506. *The Indole Alkaloids. Part V.*[1] *The Structure of Henningsoline*

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The structure of henningsoline from *Strychnos henningsii* Gilg. is shown to be 12-hydroxy-11-methoxydiaboline or a diasteroisomer. The structure is based on a study of ultraviolet, infrared, and nuclear magnetic resonance spectra and of the mass spectra of henningsoline, deoxytetrahydrohenningsoline, and diaboline.

HENNINGSOLINE, $C_{22}H_{26}N_2O_5$, the phenolic alkaloid from *Strychnos henningsii* Gilg.[1] showed an ultraviolet spectrum indicative of an *N*-acylindoline. The infrared and nuclear magnetic resonance (n.m.r.) spectra showed an aromatic methoxy-group (λ 8·0 μ, δ 3·90 p.p.m.) and an *N*-acetyl group (λ 7·28 μ, δ singlet 2·47 p.p.m.), which were confirmed by analysis. The presence of two adjacent hydrogen atoms on the aromatic ring was indicated by absorption at λ 12·50 μ whilst δ values between 6·00 and 8·30 showed two hydrogens as an AB quartet. The phenolic hydroxy-group was placed at C-12 to account for the hydrogen-bonded amide carbonyl group (λ 6·12 μ) which necessitated placing the methoxyl at C-11. Confirmation of the phenolic character was obtained by coupling of henningsoline in alkaline solution with diazotised *p*-nitroaniline to give a red azo-dye, and by the pronounced bathochromic shift[2] in alkaline solution, namely, $\lambda_{\text{max.}}$ (EtOH) 257 to $\lambda_{\text{max.}}$ (NaOH) 304 mμ.

Attempts to remove the phenolic hydroxyl group by reduction of henningsoline toluene-*p*-sulphonate gave mixtures. Methylation of the strongly hydrogen-bonded phenol was not effected with diazomethane. The alkaloid, with dimethyl sulphate in dry acetone,

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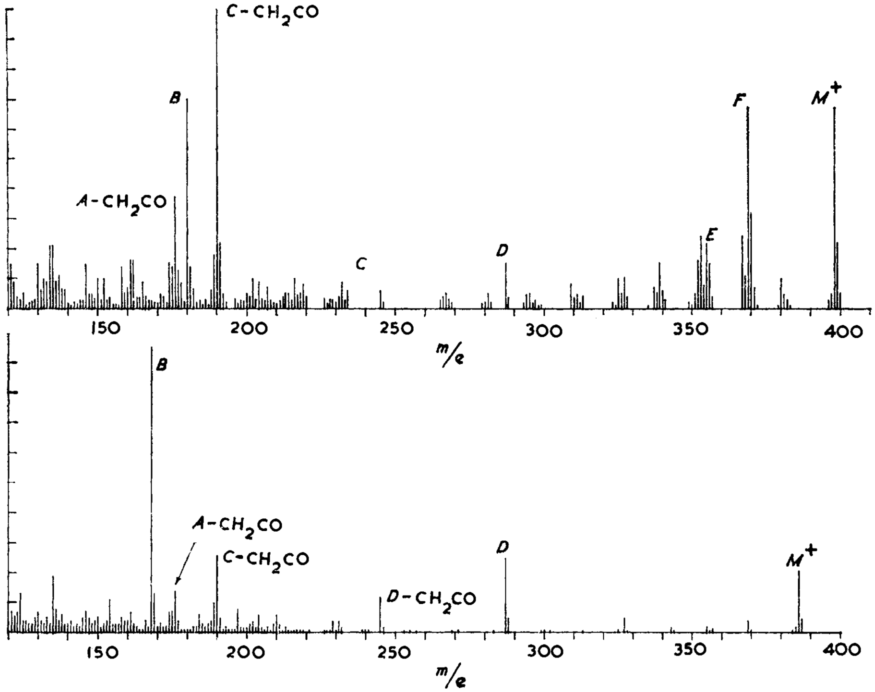
[1] Part IV, K. Biemann, J. S. Grossert, J. M. Hugo, J. Occolowitz, and F. L. Warren, preceding Paper. This Paper also forms Part XXIX of Application of Mass Spectrometry to Structure Problems, Part XXVIII, preceding Paper.

[2] N. D. Coggeshall and A. S. Glessner, *J. Amer. Chem. Soc.*, 1949, **71**, 3150.

gave intractable material, and treatment with dimethyl sulphate in alkaline solution led to *O*-methylation and quaternisation of the tertiary nitrogen to give a fully methylated methosulphate isolated as its *OO*-dimethylhenningsoline methoreineckate $C_{25}H_{33}N_2O_5[Cr(CNS)_4(NH_3)_2]$. Both these methods were used successfully for the methylation without quaternisation of the *Aspidosperma* alkaloids in which the lone pair of electrons on the nitrogen is sterically hindered. The Hofmann reaction on the methohydroxide gave a crystalline methine, $C_{25}H_{32}N_2O_5,\frac{1}{2}H_2O$, but in small yield. The ethobromide, isolated as its ethoreineckate, was prepared with a view to further methylation and removal of the ethyl group by way of the Hofmann reaction; but the ethohydroxide gave an intractable product.

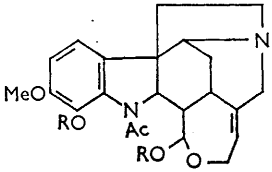


Mass spectrum of henningsoline (upper Figure) and deoxytetrahydrohenningsoline (lower Figure)

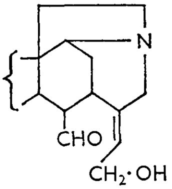
The molecular ion in the mass spectrum of henningsoline (I) at *m*/*e* 398 suggested the molecular formula $C_{22}H_{26}N_2O_5$. In contrast to henningsamine,[1] the peaks characteristic of unsubstituted dihydroindole alkaloids, namely *m*/*e* 144 and 130 were not present at significant intensity. On the other hand, intense peaks at *m*/*e* 176 and 190, *i.e.*, 130 and 144 plus 46, respectively, suggested a substituted dihydroindole structure.

On acetylation with $[^2H_6]$acetic anhydride, henningsoline gave a bistrideuteroacetyl derivative (II) (*M*, 488), *i.e.*, two hydrogens replaced by two $CD_3{\cdot}CO$ groups resulting in the observed increase of 90 m.u. This required the presence of two hydroxy groups in the molecule and was confirmed by the preparation of *O*-benzoyl-, $C_{29}H_{30}N_2O_6,H_2O$, and di-*O*-benzoyl-henningsoline, $C_{36}H_{34}N_2O_7,\frac{1}{2}H_2O$. The peaks at *m*/*e* 176 and 190 assigned to the indole moiety in henningsoline were still present in the spectrum of compound (II) but were less intense than new peaks at *m*/*e* 177 and 191, respectively, showing that one of the hydroxyl groups was present on the aromatic moiety and the elimination of $[^2H_2]$-keten (similar to the observed elimination of keten from phenyl acetate) had resulted in the transfer of deuterium to the aromatic ring.

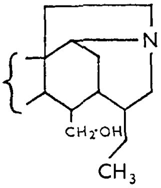
Hydrogenation of henningsoline over platinum gave a mixture of products with molecular weights 402, 400, 386, and 384; and prolonged hydrogenation (2 hr. at room temperature) followed by fractionation in the inlet of the mass spectrometer showed the component *M*, 386, deoxytetrahydrohenningsoline, to be the major product (Figure). The spectrum of this compound is similar to those obtained from strychnospermine[3] and related compounds,[4] where the ion *B* due to the alicyclic moiety forms the most intense peak of the spectrum and the ions *A* and *C* (or their products after elimination of keten from an *N*-acyl group) occur at lower intensity. The loss of 12 m.u. (398 ⟶ 386) upon hydrogenation of henningsoline and the formation of a smaller amount of a compound which had absorbed two moles of hydrogen without loss of oxygen suggested, as it did in the case of henningsamine,[1] the presence of a Wieland–Gumlich type structure. The most intense peak in the spectra of deoxytetrahydrohenningsoline and of deoxytetrahydrodeacetylhenningsamine[1] both occur at *m/e* 168 (*B*) indicating a similar alicyclic moiety. On this evidence structure (I or Ia) is suggested for henningsoline. Formula (Ia) accounts for the formation of an oxime, and the secondary nature of the hydroxyl group in (I) was revealed by the comparison of the n.m.r. spectra of henningsoline and diacetylhenningsoline when it was seen that one proton was shifted downfield by 1 p.p.m.[5] On hydrogenation over platinum simultaneous hydrogenation and hydrogenolysis of the vinyl alcohol group would result in (III) which lacks the unsaturated seven-membered ether ring and thus gives a more typical demethoxycarbonyldihydroakuammicine or strychnospermine spectrum.[3,4]



(I : R = H)
(II : R = CO·CD$_3$)



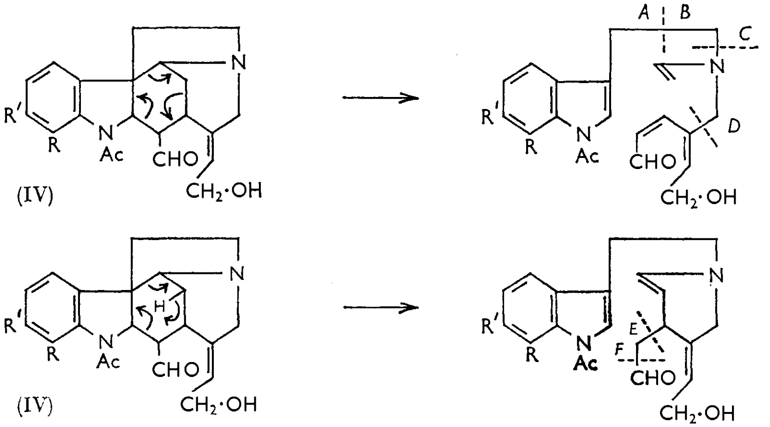
(Ia)



(III)

The presence of an acetyl group at the indole nitrogen was confirmed by a peak at *m/e* 43 (Ac) in the spectra of henningsoline and of compound (III), the (*D* — 42) ion in the spectrum of the latter, and the low carbonyl stretching frequency, 1653 cm.$^{-1}$.

Further confirmation of the assigned structure for henningsoline (IV; R = OH, R′ = OMe) was obtained by the comparison of its spectrum with diaboline (IV; R = R = H) (Figure) both of which may fragment as follows.



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[3] K. Biemann, " Mass Spectrometry, Organic Chemical Applications," McGraw-Hill, New York, 1962, ch. 8.

[4] K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *Tetrahedron Letters*, 1961, 485; H. Budzikiewicz, J. M. Wilson, C. Djerassi, J. Levy, J. LeMen, and M.-M. Janot, *Tetrahedron*, 1963, **19**, 1265.

[5] L. M. Jackman, " Applications of Nuclear Magnetic Resonance Spectra in Organic Chemistry," Pergamon, London, 1959.

The peak at $M - 18$ (loss of H_2O) in the spectrum of henningsoline increases in intensity with increasing inlet temperature and is probably due wholly to pyrolysis.

EXPERIMENTAL

Colour Reactions of Henningsoline.—Henningsoline gave typical colour reactions[6] of the indoline chromophore, the Otto colour test similar to strychnine, a pale olive green with Ehrlich's reagent suggestive of α- or β-hydrogen in the indole nucleus, and a ceric sulphate[7] test similar to strychnine (red to brown) but different in that the colour eventually turned bright green.

Henningsoline.—This had $\lambda_{\text{max.}}$ 223, 256 mμ (log ε 4·42, 3·84), $\lambda_{\text{infl.}}$ 285 mμ (log ε 2·9); λ (KBr) 2·85 (aliphatic OH), 6·12 (hydrogen-bonded amide CO), 7·28 (Me of NAc), 8·0 (aromatic OMe), 12·50 μ (aromatic ring, 2 adjacent hydrogens), δ ($CDCl_3$), 0—2·40 (5H), 2·40—3·00 (5H: NAc singlet 2·47, singlets, 2·59, 2·84), 3·00—3·70 (3H), 3·70—4·00 (5H: OMe singlet, 3·90), 4·00—5·2 (3H), 5·20—6·00 (2H: singlet 5·3; 1H triplet, 5·93, J = 8 c./sec.), 6·00—8·30 (2H: AB quartet, 6·59, 6·80, J = 8 c./sec.).

Deoxytetrahydrohenningsoline.—(*a*) Henningsoline (480 mg.) in glacial acetic acid and platinum oxide absorbed hydrogen (79 ml., 2 mol.) in 7 hr. The product, crystallised from acetone, gave *deoxytetrahydrohenningsoline* as needles, m. p. 248—249° (Found: C, 67·9; H, 7·7. $C_{22}H_{30}N_2O_4,\frac{1}{4}H_2O$ requires C, 67·6; H, 7·9%).

(*b*) Henningsoline (890 mg.), ethanol, and B.D.H. Raney nickel stabilised in paraffin wax were refluxed for 2 hr. in a stream of hydrogen. The product, crystallised from acetone, gave needles, m. p. 248—249°, undepressed on admixture with deoxytetrahydrohenningsoline, R_F 0·74, pK_a *ca.* 7·5, $\lambda_{\text{max.}}$ 225, 255, 285 mμ (log ε 4·4, 3·8, 3·3).

*Di-*O*-methylhenningsoline Methoreineckate.*—Henningsoline in 2N-sodium hydroxide was treated with dimethyl sulphate and the product, in hydrochloric acid, treated with ammonium reineckate. The di-*O*-methylhenningsoline methoreineckate crystallised from acetone–water as pink needles, λ (KBr) 3·19 (NH), 5·85 (CHO), 6·12 μ (amide CO) (Found: C, 45·6; H, 5·5; Cr, 7·2; OMe, 8·5. $C_{29}H_{39}CrN_8O_5S_4$ requires C, 45·85; H, 5·2; Cr, 6·8; 2OMe, 8·2%).

*Hofmann Reaction with Di-*O*-methylhenningsoline Methohydroxide.*—Di-*O*-methylhenningsoline methoreineckate in 80% acetone was percolated through Amberlite IRA 400 resin (OH^- form). The strongly basic product (91 mg.) was heated to 140°/0·2 mm. for 40 min. and extracted with chloroform. The chloroform extract was evaporated and chromatographed over alumina (1·2 g.) and eluted with 1% ethyl acetate in benzene to give *OO*-dimethylhenningsoline methine (15 mg.) which crystallised from light petroleum as prisms, m. p. 121—122° (Found: C, 66·8; H, 7·5. $C_{25}H_{32}N_2O_5,\frac{1}{2}H_2O$ requires C, 66·8; H, 7·4%).

Monobenzoylhenningsoline.—(*a*) Henningsoline, pyridine, and benzoic anhydride were set aside for 12 hr. and then warmed to 100° for 30 min. The product, chromatographed on alumina, eluted first with ethyl acetate, and then 1% methanol in chloroform gave *henningsoline monobenzoate* which crystallised from acetone as needles, m. p. 180—182°; λ 5·7 (ester CO), 6·0 μ (amide CO) (Found: C, 67·4; H, 6·1. $C_{29}H_{30}N_2O_6,H_2O$ requires C, 66·9; H, 6·1%).

(*b*) Benzoylation with benzoyl chloride (1 mol.) and chromatography of the product on alumina, eluted first with ethyl acetate, and then with chloroform, gave O*-benzoylhenningsoline hydrochloride* which crystallised from benzene as needles, m. p. 171—172°, and, after heating at 96°/0·1 mm., m. p. 175—177°; $\lambda_{\text{max.}}$ (EtOH), 280 mμ (log ε 4·6), $\lambda_{\text{max.}}$ (1% alcoholic KOH) 305 mμ (log ε 4·6); λ 2·9 (OH), 5·85 (ester CO), and 5·9 μ (amide CO) (Found: C, 62·7; H, 5·7; N, 5·1; O, 21·9. $C_{29}H_{31}ClN_2O_6,H_2O$ requires C, 62·5; H, 6·0; N, 5·0; O, 20·1%).

*Di-*O*-benzoylhenningsoline.*—Henningsoline (419 mg.), pyridine (15 ml.), and benzoyl chloride (2 ml.) gave a product which, chromatographed on alumina, washed with ethyl acetate and eluted with chloroform, gave *di-*O*-benzoylhenningsoline* which crystallised from benzene–methanol as rhombohedra, m. p. 214—220°, λ 5·79, 5·81 (ester CO), and 6·02 μ (amide CO) (Found: C, 70·4; H, 5·8. $C_{36}H_{34}N_2O_7,\frac{1}{2}H_2O$ requires C, 70·2; H, 5·7%).

Henningsoline oxime crystallised from methanol–acetone as prisms, m. p. 254—255°; $\lambda_{\text{max.}}$ 208, 225, 256 mμ (log ε 4·4, 4·5, 3·9); λ (KBr) 2·9, 2·99 (OH, NH), 6·14 μ (amide CO).

*Henningsoline toluene-*p*-sulphonate* crystallised from methanol–acetone as needles, m. p. 233—234°, R_F 0·85 (Found: C, 62·6; H, 6·1. $C_{29}H_{32}N_2O_7S$ requires C, 63·0; H, 5·8%).

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[6] A. Weissberger and K. W. Bentley, "Techniques in Organic Chemistry," Interscience, New York, 1963, vol. XI, p. 40.

[7] A. R. Battersby and H. F. Hodson, *Quart. Rev.*, 1960, **14**, 77.

9-(p-*Nitrophenyldiazo*)*henningsoline* crystallised from methanol–water as red prisms, m. p. >300° (decomp.) (Found: C, 56·4; H, 5·6. $C_{28}H_{29}N_5O_7,2\frac{1}{2}H_2O$ requires C, 56·8; H, 5·8%).

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