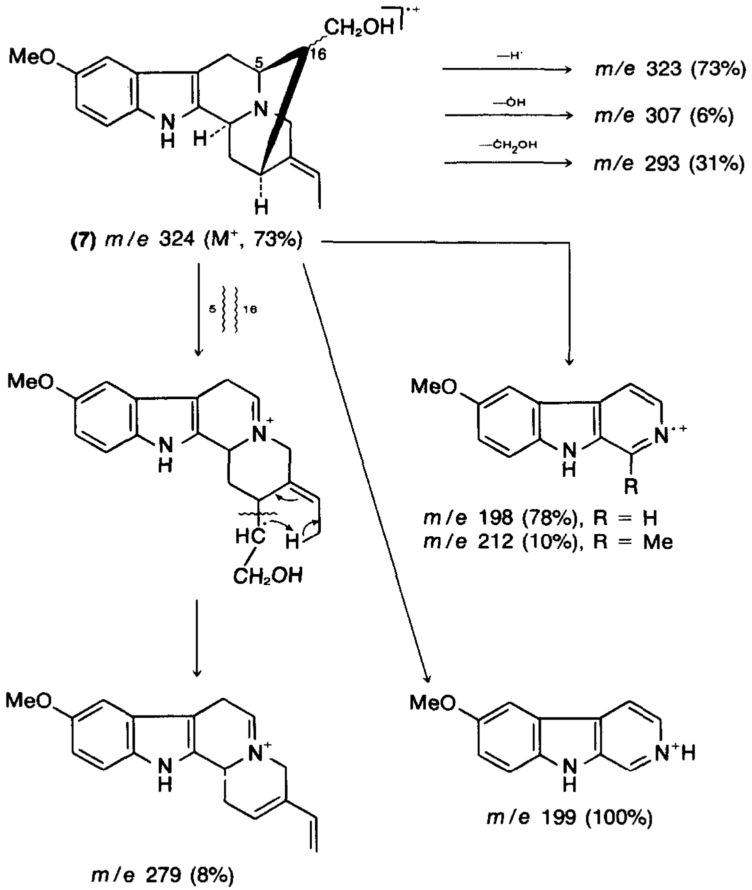
The fifth alkaloid, lochvinerine, $C_{20}H_{24}N_2O_2$ (M^+ 324), m.p. 195–197° (dec.), showed the typical UV absorption of a 10-methoxytetrahydro-β-carboline derivative ($\lambda_{\max}^{\mathrm{EtOH}}$: 228, 280 nm; log ϵ 4·40, 3·89; $\lambda_{\max}^{1\%\ \mathrm{HClO_4/EtOH}}$: 218, 271 nm). Further evidence for the presence of a 5-methoxyindole moiety was obtained from IR (877 and 805 cm^{-1}) and NMR spectra (ABX pattern: δ 7·13, 1H, *d*, *Jo* 8 Hz, C_{12}-H; δ 6·89, 1H, *d*, *Jm* 2·5 Hz, C_9-H; δ 6·75, 1H, *d* of *d*, *Jo* 8 Hz, *Jm* 2·5 Hz, C_{11}-H). The IR spectrum further revealed the presence of a hydroxyl (3550 cm^{-1}), an –NH– (3130 cm^{-1}) and a phenolic ether group (1215 cm^{-1}). In its 60 MHz NMR spectrum ($CDCl_3$) the characteristic signals were observed for an ethylidene grouping (methine quartet around δ 5·29 and methyl doublet at δ 1·57, *J* 7 Hz), an hydroxyl (δ 2·08, 1H, *s*, disappeared on deuteration) and an indole –NH– (δ 8·06, 1H, *s*, disappeared on deuteration). From the above spectral data and from its mass spectral fragmentation pattern (Scheme 1) the gross structure (7) (without any stereochemical implication at C_{16}) could be advanced for lochvinerine. The absolute configuration at C-15 is suggested from biogenetic considerations. The configuration at this centre also determines those at C_3 and C_5, as otherwise the formation of a rigid pentacyclic system in (7) would not be possible.



SCHEME 1. MASS FRAGMENTATION PATTERN OF LOCHVINERINE.

In order to settle the configuration at C_{16}, lochvinerine was compared with lochnerine (**6**), obtained by the borohydride reduction of 10-methoxyvellosimine. The physical and spectral (UV, IR, MS) properties of the two alkaloids were found to be very similar. However, on TLC comparison it was apparent that the two compounds were different in their chromatographic mobilities (vide Experimental). Hence our alkaloid should be epimeric with lochnerine at C_{16}, and the hydroxymethyl group in the former should be situated towards the aromatic nucleus. On the basis of these observations lochvinerine was, therefore, assigned the constitution and probable absolute configuration (**8**).

EXPERIMENTAL

The m.ps were determined on the Kofler block and are uncorrected. The UV spectra were measured in aldehyde-free 95% EtOH and the IR spectra in nujol mull.

Isolation of the alkaloids. The dried aerial parts (7 kg) of *Vinca major* were extracted in a Soxhlet with commercial MeOH (30 l.) for 5 days. The methanolic extract was conc. to about 1·5 l. and extracted with petrol (b.p. 60–80°) (4·5 l.). The petrol-soluble fraction was concentrated (220 ml) and churned with 5% aq. citric acid (1·5 l.) for 12 hr. The resulting mixture was filtered through a bed of celite. The filtrate was basified with NH_3 soln and extracted successively with C_6H_6 (3 × 1·5 l.) and $CHCl_3$ (3 × 1·5 l.). The benzene extract was dried over anhydrous Na_2SO_4, conc. and chromatographed over alumina. The petrol-benzene (1:3) eluates on concn yielded a yellowish solid, which on recrystallization furnished reserpinine, $C_{22}H_{26}N_2O_4$ (M^+ 382·1874), m.p. 228–230° (dec.), $[\alpha]_D^{25}$–52° ($CHCl_3$). Despite a somewhat different X-ray powder data, undepressed m.m.p., co-TLC and superimposable IR spectra with an authentic sample established its identity with reserpinine. The C_6H_6–$CHCl_3$ (1:1) eluates when concentrated yielded a solid, which was recrystallized from C_6H_6–EtOAc to yield colorless needles, $C_{20}H_{22}N_2O_2$ (M^+ 322·1686), m.p. 217–220° (dec.), characterized as 10-methoxyvellosimine from its spectral measurements (UV, IR, MS and NMR).

The petrol-insoluble portion of the methanolic extract was churned with 5% aq. citric acid (2 l.) for 14 hr. The resulting mixture was then filtered through a bed of celite. The aq. filtrate was then fully basified with NH_3 soln and extracted with C_6H_6 (3 × 1·5 l.) and $CHCl_3$ (3 × 1·5 l.). The conc C_6H_6 extract was chromatographed over alumina. Colorless crystals of majoridine, $C_{23}H_{28}N_2O_3$ (M^+ 380), m.p. 210–213° (dec.) were obtained by recrystallization from absolute alcohol of the solid obtained in the petrol–C_6H_6 (2:1) eluates. The identity of this alkaloid was established from spectral data (UV, IR, MS) and from comparison (m.m.p. and co-TLC) with an authentic sample. The later portions of the C_6H_6-eluates yielded vincamajoreine, $C_{21}H_{26}N_2O_2$ (M^+ 338·2028), m.p. 261–263° (dec.) (C_6H_6–EtOAc), which was identified from its physical and spectral data (UV, IR, MS). The combined C_6H_6–$CHCl_3$ (3:1 and 1:1) eluates furnished a solid, which was recrystallized from EtOAc to yield stout needles of lochvinerine, m.p. 195–197° (dec.), $C_{20}H_{24}N_2O_2$ (M^+ 324). It developed an indigo-blue colour with Fröhde's reagent and a deep brown colour with Hopkins-Cole reagent (Found: C, 73·77; H, 7·59; N, 8·82; OMe, 9·66. $C_{20}H_{24}N_2O_2$ requires: C, 74·04; H, 7·46; N, 8·64; OMe, 9·75%).

$NaBH_4$ *reduction of 10-methoxyvellosimine.* To a solution of 10-methoxyvellosimine (4 mg) in MeOH (2 ml) a slight excess of $NaBH_4$ was added in the cold. The mixture was then left overnight at room temp. Removal of the solvent under vacuum, followed by recrystallization of the residue from MeOH–H_2O furnished colorless needles of lochnerine, $C_{20}H_{24}N_2O_2$ (M^+ 324), m.p. 199–200° (3·5 mg). MS: M^+ 324 (100%); *m/e* 323 (88%), 307 (11%, M^+ $-$OH), 293 (40%, M^+ $-$OMe or CH_2OH), 279 (10%, M^+ $-$45), 212 (11%), 199 (65%) and 198 (58%). It differed from lochvinerine in its TLC behavior: silica gel G (Merck), MeOH, lochnerine (R_f 0·58), lochvinerine (R_f 0·66); alumina, C_6H_6–EtOAc (1:3), lochnerine (R_f 0·51), lochvinerine (R_f 0·48).

Acknowledgements—The authors are grateful to Professor (Mrs.) A. Chatterjee for providing laboratory facilities, and for her helpful discussions during the work. Thanks are also due to Dr. S. Natori, National Institute of Hygienic Sciences, Tokyo, Japan; Dr. T. Kikuchi, Kyoto University, Kyoto, Japan; Dr. A. Brossi, Hoffman La Roche & Co., Basel, Switzerland; and Dr. A. H. Jackson, University College, Cardiff, U.K. for spectral measurements. The authors also thank Dr. J. Trojánek, Research Institute for Natural Drugs, Prague, Czechoslovakia and Professor M. Shamma, the Pensylvania State University, U.S.A., for generous gifts of majoridine and reserpinine, respectively. Sincere thanks are also accorded to Dr. N. Neuss, The Lilly Research Laboratories, Indianapolis, U.S.A. for comparing the X-ray powder data and IR spectra of reserpinine with an authentic sample. One of the authors (M.C.) also thanks the C.C.R.I.M.H. (India) for financial assistance.