971. *The Alkaloids of the Amaryllidaceae. Part XI.*[1] *The Alkaloids of* Nerine krigeii *and the Structure of Krigenamine.*

By D. F. C. GARBUTT, P. W. JEFFS, and F. L. WARREN.

A new alkaloid, krigenamine (IX), has been isolated from *Nerine krigeii*, but neronine, previously isolated, is found to be absent from the plant itself. Krigenamine is shown to belong to the hemiacetal group of alkaloids by its several reactions, and is reduced to a base (VI) which is quaternised to falcatine methiodide (X). The absolute structure is accordingly established and the aromatic oxygenation is identified with that of the pyrrolo[3,2,1-*de*]-phenanthridine base falcatine.

IN a study of the alkaloids of *Nerine krigeii* we have isolated a new alkaloid, krigenamine (II; R = H), in addition to lycorine, krigeine (II; R = OH), and neronine (III; R = OH) previously obtained [2] from this plant.

Standard extraction procedures [3] afforded a crude alkaloid extract. In view of the reported ease [2] which the hemiacetal alkaloids krigeine and lycorenine disproportionated to the corresponding lactones, neronine and homolycorine, during chromatography on alumina, it was desirable to test for the presence of lactone alkaloids in the crude extract before chromatography.

A hydrochloric acid solution of the crude alkaloid extract gave no alkaloid hydrochloride soluble in chloroform and so established the absence of lactone alkaloids. Subsequent isolation of the lactones, neronine (III; R = OH) and oxokrigenamine (III; R = H), showed that they were artifacts produced by disproportionation of the corresponding hemiacetals on alumina.

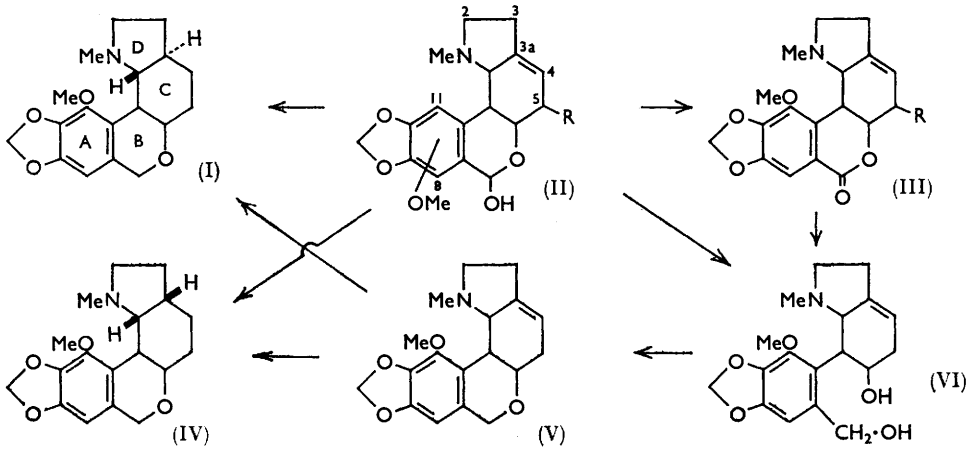
Krigenamine, $C_{18}H_{21}NO_5$, gave an infrared spectrum resembling that of its companion alkaloid, krigeine. A strong band at 10·66 μ indicated the presence of a methylenedioxy-group and this was confirmed by a positive Labat test.[4] A band at 6·18 μ was attributed, by analogy with krigeine and other alkaloids of the series, to a methoxy-methylenedioxy-phenyl chromophore. The ultraviolet spectrum ($\lambda_{max.}$ 280—285 mμ) was in accord with this formulation. Further, the alkaloid contained a tertiary nitrogen atom and one methoxyl and an *N*-methyl group. It seemed likely, in view of the similarity of the spectra of krigeine and krigenamine, that the two remaining oxygen atoms were present in a hemiacetal residue. In support of this it was found that krigenamine gave a yellow colour with concentrated hydrochloric acid, as do the known hemiacetals lycorenine and krigeine. Moreover, treatment of krigenamine with manganese dioxide in chloroform gave a lactone (III; R = H), whose spectral properties were compatible with its formulation as an aryl-conjugated δ-lactone.[2] The lactone obtained by disproportionation of krigenamine during chromatography of the crude alkaloids was identical with this oxidation product.

On the assumption that krigenamine possesses a [2]benzopyrano[3,4-*g*]indole skeleton, as in (II), analysis indicates the presence of one ethylenic double bond. Attempts to hydrogenate krigenamine and the derived lactone over palladium–carbon were unsuccessful, but krigenamine took up 2 mol. of hydrogen over Adams catalyst. The crude reduction product showed no infrared hydroxyl absorption, indicating that hydrogenolysis of the benzylic hydroxyl group had occurred with simultaneous reduction of the double bond. Chromatography of the crude reduction products afforded two isomeric bases $C_{18}H_{23}NO_4$, designated α- and β-deoxydihydrokrigenamine whose structures (I) and (IV), respectively, were established by their preparation by another route (see below).

[1] Part X, Jeffs, *Proc. Chem. Soc.*, 1962, 80.
[2] Briggs, Highet, Highet, and Wildman, *J. Amer. Chem. Soc.*, 1956, **78**, 2899.
[3] Goosen, Graham, Jeffs, Warren, and Wright, *J.*, 1960, 1088.
[4] Labat, *Bull. Soc. Chim. biol.*, 1932, **15**, 1344.

Reduction of krigenamine or the derived lactone by lithium aluminium hydride gave the diol (VI), whose ultraviolet spectrum was identical with that of krigenamine. The diol was readily converted by sulphuric acid into its oily anhydro-derivative, deoxykrigenamine (V).

Catalytic hydrogenation of deoxykrigenamine (V) gave the two deoxydihydro-compounds (I) and (IV) mentioned above.



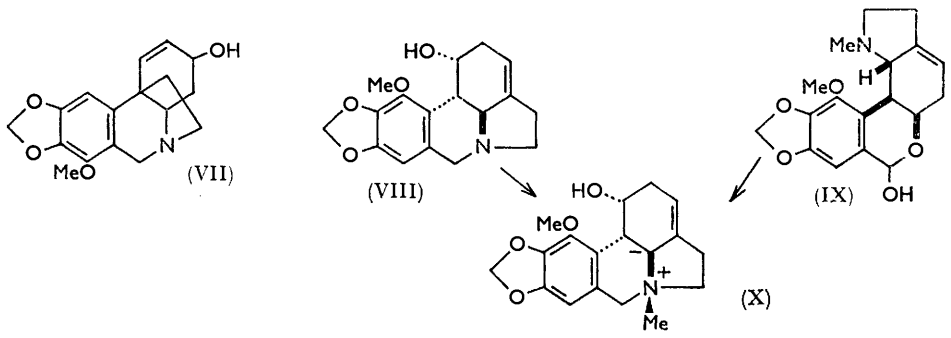
All these reactions parallel those reported for lycorenine.[5] The formulation of α- and β-deoxydihydrokrigenamine as ring C/D epimers was initially based on indirect evidence. The stability of the double bond in krigenamine to lithium aluminium hydride and the production of two isomers on catalytic reduction eliminates unsaturation at positions 2, 3 and 4, 5. The ultraviolet spectrum of krigenamine, and the fact that it is not an enol ether, leaves only the possibility of unsaturation at position 3, 3a or 3a, 4; in either case reduction could give two epimers (V) and (VI). The double bond was placed[4] tentatively in the latter position by analogy with lycorine and krigeine.

A comparison of molecular rotational differences of compounds (I)—(VI) with the analogues in the lycorenine series suggested that the two bases had the same relative and absolute stereochemistry and, further, that α- and β-deoxydihydrokrigenamine had the *trans* (I) and *cis* (IV) C/D ring fusion, respectively.

The methoxy-group in the trioxyaryl alkaloids of the Amaryllidaceae is conclusively placed as shown in powelline (VII)[6] and falcatine (VIII).[7] We previously[8] placed the methoxy-group in krigenamine at position 11, using the argument advanced by Warnhoff and Wildman[9] from consideration of the low intensity of the ultraviolet absorption of the aromatic chromophore, but this was later invalidated by chemical evidence.[7] By using the elegant method of Katagawa, Uyeo, and Yokoyama[10] for the interconversion of a hemiacetal base to a pyrrolo[3,2,1-*de*]phenanthridine base, we have now proved that the methoxyl group is at position 11 in krigenamine (II) by converting it by way of its fission product (VI) into falcatine methiodide (X). This interconversion confirms the structural features advanced above, locates the double bond and the methoxyl group conclusively, and establishes the absolute configuration of krigenamine as (IX). It also constitutes the

[5] Wildman, " The Alkaloids," ed. Manske, Academic Press, Inc., New York, 1960, Vol. VI.
[6] Lloyd, Kielar, Highet, Uyeo, Fales, and Wildman, *Tetrahedron Letters*, 1961, No. 3, 105; *J. Org. Chem.*, 1962, **27**, 373.
[7] Torssell, *Acta Chem. Scand.*, 1961, **4**, 947; see also Benington and Morin, *J. Org. Chem.*, 1962, **27**, 142.
[8] Jeffs and Warren, *Chem. and Ind.*, 1961, 468.
[9] Warnhoff and Wildman, *J. Amer. Chem. Soc.*, 1960, **82**, 1472.
[10] Kitagawa, Uyeo, and Yokoyama, *J.*, 1959, 3741.

first direct evidence for the aromatic oxygenation pattern in a member of the trioxyaryl hemiacetal and lactone alkaloids of the Amaryllidaceae as envisaged by Barton and Cohen [11] in their biosynthetic postulates.



The possibility that the biogenesis of the C_6–C_1 unit (the aromatic ring and the benzylic carbon atom) in the alkaloids based on the 5,10b-ethanophenanthridine ring system (cf. VII) differs from that in the pyrrolo[3,2,1-*de*]phenanthridine ring system (cf. VIII) has been discussed in a consideration of the incorporation of labelled aromatic amino-acids into these alkaloids.[1, 12] Although tracer experiments have not yet been reported for the hemiacetal and lactone bases it is probable that they resemble the pyrrolo[3,2,1-*de*]phenanthridine type in their mode of biosynthesis of the C_6–C_1 unit. In view of this it is tempting to speculate that those alkaloids of these two groups that have three aromatic oxygen functions should, in contrast to those alkaloids related to powelline, show the same aryl-oxygenation pattern as falcatine and krigenamine.

Experimental

Ultraviolet spectra were determined for 95% ethanol solutions, optical rotations for chloroform solutions, and infrared spectra for potassium bromide discs, unless otherwise stated.

Extraction.—Sliced bulbs of *Nerine krigeii* were extracted with hot alcohol, dried (4·9 kg.), and re-extracted with alcohol. The extract was concentrated, steam-distilled, acidified to pH 2·0 with hydrochloric acid, and filtered. The filtrate was washed with ether and then extracted with chloroform. The chloroform extract gave no alkaloid. The aqueous concentrate was basified with aqueous sodium carbonate and extracted exhaustively with chloroform–ethanol (20 : 1). The combined extracts were dried (Na_2SO_4) and concentrated. The precipitate (7·2 g.) was filtered off and crystallised from ethanol, to give lycorine, m. p. 266—268°, identified by its infrared spectrum.

The chloroform filtrate gave a brown gum (79 g.) which was chromatographed in chloroform on alumina (1·5 kg.), to give the following fractions: (*a*) (chloroform, 500 ml.) a gum (18 g.); (*b*) (chloroform, 200 ml.) a gum (4·0 g.); (*c*) (chloroform, 500 ml.) a gum (14 g.); and (*d*) (chloroform–ethanol, 20 : 1, 1 l.) a solid (11 g.).

Fraction (*b*) (4 g.) crystallised from ethyl acetate, to give neronine hydrate as prisms, double m. p. 128—130°, 196—197°, $[\alpha]_D^{20}$ +158°, $\lambda_{max.}$ 5 86, 10·66 μ (methylenedioxy) (Found: C, 59·6; H, 5·9. Calc. for $C_{18}H_{21}NO_7$: C, 59·5; H, 5·8%).

Fraction (*d*) (11 g.) crystallised from aqueous acetone as needles, m. p. 209—210° (decomp.), of krigeine, $[\alpha]_D^{25}$ +245° (*c* 0·27 in EtOH), $\lambda_{max.}$ 285 mμ (ε 990) (Found: C, 62·4; H, 6·2. Calc. for $C_{18}H_{21}NO_6$: C, 62·2; H, 6·1%) (lit.,[2] m. p. 209·5—210°).

Fraction (*a*) was rechromatographed in benzene–chloroform (4 : 1) over alumina (500 g.) and gave fractions: (i) (benzene–chloroform, 4 : 1; 700 ml.) an intractable gum (8·1 g.); (ii) (benzene–chloroform, 2 : 1; 500 ml.) a gum (2·7 g.); and (iii) (chloroform, 1 l.) a yellow resin

[11] Barton and Cohen, " Festschrift Arthur Stoll," Birkhäuser, Basle, 1957, p. 117.
[12] Battersby, Fales, and Wildman, *J. Amer. Chem. Soc.*, 1962, **84**, 681; Suhadolnik and Fischer, Amer. Chem. Soc. Meeting, Chicago, 1961, Abs. 39Q.

(4·5 g.). Trituration of fraction (ii) with ethyl acetate afforded a solid which crystallised from " wet " ethyl acetate as *didehydrokrigenamine hydrate* (III), prisms, m. p. 70—75° (Found: C, 62·4; H, 6·0; N, 4·1; OMe, 8·8, *N*-Me, 6·4. $C_{18}H_{19}NO_5,H_2O$ requires C, 62·2; H, 6·1; OMe, 8·9; *N*-Me, 8·35%). The hydrate, when dried at 25°/0·01 mm. and crystallised from ethyl acetate, gave the anhydrous *base* as prisms, m. p. 147—148°, $[\alpha]_D^{20}$ +117° (*c* 1·0) (Found: C, 65·3; H, 6·0. $C_{18}H_{19}NO_5$ requires C, 65·6; H, 5·8%), $\lambda_{max.}$ 228 (ε 22,900), 285 (ε 6020), $\lambda_{infl.}$ 310 mμ (ε 2570), $\lambda_{max.}$ 5·86 μ. Fraction (iii) crystallised from ethyl acetate to give a further quantity of neronine (4·0 g.), m. p. 196—198°.

Fraction (*c*) (14 g.) in chloroform (600 ml.) was washed with N-hydrochloric acid (3 × 50 ml.), and then 2N-ammonia (100 ml.). The chloroform solution afforded neronine (2·8 g.), m. p. 196—198° (from ethyl acetate). The hydrochloric acid washings were basified with sodium carbonate and extracted with chloroform. The chloroform extract gave a residue (11 g.) which was chromatographed in chloroform over alumina (500 g.). The first fraction (250 ml.) gave an intractable gum (6 g.), and the next (300 ml.) afforded a resin (3·1 g.). Trituration of the resin with ethyl acetate left a solid which crystallised from acetone, ethanol, or ethyl acetate, affording *krigenamine* (IX) as needles, m. p. 210—211°, $[\alpha]_D^{20}$ +210° (*c* 0·43), $\lambda_{max.}$ 280—285 mμ (ε 1385), $\lambda_{max.}$ 6·18, 9·55, and 10·66 μ (Found: C, 65·0; H, 6·5; OMe, 8·7; *N*-Me, 6·0. $C_{18}H_{21}NO_5$ requires C, 65·2; H, 6·4; OMe, 9·4; *N*-Me, 8·8%).

Krigenamine Metho-salts.—Krigenamine (50 mg.), methanol (5 ml.), and methyl iodide (0·5 ml.) were set aside at 25° for 5 hr. The product crystallised from acetone to give the *methiodide* as prisms m. p. 235—237° (decomp.), $[\alpha]_D^{20}$ +150° (*c* 1·2 in H_2O) (Found: C, 48·2; H, 5·6. $C_{19}H_{24}INO_5$ requires C, 48·2; H, 5·1%).

The methiodide (20 mg.) in methanol (1 ml.) was treated with 70% perchloric acid (1 drop), and ether was added, giving the *methoperchlorate*, prisms, m. p. 248—249° (decomp.) (Found: C, 51·5; H, 5·6. $C_{19}H_{25}ClNO_9$ requires C, 51·0; H, 5·4%).

Didehydrokrigenamine (III).—Krigenamine (100 mg.) in chloroform (50 ml.) was stirred in the presence of manganese dioxide for 3 hr. The solution was filtered and concentrated to *ca.* 10 ml., diluted with benzene (5 ml.), and passed through a short column of alumina. The product (60 mg.), crystallised from wet ethyl acetate, gave didehydrokrigenamine hydrate in prisms, m. p. 70—75° undepressed on admixture with, and showing the same infrared spectrum as, that obtained above. The *methiodide*, prepared in the usual way, crystallised from methanol in prisms, m. p. 254—255° (decomp.) (Found: C, 48·3; H, 5·3. $C_{19}H_{26}INO_5$ requires C, 48·0; H, 5·5%).

Fission Product (III).—(*a*) Krigenamine (200 mg.) in tetrahydrofuran (30 ml.) was refluxed with lithium aluminium hydride (200 mg.) for 24 hr. The solution was poured into water, the mixture was filtered, and the filtrate was extracted with chloroform (3 × 20 ml.). Recovery of the product from the chloroform extract yielded a gum which solidified on trituration with acetone. Two crystallisations from acetone gave the *alcohol* (VI) as needles, m. p. 171—172°, $[\alpha]_D^{20}$ −36° (*c* 1·0) (Found: C, 64·6; H, 6·75. $C_{18}H_{23}NO_3$ requires C, 64·85; H, 6·95%), $\lambda_{max.}$ 282 mμ (ε 1400), $\lambda_{infl.}$ 240 mμ (ε 5220), $\lambda_{max.}$ 6·18 μ.

(*b*) Didehydrokrigenamine hydrate (200 mg.) was treated with lithium aluminium hydride (200 mg.), and the product was worked up as described above, yielding a gum (185 mg.) which crystallised from acetone in needles, m. p. 171—172°, identical (mixed m. p., infrared spectrum, and $[\alpha]_D$) with the above alcohol.

Deoxykrigenamine.—A solution of the alcohol (VI) (170 mg.) in 5% sulphuric acid (25 ml.) was heated for 2 hr. on a water-bath, basified with sodium carbonate, and extracted with chloroform. The extract gave a gum which with ethanolic picric acid gave *deoxykrigenamine picrate*, needles (from ethanol, m. p. 205—206°, $[\alpha]_D$ +123° (*c* 0·82) (Found: C, 53·3; H, 4·7. $C_{24}H_{24}N_4O_{11}$ requires C, 52·9; H, 44%). The picrate in chloroform was passed through a column of alumina, affording *deoxykrigenamine* as a colourless glass which was evaporatively distilled at 110°/0·01 mm. (Found: C, 68·1; H, 6·7. $C_{18}H_{21}NO_4$ requires C, 68·55; H, 6·7%).

Hydrogenation of Krigenamine.—Krigenamine (290 mg.) in glacial acetic acid (35 ml.) was shaken with hydrogen and platinum oxide (80 mg.), 2 mol. being absorbed. The product was a gum (260 mg.), which in benzene–light petroleum (1 : 1; 20 ml.) was chromatographed over alumina (6 g.). Benzene (50 ml.) eluted α-deoxydihydrokrigenamine (I) (30 mg.) as an oil, $[\alpha]_D^{20}$ +23°, which furnished a *perchlorate*, prisms, m. p. 201° (decomp.) (Found: C, 51·6; H, 5·7. $C_{18}H_{24}ClNO_8$ requires C, 51·7; H, 5·8%). Continued elution with benzene (75 ml.) gave a gum which on crystallisation from ether gave β-*deoxydihydrokrigenamine* (IV) as prisms, m. p.

160—162°, $[\alpha]_D^{20}$ +48° (*c* 1·02) (Found: C, 67·95; H, 7·15. $C_{18}H_{23}NO_4$ requires C, 68·1; H, 7·3%).

Catalytic Hydrogenation of Deoxykrigenamine.—Deoxykrigenamine (87 mg.) in acetic acid (5 ml.) absorbed hydrogen (1·1 mol.) in the presence of a platinum catalyst (25 mg.). Working up the product as described above afforded α- (10 mg.) and β-deoxydihydrokrigenamine, m. p. 161—162°, both identified by their infrared spectra.

Conversion of the Alcohol (VI) *into Falcatine Methiodide* (X).—Toluene-*p*-sulphonyl chloride (500 mg.) was added to a solution of the alcohol (500 mg.) in pyridine (7 ml.), and the mixture left for 15 hr. at room temperature. The pyridine was removed under reduced pressure and the residue, dissolved in water, was passed through a column of Amberlite I.R.A.-400 resin (OH^- form). The column was washed with water, and the combined eluate and washings were extracted with ether (3 × 10 ml.), then acidified with dilute hydriodic acid, filtered, and concentrated under reduced pressure. The product crystallised from ethanol, yielding falcatine methiodide as needles (300 mg.), m. p. 262—265° (decomp.) (Found: C, 48·95; H, 4·8. Calc. for $C_{18}H_{22}INO_4$: C, 48·8; H, 5·0%), identical (m. p., mixed m. p., and infrared spectrum) with a sample prepared from falcatine from *N. falcata*.

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C.S.I.R. Natural Products Research Unit,
Department of Chemistry, University of Natal,
Pietermaritzburg, South Africa.

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