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THE STRUCTURE AND STEREOCHEMISTRY OF CLIVONINE

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Abstract—The structural and stereochemical features of the lactone alkaloid clivonine (**9**, R = O, R′ = H) are elucidated from a detailed analysis of the PMR spectra of the base and its O-acetyl derivative. Complimentary evidence for the assigned structure is presented from the results of the study of the Von Braun reaction on clivonine and some of its derivatives. A simple sequence of reactions which involves the conversion of clivonine to the known lactone alkaloid **8** by treatment the chlorolactone **16** with base then acid is described. This conversion substantiates the relative stereochemistry provided by the PMR spectral studies and establishes the absolute stereochemistry of clivonine as depicted

THE lactone and hemi-acetal alkaloids of the *Amaryllidaceae* which are based upon the [2]benzopyrano[3,4*g*]indole skeleton form one of the major sub-groups of alkaloids produced by this botanical family.[1] Recent structural investigations of these alkaloids by chemical procedures have generally followed methods pioneered by Uyeo and *et al.*[2] in which conversion of the lactone or hemi-acetal base to a derivative possessing the lycorine-type skeleton is accomplished. The success of this approach relies upon the fact that the lycorine-type alkaloids are not only more extensive as a group but also better characterized than alkaloids of the other sub-groups. Homolycorine (**1**) and hippeastrine (**2**), two of the most abundant lactones in this family, were the first to have their structure placed upon a firm basis using this approach by their conversion to the methiodides of pluviine (**3**) and lycorine (**4**), respectively.[2]

Although the usefulness of the interconversion of the lactone series to the lycorine type as an approach to structural elucidation is attested to by the success achieved in the past, it has obvious limitations.‡ It was with this in mind that we set out some years ago to make a systematic study of the application of mass spectral[3] and PMR methods[4] for structure elucidation in the lactone–hemi-acetal sub-group. The results obtained have shown that these two spectral methods are of tremendous utility for characterization of alkaloids belonging to this series. Of the members which have been subjected to critical examination by these methods, the [2]benzopyrano[3,4*g*]indole skeleton in these alkaloids is found to exist in a single stereochemical modification in which a *cis* B:C ring and a *trans* arrangement of the C-11b and C-11c hydrogens prevails (*cf* **1** and **2**). The studies

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‡ A case in point is the alkaloid clivonine, the subject of this paper, in which the corresponding base in the lycorine series is not known (*vide infra*).

reported in this paper demonstrate that the lactone alkaloid clivonine belongs to a different stereochemical series.[5]

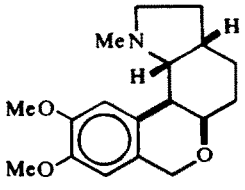
In an early investigation of the plant *Clivia miniata* Regel, Wildman *et al.*[6] isolated a new lactone alkaloid, clivonine, $C_{15}H_{19}NO_5$ which was assigned the tentative structure **5**. Subsequently, Mehlis presented evidence for the *cis* relation of the C-5 and C-5a oxygen functions in clivonine. This assignment was based on the relatively fast rate of periodate cleavage of the LAH reduction product of clivonine in comparison to the cleavage of the corresponding compound derived from hippeastrine. A *cis* B:C ring juncture was assumed, and a structure portraying the absolute stereochemistry as depicted in **6** was derived by application of the empirical Hudson–Klyne lactone rule. The co-occurrence of clivonine with hippeastrine and three new bases, clivatine $C_{21}H_{25}NO_7$, clivimine $C_{43}H_{43}N_3O_{12}$ and minatine $C_{45}H_{47}N_3O_{15}$ in *C. miniata* was reported from a further study of the alkaloids of this plant.[8] The presence of three N atoms in the latter two bases places them in a novel category in this family since all other Amaryllidaceae alkaloids possess a single N atom and a central carbon skeleton embodying 15 C atoms.

Our interest in clivonine arose from the fact that it was reported as the ester component of clivimine, which was attributed[7] the symmetrical 2,6-lutidine-3,5-dicarboxylic ester structure **7**. This proposal was supported by the alkaline hydrolysis of the alkaloid which led to isolation and characterization of 2,6-dimethyl-3,5-dicarboxypyridine, together with a hydroxy amino acid which afforded clivonine on treatment with acid. The products derived from LAH reduction of clivimine also gave products in accord with this structure.

The diagnostic utility of mass spectrometric methods for characterization of the gross structural features of alkaloids of the [2]benzopyrano]3,4*g*]indole series has been previously demonstrated.[3] The close similarity of the mass spectral fragmentation pattern of clivonine and 5α-hydroxymasan-7-one (**8**) (α-dihydrohippeastrine)* has

* The conventions for naming the compounds derived from the [2]benzo-pyranol 3,4-*g*]indole ring system have evolved as a result of the interrelationship of the stereochemistry of the compounds of this series with the stereochemistry of the alkaloids of the lycorine series.[2] While these conventions were reasonable when only a few compounds were involved, they have become somewhat cumbersome with the recent expansion of the number of compounds in the series. In particular, the designation of the stereochemistry at C-3a is in direct conflict with the normal steroid convention. In this paper a new convention will be used which is consistent with that in more general use.

Compounds in this series incorporate aryl oxygen functions at both the C-9 and the C-10 positions either as a methylenedioxy group or a pair of OMe groups. It is proposed that two series may account for most of the known compounds of the family and their derivatives. The parent systems for each series will be the cyclic ethers **i** and **ii**, referred to as homolycorane and masanane, respectively.



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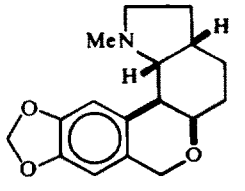
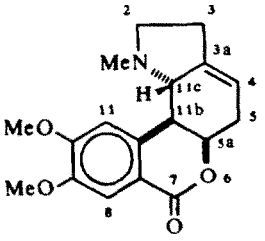


ii

Inversion of the stereochemistry at a ring juncture will be noted by the prefix x-*epi*-, where x is the ring position of concern. Unsaturation will be denoted, as in steroidal systems, by the suffix y, where y denotes the location of the double bond. Configuration at positions other than the ring junctures will be indicated by the normal convention, where α- indicates that the group is below the plane of the ring, while a group above the plane of the ring is designated by β-.



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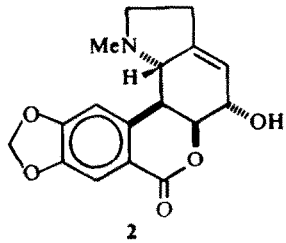

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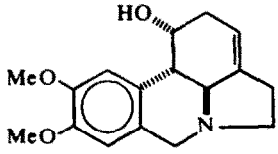

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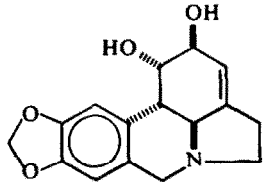

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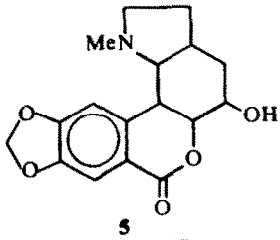

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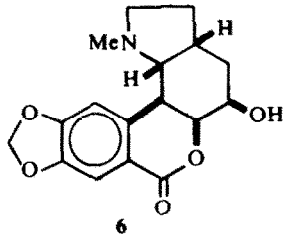

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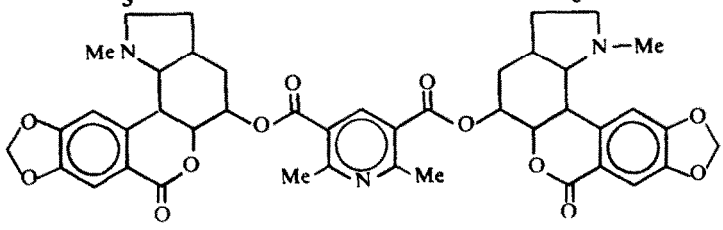
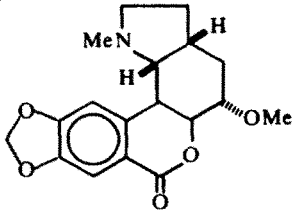


For derived systems in which ring fission has occurred, the normal IUPAC nomenclature may be applied. Ring fission with addition of a hydrogen atom at each new terminal group thus created will be indicated by prefacing the name of the parent compound with m,n-*seco*-, where the bond between positions m and n has been broken. The numbering in the derived system will be the same as in the parent compound. Hence, LAH reduction of 5α-methoxymasan-7-one, (iii), would produce the diol, iv, which would be properly named 7-hydroxy-5α-methoxy-6,7-*seco*masanane. Note here that although the starting material was a lactone, the product, by convention, is named as the dihydro-cleavage product of the hemi-acetal.



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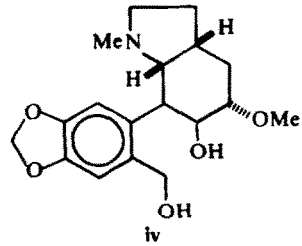
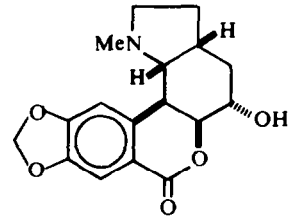


iv

In those cases where a trivial name has been in common use for some time and is unambiguous in its designation, it will be retained for use in this and subsequent papers i.e., candimine and hippeastrine will be used. However, where the old designation is misleading, or when a trivial name has been applied, the new nomenclature will be used, i.e., 5α-hydroxymasan-7-one is used instead of α-dihydrohippeastrine.



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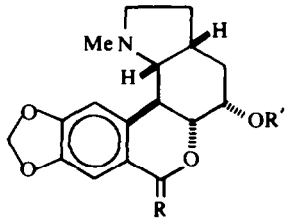

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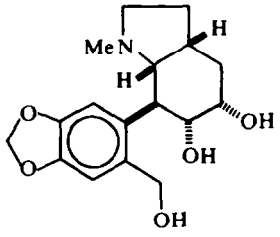

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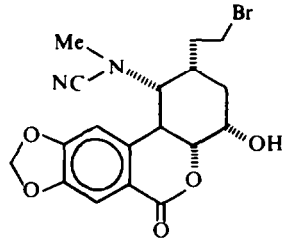

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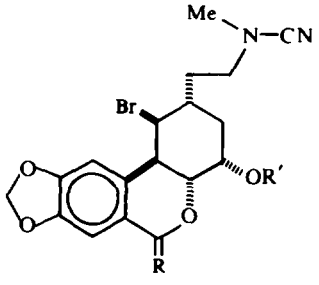

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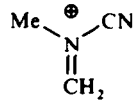

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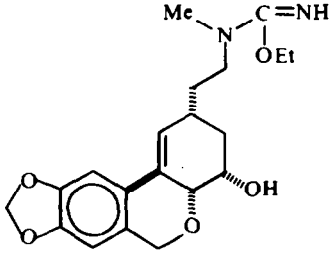

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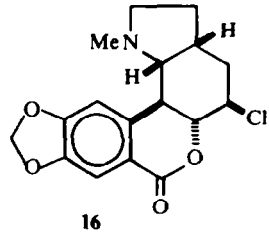

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provided clear evidence that the tentative structural assignment of clivonine made by Wildman and co-workers is correct. Thus, the questions remaining concern its relative and absolute stereochemistry. PMR spectral studies of clivonine and its O-acetyl derivative (5, R = Ac) have been undertaken in an effort to define the relative stereochemistry of this alkaloid.

The 100 MHz spectra of clivonine and O-acetylclivonine are shown in Figs 1 and 2, respectively. The spectra show typical signals associated with N-Me, methylenedioxy

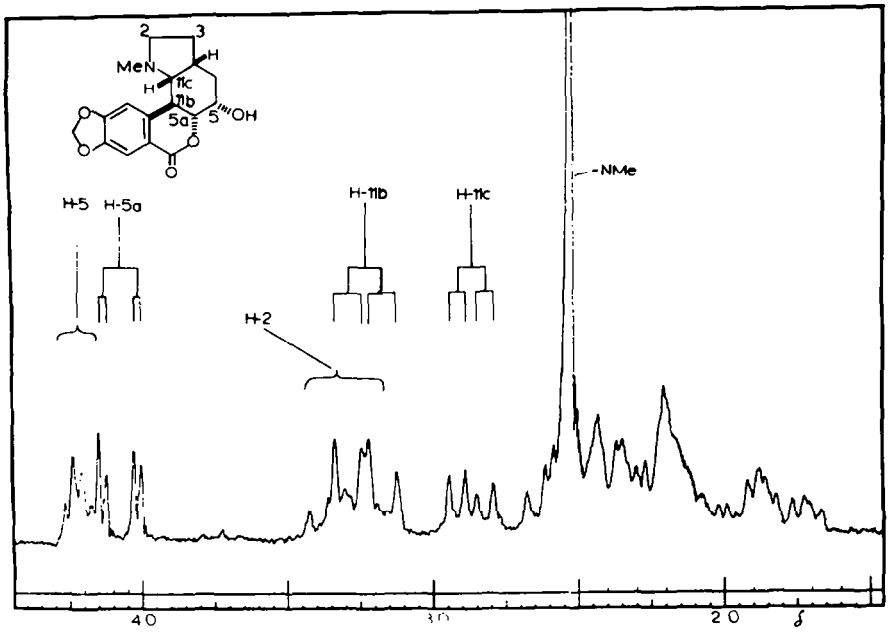


FIG 1. Partial PMR Spectrum of Clivonine (9, R = O, R′ = H).

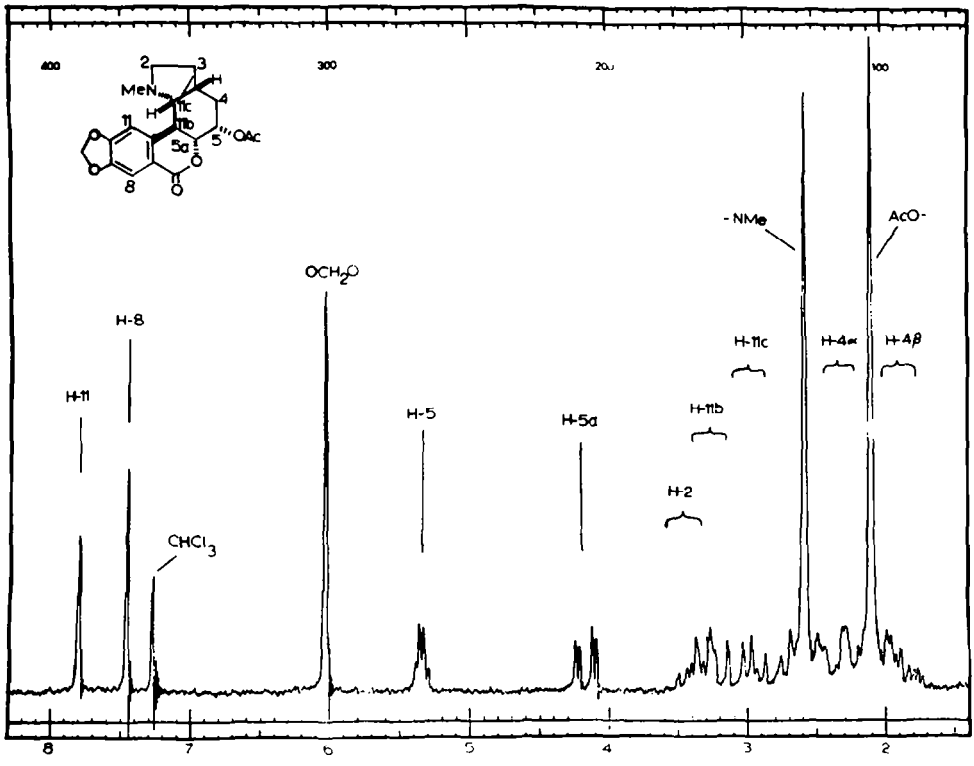


FIG 2. PMR Spectrum of O-Acetyl Clivonine (9, R = O, R′ = Ac).

groups and of two isolated aryl hydrogens located next to an oxygen function. Furthermore the general features of the spectra are characteristically similar to the spectra of other lactones of the sub-group.[4]

Assignment of the C-5 H signal to the multiplet at 4·18 δ in clivonine is supported by the paramagnetic shift of this signal to 5·35 δ in the spectrum of the acetate. In both spectra the width at half-height of this signal is less than 10 Hz and attest to its equatorial nature. Of considerable significance is the nature of the signal attributable to the C-5a H resonance which appears as a double doublet at 4·06 δ in the spectrum of clivonine and at 4·10 δ in the acetate. The appearance of the signal indicates it is weakly coupled to one proton and strongly coupled to another. Irradiation of the C-5 H resonance of the acetate at 5·35 δ in a frequency sweep decoupling experiment (Fig 3) removed the small coupling and left the signal at 4·16 δ as a doublet. This result confirms the assignment of the C-5a H signal and affords the important couplings $J_{5,5a} = 3{\cdot}0$ Hz and $J_{5a,11b} = 12{\cdot}5$ Hz. The 12·5 Hz coupling between the hydrogens at C-5a and C-11b clearly implies that they are *trans* diaxial and consequently clivonine must possess a *trans* B:C ring juncture.

The *cis* relationship between the C-5 and C-5a oxygen functions in clivonine suggested from the rates of periodic cleavage of the LAH_4 reduction products cited earlier is confirmed by the 3·0 Hz coupling between the hydrogens at these positions in the O-acetyl clivonine spectrum.

Information on the stereochemical relationship of the C-11b, C-11c, and C-3a hydrogens was most readily obtained from the spectrum of clivonine in which the C-11b H appears as a double doublet at 3·23 δ with apparent couplings of 12·0 and 9·5 Hz. The larger value is reflected in the C-5a H signal at 4·09, so the 9·5 Hz coupling may be assigned confidently to $J_{11b,11c}$. A one proton signal is observed at 2·87 δ with apparent couplings of 9·5 Hz and 5·8 Hz and this is most reasonably assigned to the C-11c H resonance. Since the 9·5 H coupling is reflected in the C-11b signal a *trans* relationship of the hydrogens at C-11b and C-11c is implied. It follows that since the C-11c hydrogen is axial in character the 5·8 Hz coupling represents $J_{11c,3a}$ and may be interpreted as being indicative of the equatorial nature of a *cis* oriented C-3a hydrogen. Corroborative evidence for these assignments which lead to the stereochemical expression (**9**, R = 0, R = H) for clivonine was obtained from the spectrum of O-acetylclivonine (Fig 3) in which decoupling of C-5 H permitted an analysis of the ABX sub-spectrum originating from C-3a and the C-4 methylene protons giving $J_{4\alpha,4\beta} = 15{\cdot}5$ Hz, $J_{4\alpha,3\alpha} = 3{\cdot}5$ Hz and $J_{4\beta,3\alpha} = 6{\cdot}5$ Hz. These values are in best accord with an equatorial orientation of the C-3a hydrogen.

Chemical shift values for the C-8 and C-11 aromatic hydrogens in the clivonine series occur at significantly lower field than for the corresponding hydrogens in the other lactone members of this family. This is particularly true of the C-11 hydrogen which appears at even lower field than the C-8 hydrogen in clivonine, despite the fact that latter experiences a strong deshielding from the lactone carbonyl. The assignment of the C-11 H to the lower field signal is supported by a comparison of the chemical shifts of the C-8 and C-11 hydrogens in the cyclic ether **9** (R = H_2, R′ = H), which is obtained by acid catalyzed cyclization of the LAH reduction product **10**, with the analogous signals in clivonine spectrum.

We feel that the most reasonable explanation of the strong deshielding of the C-11 hydrogen is occasioned by its close proximity to the nitrogen atom in ring D. Some

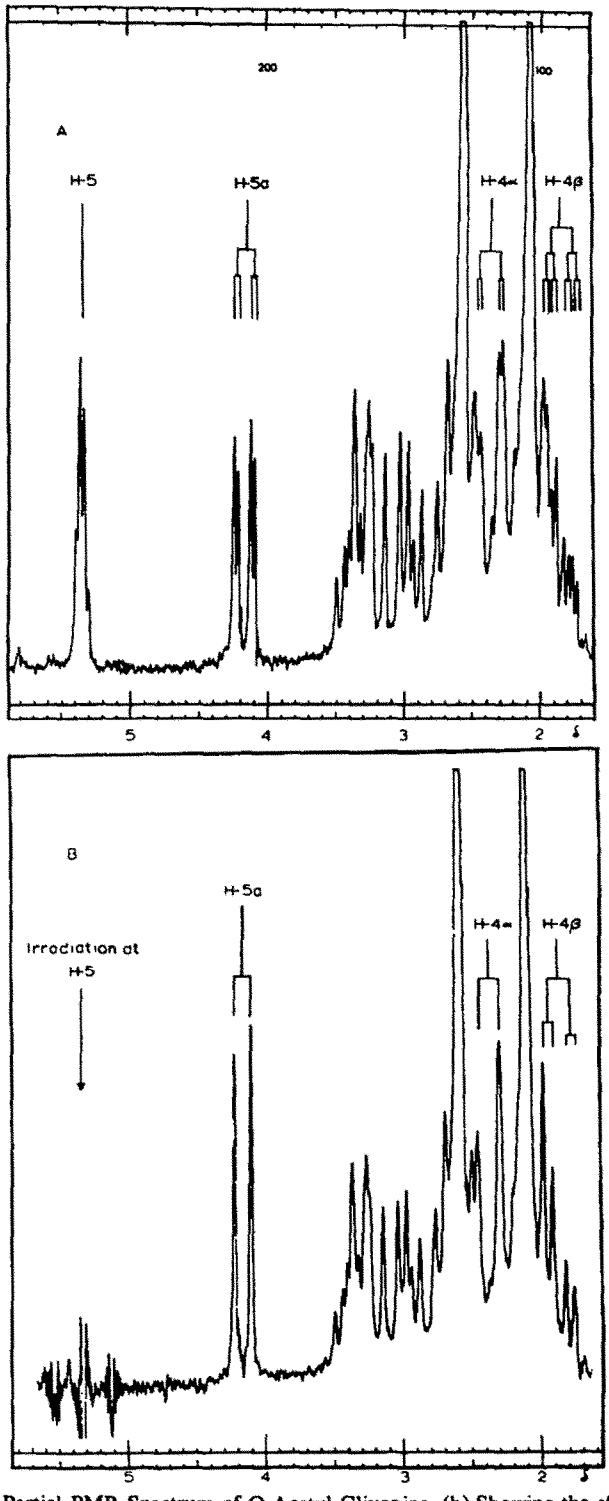
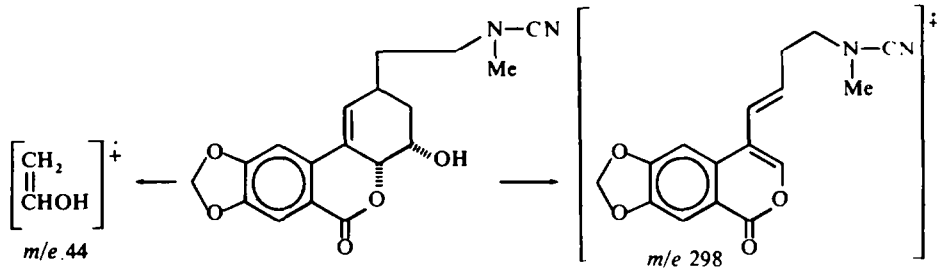


FIG 3. (a) Partial PMR Spectrum of O-Acetyl Clivonine. (b) Showing the effects of Spin decoupling the C-5 hydrogen signal.

support for this suggestion may be inferred from the PMR spectrum* of the ring B *seco* compound **10** in which the aryl hydrogen signals appear at 6·82 δ and 6·85 δ.

In an effort to gain chemical evidence to support the stereochemical assignments of clivonine derived from the foregoing PMR spectral studies several approaches were tried. The first of these involved a study of the Von Braun reaction of clivonine and several of its derivatives. Clivonine reacts with cyanogen bromide in benzene to give a single product in good yield. Of the three possible structures which might originate from this reaction one of these is readily eliminated by the observation of an N-Me signal at 2·87 δ in the spectrum of the product. This leaves structures **11** and **12** (R = O, R′ = H) as the only obvious candidates.

The mass spectrum of the Von Braun product does not show a molecular ion and the ions of highest mass occur at *m/e* 343 and 342. These correspond to the loss of Br and HBr, respectively, from the molecular ion. An accurate mass measurement of the peak at *m/e* 342 confirmed its predicted elemental compostion as $C_{18}H_{20}N_2O_4$ and a scan of the mestastable spectrum of this ion showed a weak parent ion which appeared at *m/e* 422·4, in accord with the value calculated for structures **11** or **12** (R = O, R′ = H). A strong fragment ion appears at *m/e* 69 and consideration of its structure as the even electron species **13** is supported by a high resolution mass measurement which establishes its elemental composition as $C_3H_5N_2$. The presence of this ion is readily explained by the occurrence of a simple α-cleavage process from the molecular ion derived from **12** (R = 0, R′ = H) and constitutes firm evidence for representing the Von Braun product as having this structure rather than the alternative **11**. The base peak at *m/e* 44 and the abundant ion at *m/e* 298 are accounted for by separate pathways corresponding to the retro-Diels–Alder processes represented below.



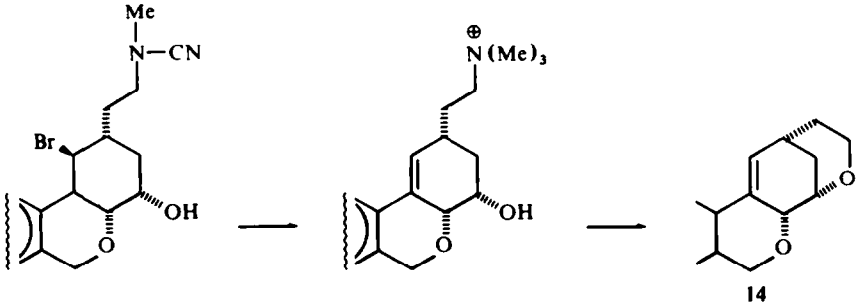
The PMR spectrum of this compound lends strong support for structure **12** (R = 0, R′ = H) and provides evidence for the stereochemical features. A one-proton doublet at 4·70 δ with apparent couplings of 2·5 and 11·5 Hz together with a second one-proton double doublet at 3·62 δ with splittings of 3·5 and 11·5 Hz are assigned to C-5a and C-11b protons, respectively. It will be noted that the C-5a H-signal is located further

* The chemical shift of the *N*-methyl signal in this compound occurs at abnormally high field (1·84 δ) and suggests that the preferred conformation is one in which the *N*-Me group is situated over the center of the aromatic ring. This result is in accord with previous studies[4] in which kinetic data on the rates of methiodide formation of ring B *seco*-compounds in this series led to the conclusion that the N-Me group is less hindered than in the parent compounds in which ring B is intact.

downfield in the Von Braun product than the corresponding signal in clivonine. This paramagnetic shift is readily explained on the basis of deshielding occasioned by the C-11 α-oriented bromo-substituent, which is in a 1,3-diaxial relation to the C-5 α hydrogen. The relative stereochemistry at the C-11c-position with respect to the adjacent C-11b position in **12** (R = O, R′ = H) is uniquely determined from the C-11c H-signal which appears as a narrow multiplet (W_+ 7·0 Hz) at 4·82 δ. Irradiation at 4·82 δ causes a collapse of the C-11b signal to a doublet $J_{5a,11b} = 11{\cdot}5$ Hz in agreement with the *trans*-diaxial arrangement of the hydrogens at C-5a and C-11b. Hence the smaller coupling noted earlier in the C-11b H-signal, 3·5 Hz, may now be confidently assigned to $J_{11b,11c}$ and attests to the *cis*-relation of the hydrogens at C-11b and C-11c. Unfortunately the small coupling which exists between the hydrogens at C-11c and C-3a does not permit an assignment of their relative stereochemistry. However, the fact that inversion of configuration at C-11b which must occur in the Von Braun reaction leads to only a small reduction in the magnitude of $J_{11c,3a}$ in **12** (R = O, R′ = H) as compared to this coupling in clivonine itself is circumstantial evidence in favor of the stereochemistry portrayed.

The Von Braun reaction with O-acetyl clivonine (**9**, R = O, R′ = Ac) and the cyclic ether, 5α-hydroxy-5a-*epi*masanane (**9**, 4 = H_2, R′ = H) afforded analogous products to that produced from clivonine and are formulated as **12** (R = O, R′ = Ac) and **12** (R = H_2, R′ = H), respectively.

The Von Braun product **12** (R = H_2, R′ = H) was of particular interest in that it seemed to offer a means of corroborating the stereochemical relationship of the C-5 OH and the C-3a—C-2 bond which had been derived from the PMR studies on clivonine. Conversion of the N-cyano function to an —$\dot{N}(Me_3)$ group and treatment of the product with base would be expected to lead to internal nucleophilic displacement at C-2 by the C-5 OH to give the ether **14**. In view of the conformational restrictions imposed on ring C by a *trans* B:C ring junction the formation of **14** would require a *cis* relation of the C-5 hydroxyl and the C-3a ethanamine side chain.



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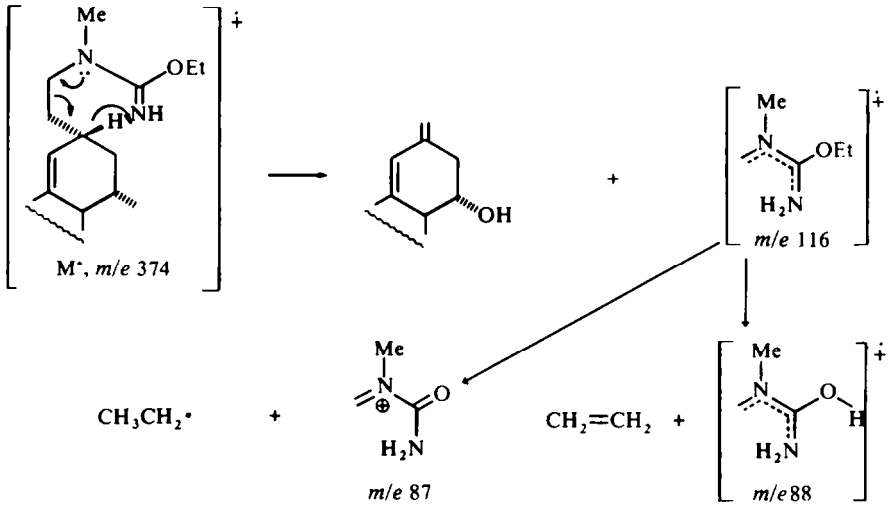
Treatment of the bromocyanamide **12** (R = H_2, R′ = H) with alcoholic potassium hydroxide effected rapid dehydrobromination as evidenced by the immediate precipitation of potassium bromide. The product, m.p. 115–7°, however, was shown to possess the formula $C_{20}H_{26}N_2O_5$ by a high resolution mass measurement of the molecular ion, and indicated that the reaction had occurred with loss of HBr and addition of ethanol. The presence of an O-Et group was readily ascertained from the PMR spectrum. The UV

spectrum possessed absorptions characteristic of a methylenedioxystyrene chromophore,[9] which confirmed that the double bond is at the expected C11b-C11c position. Corroborative evidence for this assignment is provided by the paramagnetic shift (0·3 δ) observed for the C-11 aryl hydrogen resonance upon generation of the double bond in this compound and by the complimentary deshielded value (6·07 δ) of the C-11c olefinic hydrogen signal, which is approximately coplanar with the aromatic ring in this system. The foregoing data suggested that the hydrolysis had proceeded to give the O-ethylisourea **15**. The mass spectrum provides firm support for this structure. The base peak occurs at *m/e* 116, and an accurate mass measurement shows this to correspond to $C_5H_{12}N_2O$. Aside from the molecular ion the only other peaks of any appreciable abundance occur at *m/e* 88 and *m/e* 87. The origin of these three major fragments is readily explained on the basis of structure **15** by unexceptional processes as summarized in Scheme 1. The direct decomposition of the ion *m/e* 116 to the ion *m/e* 88 is supported by the observation of a metastable peak at 66·8 (calc. $88^2/116 = 66·7$).

SCHEME 1



Unfortunately, attempts to hydrolyse the imino ether with acid were unsuccessful and led to recovery of starting material. Acid hydrolysis of the Von Braun products **12** (R = O, R′ = H) and **12** (R = H_2, R′ = H) with 30% sulfuric acid afforded complex mixtures and LAH reduction gave a product in which the N-cyano group was still present. Further attempts to correlate the stereochemical relation of the C-5 OH and the C-3a side chain in these compounds by this approach was abandoned in favor of the successful alternative described below.

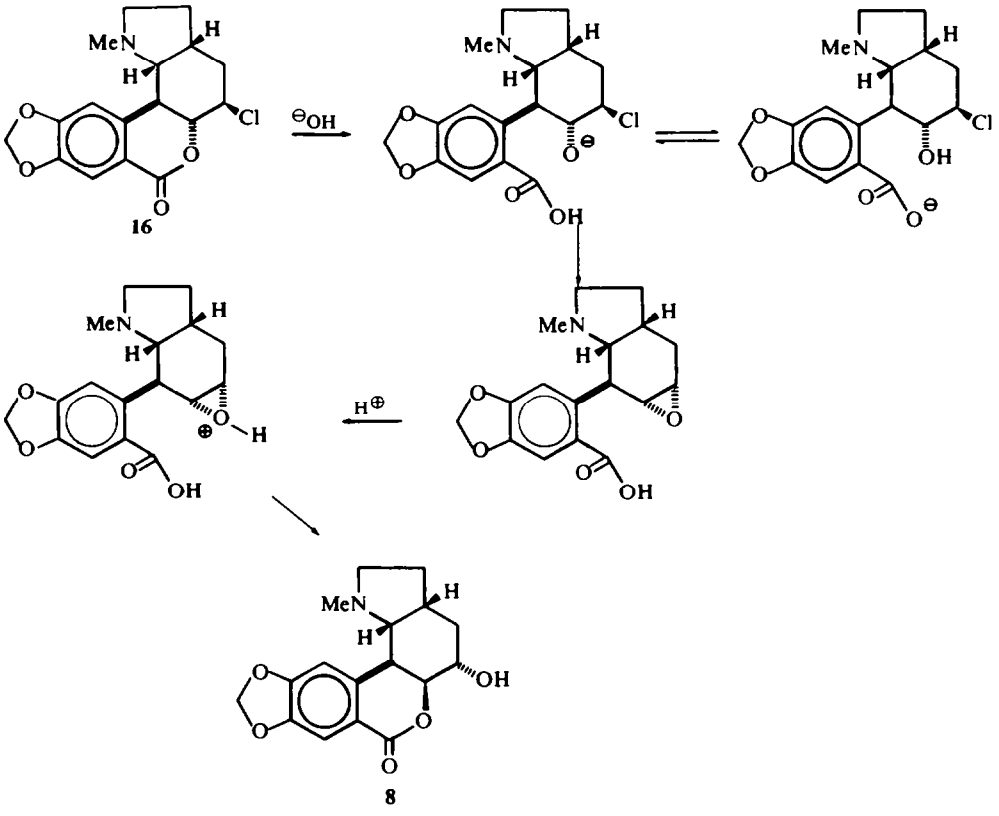
A unique solution of the structure and stereochemistry of clivonine was provided in an unexpected fashion by a series of reactions which led to its conversion to the known compound 5α-hydroxymasan-7-one (**8**) (α-dihydrohippeastrine).

Reaction of clivonine with phosphorous oxychloride effected the replacement of the C-5 OH by Cl to afford 5β-chloro-5α-*epi*masan-7-one (**16**). Evidence that the anticipated inversion of stereochemistry at C-5 had occurred in this reaction was provided by the PMR spectrum of **16** in which the C-5 signal has a $W_{\frac{1}{2}}$ 25Hz, indicating its axial

nature. Further, a first order analysis of the signal gives coupling constants of 4·5, 10·0, and 11·0 Hz in accord with two *trans* diaxial couplings and an axial-equatorial coupling. The other signals in the spectrum are in full accord with the proposed structure and parallel those reported earlier for clivonine.

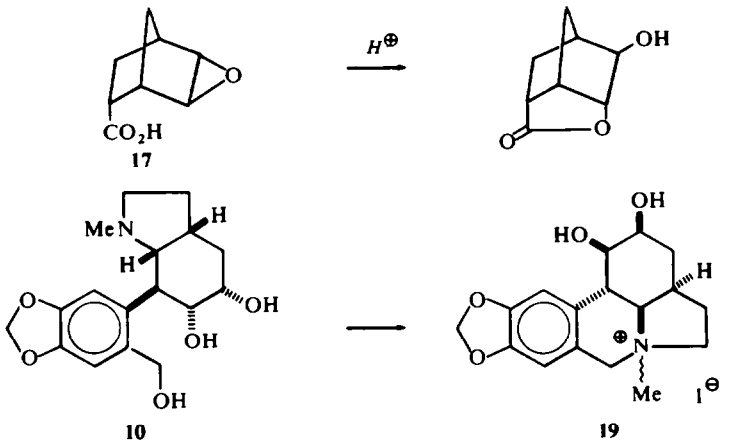
Treatment of the chlorolactone **16** with hot dilute alcoholic KOH gave a water soluble salt which upon acidification with 2N H_2SO_4 and subsequent rebasification with NH_4OH afforded the crystalline lactone **8** in a low melting polymorphic form, m.p. 178·5–179·5°. In view of the unexpected nature of this reaction, which requires an inversion of configuration at two centers, C-5 and C-5a, some care was taken to ensure that the product was indeed the lactone **8**. The comparisons that were made to verify this are detailed in the Experimental. Despite the serendipitous manner in which this transformation was effected it is readily accounted for (*post facto*) as shown in Scheme 2.

SCHEME 2



Nucleophilic attack at the CO of the lactone in **16** will afford the alkoxide as the initial product. The *trans* relation of the chlorine and the alkoxy anion in this compound fulfills the requirement to permit the facile formation of the epoxide. We have chosen to represent the conversion of the epoxide to the final product as an acid catalysed reaction since there is a clear precedent for this in the reported conversion[10] of 2,3-*exo*-epoxynorbornane-5-*endo*-carboxylic acid (**17**) to the lactone **18** under acidic conditions.

An attempted Hofmann degradation on the chlorolactone **16** also led to the formation of 5α-hydroxymasan-7-one in 25% yield, together with recovered starting material.



17 10 19

Since the structure and absolute configuration of 5α-hydroxymasan-7-one is known by virtue of the correlation[2] of hippeastrine (**2**) with lycorine (**4**)*, the above conversion **8** → **16** leads to the establishment of the relative and absolute stereochemistry of clivonine as represented by structure **9** (R = O, R′ = H).

Clivonine provides an example of where conversion to the corresponding lycorine-type base methiodide **19** fails to provide structural information since this represents one of the few instances in which the product in this stereochemical modification has not been reported previously. This transformation has been carried out and the physical properties of **19** are described in the Experimental.

EXPERIMENTAL

M.ps. were determined on a microscope hot stage and are corrected. Optical rotations were measured in a Winkler polarimeter in 1 dm tubes. IR spectra were obtained on a Zeiss double beam of spectrometer UR10 and the UV spectra were recorded in EtOH on a Beckman DK 2A spectrometer. PMR spectra were obtained in $CDCl_3$ using TMS as an internal reference on a Varian HA-100 spectrometer. Mass spectra were obtained with an Associated Electrical Industries MS-902 double focussing high resolution mass spectrometer as direct probe sample using an ionising voltage of 70 eV.

Reaction of clivonine with cyanogen bromide. Clivonine (100 mg), in 8 ml dry benzene, was treated with 60 mg CNBr contained in 2 ml dry benzene and the resulting mixture was refluxed for 2 hr. The soln was diluted with 10 ml benzene and washed successively with H_2O, dilute HOAc, H_2O and then dried (Na_2SO_4). Removal of the solvent afforded **12** (R = O, R′ = H) as a pale yellow resin (102 mg), $[\alpha]_D^{22}$ + 160° (*c* = 0·2; $CHCl_3$); spectra: IR ($CHCl_3$), 2210 cm^{-1} (CN), 1720 cm^{-1} (aryl conjugated δ-lactone); MS, *m/e* rel. inten.) no M⁺, 343 (7·3), 342 (26·1), 341 (10), 323 (13·4), 299 (40), 298 (10·7), 201 (20·2), 149 (14), 108 (25·4), 93 (16), 96 (11·3), 89 (12·7), 83 (21·4), 82 (37·4), 81 (15·4), 80 (41·4), 79 (14), 69 (41·4), 57 (26), 56 (14), 55 (16·4), 45 (40), 44 (100), 43 (54·2), 42 (18·1), 41 (19·4); PMR δ7·48 (bs, 1H, H-8), 6·78 (s, 1H, H-11), 6·04 (s, 2H, CH_2O_2), 4·82 (d, 1 H, *J* = 3·5Hz, H-11c), 4·70 (dd, 1H, *J* = 3·0, *J* = 11·5 Hz), 4·31 (m, 1H, H-5), 3·62 (dd, 1H, *J* = 11·5, 3·5 Hz), 2·88 (s, 3H, N—CH_3). (Found: C, 51·44; H, 4·46; N, 6·63. $C_{18}H_{19}N_2O_5$ Br requires: C, 51·18; H, 4·52; N, 6·62%. Found: *m/e* 342 1222 m* from *m/e* 342 with parent at *m/e* 422·4. Calc. $C_{18}H_{18}N_2O_5$ (M-HBr) 342.1216; M⁺, *m/e*, 424, 422).

* The absolute configuration of lycorine is fully established by the results of the x-ray crystal structure of dihydrolycorine hydrobromide.[11]

Von Braun product from O-*acetylclivonine*. The acetate **9** (R = O, R′ = Ac), (40 mg), in dry benzene (3 ml) was heated under reflux for 3 hr with CNBr (30 mg) in 1 ml of dry benzene. The soln was treated in the same manner as described above to leave the product as a gum (30 mg). Crystallization from acetone afforded 21 mg of the pure acetate **12** (R = O, R′ = Ac), m.p. 150–151°, which was homogeneous by TLC.* (R_f = 0·75, silica gel G; EtOAc : $CHCl_3$: MeOH, 1 : 1 : 1); PMR, δ 7·53 (s, 1H, H-8), 6·77 (s, 1H, H-11), 6·06 (s, 2H, CH_2O_2), 5·47 (m, 1H, H-5) 4·86 (m, 1H, H-5a), 3·60 (m, 1H, H-11c), 2·90 (s, 3H, N—CH_3), 2·13 (s, 3H, O—Ac). (Found: C, 51·44; H, 4·47; N, 6·10. $C_{20}H_{21}N_2O_6Br$ requires: C, 51·60; H, 4·51; N, 6·02%).

Von Braun product from 5-*hydroxy*-5-*epimasanone* (**9** R = H_2, R = H). Treatment of the ether (184 mg) in benzene (15 ml) with a benzene soln of CNBr (120 mg) in analogous manner to the two preceeding experiments afforded a non-crystalline gum (218 mg), $[\alpha]_D^{22}$ + 150° (*c* = 0·2; $CHCl_3$) which was homogeneous by TLC. (R_f 0·70); spectra: IR ($CHCl_3$), 2210 cm^{-1} (C≡N), PMR, δ 6·72 (s, 1H, H-11), 6·42 (s, 1H, H-8), 5·94 (s, 2H, CH_2O_2), 4·83 (d, 1H, *J* = 15 Hz, H-7), 4·74 (d, 1H, *J* = 15 Hz, H-7), 4·82 (m, 1H, H-11c), 4·22 (m, 1H, *J* = 2·5 Hz, H-5), 3·84 (dd, 1H, *J* = 10·0, 2·5 Hz, H-5a), 3·34 (bd, 1H, *J* = 10·0 Hz, H-11b), 2·90 (s, 3H, N—CH_3) (Found: C, 52·45; H, 5·11; N, 6·82. $C_{18}H_{21}N_2O_4Br$ requires: C, 52·84; H, 5·17; N, 6·85%).

Attempted hydrolysis of the Von Braun product **12** (R = O, R′ = 4). A soln of 100 mg of **12** (R = O, R′ = H) in 5 ml of abs EtOH containing 300 mg was allowed to stand, the KBr which precipitated was filtered off, and the filtrate kept for 3 hr before removing the solvent *in vacuo*. The residue was neutralized with 5 ml 2N, HOAc, extracted with $CHCl_3$ and the extract discarded. The aqueous acetic acid phase was basified with aqueous NH_3 and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ gave 50 mg of a gum which was crystallized twice from acetone to give the iminoether **15**, (51 mg, m.p. 155–7°, $[\alpha]_D^{22}$ + 106° (*c* = 0·348; $CHCl_3$); spectra: UV, λ_{max}^{EtOH} 264 mμ (5240), 312 (2960); IR ($CHCl_3$), 1610 cm^{-1} (—N=C—OR); MS, M⁺, 374 (72), 299 (8), 117 (19), 116 (100), 115 (18), 88 (60), 87 (86), 44 (10); PMR, δ 7·03 (s, 1H, H-11), 6·42 (s, 1H, H-8), 6·07 (d, 1H, *J* = 4·0 Hz, H-11 c), 5·87 (s, 2H, CH_2O_2), 4·86 (d, 1H, 3 = 15 Hz, H-7), 4·76 (d, 1H, *J* = 15 Hz, H-7), 4·14 (q, 2H, *J* = 7·0 Hz, O-C<u>H</u>$_2$CH$_3$), *ca* 4·20–4·10 (om, 2H, H-5 and H-5a), 3·62 (bs, 1H, OH, 3·39 (t, 2H, *J* = 7.OHz, H-2), 2·87 (s, 3H, N-CH_3), 1·88 (m, 4H, 2 H-3 and 2 H-4), 1·34 (t, 3H, *J* = 7·0 Hz, —$OCH_2C\underline{H}_3$). (Found: M⁺, 374·183, $C_{20}H_{20}N_2O_5$ requires 374·184).

Attempted acid hydrolysis of the imino-ether **15**. A soln of **15** (70 mg) in 3 ml of 3% HCl was heated for 5 hr on a water bath. The resulting red soln was extracted with chloroform and the aqueous phase basified with NH_4OH. Extraction of the basic soln with chloroform gave 53 mg of gum which crystallized from acetone as prisms, m.p. 154–7 (32 mg). This was shown to be unreacted starting material by the usual chromatographic and spectral comparisons.

Acid hydrolysis of the lactone **12** (R=O, R′=H). The lactone **12** (R=O, R′=H) (100 mg) was dissolved in 3 ml of 30% H_2SO_4 and heated on a water bath for 1 hr. The soln was cooled, diluted with 10 ml of H_2O and after extraction with $CHCl_3$, the aqueous phase was basified and reextracted with $CHCl_3$. Evaporation of the $CHCl_3$ left a brown gum (25 mg) which showed a large number of components on examination by TLC and therefore was not investigated further.

Hydrolysis of lactone **12** (R = O, R′ = H) *with aqueous base*. A sample (100 mg) of the lactone **12** (R = O, R′ = H) was heated with 3 ml of 1N KOH on a water bath for 4 hr. The soln was cooled and a slight excess of 10% HCl added to effect relactonization. A chloroform extract of this soln afforded a small quantity (17 mg) of neutral material. Basification with NH_4OH and extraction with $CHCl_3$ gave 30 mg of brown gum which showed 5 components by TLC.

5β-*Chloro*-5a-epi*masan*-7-*one* (**16**).

(a) A soln of clivonine (500 mg) in 7 ml freshly distilled pyridine was coooled in an ice bath and $POCl_3$ (2g) added dropwise. After standing 20 hr at room temp the mixture, which contained a crystalline ppt, was poured into ice water, basified with NaOH or NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4) and the solvent removed *in vacuo*. After removal of residual pyridine by azeotropic distillation the residue (470 mg) was chromatographed on 30 g of neutral alumina. Elution with benzene: EtOAc (8 : 2) gave **16** (440 mg) a colorless gum $[\alpha]_D^{22}$ + 22° (c = 0·8; $CHCl_3$); spectra: IR ($CHCl_3$), 1720 cm^{-1} (aryl conjugated δ-lactone), no OH band; MS, M⁺ 337 (^{37}Cl) (17), 335 (^{35}Cl) (35·5), 300 (10·7), 299 (12·8), 254 (6·8), 162 (2·5), 149 (10·7), 120 (5·1), 115 (3·2), 96 (81·0), 85 (25·5), 83 (100), 82 (27·6), 71 (53), 69 (6·8), 57 (15·0), 55 (9·1); PMR, δ7·50 (s, 1H, H-11c), 7·44 (s, 1H, H-8), 6·00

* The R_f values reported subsequently refer to this solvent system using silica gel G as a support.

(s, 2H, CH_2O_2), 4·26 (oct, 1H, J = 4·5, 10·0 and 11·0 Hz, H-5), 4·03 (dd, 1H, J = 10 and 11 Hz, H-5a), 3·32 (oct, 1H, J = 4·5, 10 and 12 Hz, H-2), 3·00 (4 lines, 1H, J = 5·5 and 10 Hz, H-11c), 2·78 (t, 1H, J = 10 and 10 Hz, H-11b), 2·56 (s, 3H, N-CH_3). (Found: C, 60·80; H, 5·35; N, 4·14; M^+, 335·0930. $C_{17}H_{18}NO_4Cl$ requires: C, 60·81; H, 5·40; N, 4·17%; M^+, 335·0929.

(b) Thionyl chloride (150 mg) was added to a soln of clivonine (100 mg) in 2 ml pyridine and the mixture allowed to stand at room temp for 15 hr. The soln, which after this time contained a crystalline ppt, was poured into ice water and worked up as described in procedure (a). The 85 mg of gummy solid which was obtained crystallized from acetone to give 76 mg of the pure chloro-compound **16**, m.p. 198–9°.

Conversion of **16** *to 5α-hydroxymasan-7-one* (**8**)

(a) The chloro-compound **16** (100 mg) was dissolved in 2 ml hot EtOH and 5 ml 1N KOH was added. After heating this mixture on a water bath for 15 min sufficient solvent was removed *in vacuo* to give a homogeneous soln. Water (5 ml) was added and the soln heated for 6 hr. The soln was then acidified with 2N H_2SO_4 and heated for a further hr on a water bath. The acidic soln was then cooled and extracted with $CHCl_3$. Basification of the aqueous phase with NH_4OH and $CHCl_3$ extraction gave 72 mg of a pale yellow gum upon evaporation of the $CHCl_3$ extract ; this showed two spots on TLC (R_f 0·8 and 0·52) in a ratio of 1 : 4. Treatment of the gum with acetone afforded the lactone **8**, m.p. 178·5–179·5°, $[\alpha]_D^{22}$ + 50° (c = 0·2; $CHCl_3$) R_f = 0·52; spectra: IR ($CHCl_3$), 1720 cm^{-1} (δ-lactone), 2595 cm^- (OH). MS and PMR were identical with corresponding spectra of an authentic sample of 5α-hydroxymasan-7-one. A soln of the polymorph (m.p. 178·5–179·5°) when seeded with a crystal of **8** (M.p. 196°) gave crystals (m.p. 196°). The minor product (R_f 0·8) was tentatively identified as starting material from its TLC behavior.

(b) A soln of **16** (100 mg) in 2 ml EtOH and 3 ml H_2O was treated with 500 mg freshly precipitated Ag_2O. After keeping the mixture at 60° for 10 hr the soln was filtered and the filtrate acidified with 2N H_2SO_4. Work up as in (a) gave a gum which on TLC was shown to be mainly starting material together with a minor product. This minor product was isolated by thick layer chromatography (R_f 0·52) and crystallized from acetone, m.p. 178–180 (25 mg) and was identified by the usual spectral and chromatographic comparisons as the low melting form of 5α-hydroxymasan-7-one.

Lithium aluminium hydride reduction of **8**. The product **8**, m.p. 178° (100 mg) obtained from **16** was dissolved in 5 ml THF and added to a stirred suspension of LAH (50 mg) in 5 ml THF. After one hr the reaction was worked up in the customary manner to afford 50 mg of a clear gum. Purification by thick layer chromatography gave 39 mg of the major product, m.p. 98–103° dec. $[\alpha]_D^{22}$ + 20° (c = 0·2; $CHCl_3$), R_f = 0·22, which was identical in every respect with an authentic sample of 5α,7-dihydroxy-6,7-*seco*masane (α-dihydro-tetrahydrohippeastrine) (Lit.[12] m.p. 99–101°). A minor product from the reduction, m.p. 200° dec., R_f 0·48, was not identified.

Cryclization of the LAH reduction product of lactone **8**. The reduction product from the preceding experiment, m.p. 98–103° (30 mg), was dissolved in 3 ml 5% HCl and the resulting soln heated on a water bath for 2 hr. The soln was evaporated to dryness *in vacuo* and the residue crystallized from acetone to give 5α-hydroxymasanane hydrochloride (α-dihydrodeoxyhippeastrine hydrochloride, m.p. 285–8 (in vac.) (Lit[12] m.p. 285–7°). The m.p. was not depressed when the product was admixed with an authentic sample of 5α-hydroxymasanane hydrochloride, m.p. 285–7°.

LAH reduction of clivonine. Clivonine (200 mg) was dissolved in 20 ml THF and added dropwise to a suspension of LAH (50 mg) in 20 ml THF over a period of 30 min. EtOAc and water were added to destroy the excess reagent and the ppt of aluminium oxide which formed was filtered and washed well with hot EtOAc. The EtOAc extracts and washings were combined and the solvent evaporated *in vacuo* to leave a non-crystalline residue which was dissolved in $CHCl_3$ and the soln filtered again to remove traces of aluminium oxide. Evaporation of the $CHCl_3$ from this solution left 185 mg of a gum which crystallized from acetone to give 150 mg of pure **10**, m.p. 172·5–173·5°, $[\alpha]_D^{22}$ + 21·5° (c = 0·325; $CHCl_3$); PMR, δ 6·85 (s, 1H, H-11), 6·82 (s, 1H, H-8), 5·95 (s, 2H, CH_2O_2), 4·75 (d, 1H, J = 12 Hz, H-7) 4·16 (d, 1H, J = 12 Hz, H-7), 4·10 (m, 1H, H-5), 3·80 (dd, 1H, J = 3 and 11 Hz, H-5a), 3·36 (t, 1H, J = 11 Hz), 1·84 (s, 3H, N–CH_3); MS*, M^+, 321, 304, 303, 286, 156, 149, 126, 96, 83. (Found: C, 63·57; H, 7·39; N, 4·71. $C_{17}H_{23}NO_5$ requires: C, 63·53; H, 7·21; N, 4·36%).

Conversion of the triol **10** *to the methiodide* **19**. *p*-Toluenesuphonyl chloride (350 mg) was added to a soln containing 500 mg of **10** in 7 ml freshly distilled pyridine. After allowing the mixture to stand at room temp

* The intensities of the ions in the spectrum of this compound were very sensitive to operating conditions and are therefore not reported.

for 20 hr the solvent was removed under reduced pressure and the residue dissolved in 40 ml H_2O. The aqueous phase was extracted with $CHCl_3$ (×3) and then passed through a column of Amberlite IRA 400 resin (OH form). The combined eluates from the column were extracted with ether (×5) to remove pyridine and since the aqueous phase showed a significant amount of starting material on examination by TLC it was extracted a further 5 times with $CHCl_3$. 1N AcOH was added to neutralize the aqueous phase and the resulting soln concentrated to 3 ml under vacuum. Addition of a saturated soln of NaI to this soln gave a crystalline ppt, m.p. 280–88° (d) which after two crystallizations from MeOH afforded 120 mg of the pure *methiodide* **19**, m.p. 192–7° (d) or m.p. 301–3° (d.; vac).

5α-Hydroxy-5α-epimasanane [**9**, R = H_2, R′ = H). A finely pulverized sample of 200 mg of **10** was added to 2 ml 80% phosphoric acid and the mixture heated for 15 min at 80–90°. The dark brown mixture was diluted with water, basified with NH_4OH, and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with water and dried (Na_2SO_4). Evaporation of the $CHCl_3$ left 162 mg gum which crystallized from acetone to give 134 mg of the pure *ether* **9** (R = H_2, R′ = H), m.p. 136–8°, R_f 0·53; spectra: PMR 7·94 (s, 1H, H-11), 6·38 (s, 1H, H-8), 5·87 (s, 2H, CH_2O_2), 4·74 (q, 2H, center of AB pattern, H-7), 4·13 (m, 1H, H-5) 3.12 (dd, 1H, H-11b), 2·69 (dd, 1H, H-11c), 2·48 (s, 3H, N-CH_3). The sample was identical, mixed m.p., spectral and chromatographic properties with an authentic sample.[7]

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FEFERENCES

[1] For a recent review of these alkaloids see, W. C. Wildman. *The Alkaloids Edited by R. H. F. Manske*] Vol. XI. p. 308, Academic Press, N.Y. (1968)
[2] T. Kitagawa, S. Uyeo and N. Yokoyama, *J. Chem. Soc.* 3741 (1959)
[3] H. K. Schnoes, D. M. Smith, A. L. Burlingame, P. W. Jeffs and W. Döpke, *Tetrahedron* **24**, 2824 (1968)
[4] P. W. Jeffs, W. A. Hawksworth, B. K. Tidd and T. P. Toube, *J. Chem. Soc.* 1491 (1965)
[5] A preliminary account of a portion of this work has appeared. W. Dopke. M Bienert, A. l. Burlingame, H. K. Schnoes, P. W. Jeffs, and D. S. Farrier, *Tetrahedron Letters* 451 (1967)
[6] C. K. Briggs, P. J. Highet, R. J. Highet and W. C. Wildman, *J. A. Chem. Soc.* **78**, 2899 (1956)
[7] B. Mehlis, *Naturwissenschaften* **52**, 34 (1965)
[8] H. G. Boit and B. Mehlis, *Ibid.* **48**, 603 (1961)
[9] See isosafrole, L. Lang. *Absorption Spectra*, Vol. 2, Academic Press, N.Y. (1961)
[10] H. B. Henbest and B. Nicholls, *J. Chem. Soc.* 221 (1959)
[11] M. Shiro, T. Sato, and H. Koyama, *Chem. & Ind.* 1229 (1966)
[12] K. Kotera, Y. Hamada, and R. Nakane, *Tetrahedron* **24**, 759 (1968)

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