Pergamon 0031-9422(95)00391-6 *Phytochemistry*, Vol. 40, No. 4, pp. 1291–1293, 1995

0031-9422/95 $9.50 + 0.00

ALKALOIDS FROM *CRINUM KIRKII*

J. BASTIDA,* C. CODINA, P. PEETERS, M. RUBIRALTA,† M. OROZCO,‡ F. J. LUQUE‡ and S. C. CHHABRA§

Departament de Productes Naturals, †Laboratori de Quimica Orgànica, ‡Laboratori de Química-Física, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona, Spain; §Chemistry Department, Kenyatta University, PO Box 43844, Nairobi, Kenya

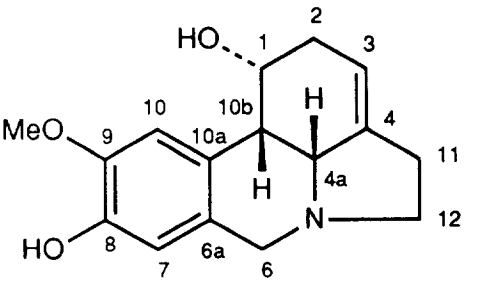
(*Received* 15 *February* 1995)

Key Word Index—*Crinum kirkii*; Amaryllidaceae; bulbs; alkaloids; kirkine; 8-*O*-demethylvasconine; crinine; hamayne; 3-*O*-acetylhamayne.

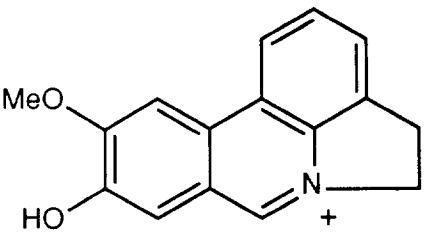
Abstract—The new alkaloids, kirkine and 8-*O*-demethylvasconine, have been isolated from bulbs of *Crinum kirkii*. Their structures were established by physical and spectroscopic methods.

INTRODUCTION

The genus *Crinum* (Amaryllidaceae) is one of about 10 genera of the tribe Amaryllideae which has a wide distribution in the tropics and warm temperature regions [1]. *Crinum kirkii*, a common grassland plant of East Africa, is used in Kenya for marking shamba boundaries [2] and for the treatment of sores [3]. In Tanzania, the fruit and inner parts of the bulb are used as a purgative and the outer scales are used as a rat poison [4]. In this paper, we report the isolation and characterization of five alkaloids from *C. kirkii*: kirkine (**1**), 8-*O*-demethylvasconine (**2**), crinine, hamayne and 3-*O*-acetylhamayne.



1



2

RESULTS AND DISCUSSION

Separation and isolation of individual compounds from the ethanol extract of the fresh bulbs of *Crinum kirkii* followed the steps described in the Experimental.

Compound **1**, $C_{16}H_{19}NO_3$ from HR mass spectrometry, was isolated from fraction III, and the name, kirkine, is proposed for this alkaloid. Its EI mass spectrum showed a $[M]^+$ at *m/z* 273 and important fragments at *m/z* 272, 253, 252 (base peak) and 226. Although some fragments were similar to the alkaloid caranine [5], fragmentation followed a different pattern which could be explained by aromatization of the A, B and C rings, as a consequence of a different B/C ring fusion stereochemistry. The peak stability at *m/z* 252 (100%) is in accordance with the isolation of compound **2** in this plant species. In the ^{1}H NMR spectrum of **1**, recorded in CD_3OD, three singlets at δ7.08, 6.60 and 3.87 were assigned to the two aromatic protons (H-10 and H-7) and a MeO group, respectively. The assignment of the aromatic protons was made according to the benzylic couplings with H-10b and H-6β, respectively, observed in a COSY experiment. The location of the methoxy group on C-9, was deduced from the 2D NOESY spectrum. The H-3 proton resonated as a broad double triplet at δ5.87; a transoid allylic coupling between H-3 and H-4a, and a cisoid one between H-3 and H-11β, were observed in the COSY spectrum. A double triplet at δ4.61 and a doublet at δ4.40 were assigned to the methylene protons of the benzylic position. The H-1 proton was observed as a quartet at δ4.37 with a coupling constant of 3 Hz, allowing us to assign the hydroxyl group in an axial disposition. The two methine protons, H-4a and H-10b,

* Author to whom correspondence should be addressed.

were ascribed to the broad doublet at δ4.24 and the double doublet at δ3.52, respectively; their smaller coupling constant with respect to caranine [6], an analogous alkaloid with a *trans*-diaxial configuration, was in agreement with a *cis*-fusion of B/C rings. Moreover, this data could explain the shielding effect of H-4a and H-10b protons on caranine due to the *anti*-position of H-4a with respect to the nitrogen lone pair, and the geometrical perpendicularity of H-10b with respect to the aromatic ring. Finally, the chemical shifts for the H-2 protons were observed as a *ddd* at δ2.46 for H-2α and a *dddd* at δ2.34 for H-2β. The very small vicinal coupling constant between H-2β and H-3 was in accordance with an angle of *ca* 90° (from Dreiding models). On the contrary, homoallylic couplings between H-2β and H-11β, as well as H-2β and H-4a, were observed in the COSY experiment.

The conformational mobility of both B- and C-rings, and also the chirality of C-1 position, promote eight different conformers. Geometries and conformational energies of the different conformers in the gas phase were determined from molecular mechanics calculations (see Experimental). The conformational free energies in water and methanol were also computed and strongly suggest that the solvent plays only a small influence on the conformational preferences of this structure. The conformer having the *R*-stereoisomerism at C-1, together with a pseudochair disposition in B- and C-rings was the most stable and confirm the results previously obtained by ^{1}H NMR.

The ^{13}C NMR spectrum of **1** showed 16 carbon atoms, eight of which showed resonances in the shift range $\delta > 90$, corresponding to the aromatic and olefinic carbons. The aliphatic shift range was characterized by three doublets (C-4a, C-1 and C-10b), four triplets (C-12, C-6, C-2 and C-11) and one quartet for the aromatic methoxy carbon. The assignments were corroborated by a HMQC experiment.

Compound **2**, $C_{16}H_{14}NO_2$ from HR mass spectrometry, was similar to vasconine, an alkaloid isolated from *Narcissus vasconicus* [7]. Its EI mass spectrum showed a $[M]^+$ at *m/z* 252 (also a significant peak for **1**). The ^{1}H NMR of **2**, recorded in CD_3OD, exhibited (i) a singlet at δ8.85 belonging to the proton of the iminium salt; (ii) two doublets at δ7.95 and 7.52 and one triplet at δ7.67, belonging to a 1,2,3-three-substituted aromatic ring; (iii) two singlets at δ7.21 and 6.92 for the aromatic protons of ring A; (iv) two pseudo-triplets at δ4.96 and 3.64 for the protons at the positions 12 and 11, respectively; and (v) a singlet at δ3.85 assignable to the MeO group. A 2D NOE experiment allowed both unambiguous assignment of the aromatic protons of ring A and confirmation of the position of the MeO group in this aromatic ring. Thus, the assignment of H-10 was made on the basis of the NOE contour with H-1. After that, the signal at δ3.85 was easily assigned to the MeO group at the C-9 position by NOE contour with H-10. Furthermore, the singlet at δ8.85 (H-6) showed a NOE correlation with the singlet at δ6.92, providing evidence for its assignment to H-7.

The ^{13}C NMR spectrum of **2** showed 16 carbon atoms. A DEPT spectrum revealed the presence of seven quaternary carbons, six methine carbons, two methylene carbons and one methyl carbon. The ^{13}C NMR signals were assigned considering the connectivities from HMQC and HMBC spectra. The singlets at δ161.9 and 161.7 were assigned to C-9 and C-8, respectively, because of their three-bond correlation with H-7 and H-10 in the HMBC spectrum. H-1, H-6 and H-7 gave a three-bond correlation with the carbon singlet at δ124.2, leading us to assign this signal to C-10a. The carbon singlet at δ134.9 was ascribed to C-4a due to its three-bond connectivities to H-1, H-3, H-6 and H-11. Also, H-11 gave a three-bond correlation with C-3 and two-bond connectivity with the carbon singlet at δ137.1, which was assigned for the C-4 resonance. Deshielding of the methine carbon C-6 as a consequence of an imminium salt effect, was similar to that observed for vasconine [7] in relation to assoanine [8]. Likewise, a deshielding effect on the C-12 position, α with respect to the quaternary nitrogen, was also observed.

EXPERIMENTAL

General. Mp uncorr. IR were recorded in KBr discs and EIMS at 70 eV. NMR were recorded in the solvent specified with TMS as int. standard; chemical shifts are reported in δ units (ppm). Silica gel 60G (Merck) was used for VLC and Sephadex LH-20 (Pharmacia) for gel filtration. Silica gel 60 F_{254} (Merck) was used for TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

Plant material. Bulbs were collected in May 1991 from Muiga, Nyeri District, Central Province, Kenya. A voucher specimen (no 91/230) is deposited at the Herbarium of the Botany Department (University of Nairobi, Kenya).

Extraction and isolation of alkaloids. Fresh bulbs of *C. kirkii* Bak. (3 kg) were macerated with EtOH for 48 hr × 4. The extract was evapd under red. pres. and acidified to pH 4. After removing neutral material with Et_2O, the acidic soln was made alkaline (pH 8–9) and extracted with $CHCl_3$. The extract was concd under red. pres. to a brown gummy solid (1.5 g). This extract was chromatographed by VLC [9, 10] giving 4 different frs. Fr. I was purified by prep. TLC using CH_2Cl_2–MeOH (19:1). After final purification on Sephadex LH-20, 3-*O*-acetylhamayne (37 mg) was isolated. Fr. II was purified on Sephadex LH-20 yielding crinine (16 mg). Compound **1** recrystallized directly from fr. III; recrystallization from MeOH afforded 53 mg. Finally, from fr. IV, after prep. TLC eluting with MeOH, two alkaloids were isolated, hamayne (19 mg) and **2** (15 mg).

Molecular mechanics calculations. Energy minimizations were performed for each conformer with the standard CVFF force field [11, 12] implemented in the program DISCOVER [13], as well as with the MM2 [14, 15] program. The results obtained are available by request.

Table 1. ^{1}H NMR (500 MHz, CD_3OD) spectral data of kirkine (**1**)*

Proton	δ
1	4.37 *q* (3)
2α	2.46 *ddd* (16.5, 7.5, 3)
2β	2.34 *dddd* (16.5, 3, 2.5, 2.5)
3	5.87 *br dt* (7.5, 2.5)
4a	4.24 *br d* (8)
6α	4.40 *d* (14)
6β	4.61 *dt* (14, 1)
7	6.60 *s*
10	7.08 *s*
10b	3.52 *dd* (8, 3)
11α	2.87 *ddd* (16, 8.5, 2.5)
11β	2.81 *dddd* (16, 10.5, 3, 2.5)
12α	3.71 *ddd* (11, 8.5, 3)
12β	3.83 *ddd* (11, 10.5, 2.5)
MeO	3.87 *s*

*Coupling constants in parentheses are in Hz.

(+)-*Kirkine* (**1**). HRMS *m/z* 273.1372, $C_{16}H_{19}NO_3$ requires 273.1365. Mp 170–172°. $[\alpha]_D^{22}$ + 59.6° (MeOH; *c* 0.57). IR ν_{max} cm^{-1}: 3520, 2927, 2360, 1612, 1526, 1447, 1328, 1243, 1208, 1116, 1067, 1039, 1003, 917, 883, 855, 792. EIMS 70 eV, *m/z* (rel. int.): 273 $[M]^+$ (13), 272 (17), 254 (11), 253 (59), 252 (100), 237 (24), 226 (12), 209 (23). ^{1}H NMR (500 MHz, CD_3OD): see Table 1. ^{13}C NMR (50 MHz, CD_3OD): δ26.2 (*t*, C-11), 34.7 (*t*, C-2), 42.1 (*d*, C-10b), 56.2 (*q*, OMe), 65.4 (*d*, C-1), 66.2 (*t*, C-6), 67.5 (*t*, C-12), 71.2 (*d*, C-4a), 111.9 (*d*, C-10), 115.3 (*d*, C-7), 123.2 (*d*, C-3), 123.7 (*s*, C-10a), 124.8 (*s*, C-6a), 133.9 (*s*, C-4), 149.1 (*s*, C-8), 150.2 (*s*, C-9).

8-O-*Demethylvasconine* (**2**). HRMS *m/z* 252.1037, $C_{16}H_{14}NO_2$ requires 252.1024. Mp 200–202°. IR ν_{max} cm^{-1}: 3420, 2364, 1559, 1506, 1386, 1323, 1297, 1225, 1168, 1026, 919, 754. EIMS 70 eV, *m/z* (rel. int.): 252 $[M]^+$ (100), 251 (21), 250 (32), 237 (33), 209 (36), 207 (14), 180 (18), 178 (14), 129 (25). ^{1}H NMR (500 MHz, CD_3OD): δ3.64 (2H, *t*, *J* = 6.5 Hz, H-11), 3.85 (3H, *s*, OMe), 4.96 (2H, *t*, *J* = 6.5 Hz, H-12), 6.92 (1H, *s*, H-7), 7.21 (1H, *s*, H-10), 7.52 (1H, *d*, *J* = 7.5 Hz, H-3), 7.67 (1H, *t*, *J* = 7.5 Hz, H-2), 7.95 (1H, *d*, *J* = 7.5 Hz, H-1), 8.85 (1H, *s*, H-6). ^{13}C NMR (50 MHz, CD_3OD): δ28.0 (*t*, C-11), 55.7 (*t*, C-12), 56.2 (*q*, OMe), 100.8 (*d*, C-10), 112.9 (*d*, C-7), 119.4 (*d*, C-1), 123.7 (*s*, C-10b), 123.7 (*s*, C-6a), 123.7 (*d*, C-3), 124.2 (*s*, C-10a), 130.8 (*d*, C-2), 134.9 (*s*, C-4a), 137.1 (*s*, C-4), 142.2 (*d*, C-6), 161.7 (*s*, C-8), 161.9 (*s*, C-9).

(−)-*Crinine*, (+)-*hamayne and* (+)-3-O-*acetylhamayne*. Analytical data in agreement with lit. [16, 17].

Acknowledgements—Part of this work was supported financially by CIRIT-CICYT (project QFN91-4205). Thanks are due to Mr Simon Mathenge, Department of Botany, University of Nairobi, for collection and authentication of plant material. One of the authors (P.P.) thanks the 'Erasmus Project' (ICP-92 B-1110/13) for a scholarship grant.

REFERENCES

1. Dahlgren, R. M. T., Clifford, H. J. and Yeo, P. F. (1985) in *The Families of the Monocotyledons*. Springer-Verlag, Berlin.
2. Gachathi, F. N. (1989) in *Kikuyu Botanical Dictionary of Plant Names and Uses*. AMREF, Nairobi.
3. Kokwaro, J. O. (1976) in *Medicinal Plants of East Africa*. East Africa Literature Bureau, Kampala.
4. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) in *Medicinal and Poisonous Plants of Southern and Eastern Africa*. E. & S. Livingstone Ltd, Edinburgh.
5. Kinstle, T. H., Wildman, W. C. and Brown, C. L. (1966) *Tetrahedron Lett.* **39**, 4659.
6. Evidente, A., Cicala, M. R., Giudicianni, I., Randazzo, G. and Riccio, R. (1983) *Phytochemistry* **22**, 581.
7. Bastida, J., Codina, C., Viladomat, F., Rubiralta, M., Quirion, J. C. and Weniger, B. (1992) *J. Nat. Prod.* **55**, 122.
8. Llabrés, J. M., Viladomat, F., Bastida, J., Codina, C. and Rubiralta, M. (1986) *Phytochemistry* **25**, 2637.
9. Pelletier, S. W., Chokshi, H. P. and Desai, H. K. (1986) *J. Nat. Prod.* **49**, 892.
10. Coll, J. C. and Bowden, B. F. (1986) *J. Nat. Prod.* **49**, 934.
11. Dauber-Osguthorpe, P., Roberts, V. A., Osguthorpe, D. J., Wolff, J., Genest, M. and Hagler, A. T. (1988) *Proteins* **4**, 31.
12. Hagler, A. T., Stern, P. S., Sharon, R., Becker, J. M. and Nadjer, F. (1979) *J. Am. Chem. Soc.* **101**, 6842.
13. BIOSYM (1991) Insight II and Discover. BIOSYM Technologies, Co.
14. Allinger, N. L. (1987) in *MM2 Users Manual*. University of Georgia, Athens.
15. Allinger, N. L. (1977) *J. Am. Chem. Soc.* **99**, 8127.
16. Kobayashi, S., Tokumoto, T., Kihara, M., Imakura, Y., Shingu, T. and Taira, Z. (1984) *Chem. Pharm. Bull.* **32**, 3015.
17. Viladomat, F., Bastida, J., Codina, C., Campbell, W. E. and Mathee, S. (1994) *Phytochemistry* **35**, 809.