Tetrahedron Letters No. 5, pp. 451–457, 1967. Pergamon Press Ltd. Printed in Great Britain.

STRUCTURES AND STEREOCHEMISTRY OF CLIVONINE AND CLIVIMINE.

W. Döpke and M. Bienert, Humboldt University, Berlin, Germany

A. L. Burlingame and H. K. Schnoes, University of California
Berkeley, California, USA

P. W. Jeffs[+] and D. S. Farrier[++], Duke University
Durham, North Carolina, USA

(Received 22 October 1966)

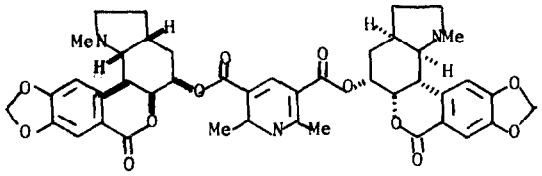
Lactone and hemiacetal alkaloids of the Amaryllidaceae possess, in common with most other alkaloids of this family, a single nitrogen atom and a central carbon skelton embodying fifteen carbon atoms (1). In a recent note the alkaloid clivimine (2) which occurs in Clivia miniata Regel (3), was ascribed the molecular formula $C_{43}H_{43}O_{12}N_3$. Its structure was assigned as shown in I, mainly on the basis of its hydrolysis to 2,6-lutidine-3,5-dicarboxylic acid and the known alkaloid clivonine.

The structure of clivonine, which had been proposed tentatively by Wildman and coworkers (4), was extended by Mehlis to include both the relative and absolute stereochemistry shown in II. However, the assignments of stereochemistry at C(11b) and C(11c), relative to the C(5a)-position, were based only on analogy with other lactones of this series (5). In view of this lack of evidence supporting the stereochemistry of II we have undertaken a further investigation of both clivonine and clivimine. We report in this note the results of mass spectral studies which support the gross structures assigned previously to these alkaloids, together with evidence from NMR studies which leads to a reassignment of their stereochemistry.

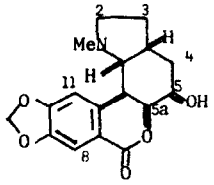
The mass spectrum of clivonine exhibited a fragmentation pattern typical of a 3a,4-dihydro-5-hydroxylactone of a benzopyrrolo[2,4g] indole series (6), and in fact it was very similar to the spectrum of α-dihydrohippeastrine (III, R=H). Such differences that exist in the spectra are mainly small variations in the relative abundances of the fragment ions. This suggests a close structural relationship between these two compounds.

[+] To whom inquiries regarding this paper should be addressed.

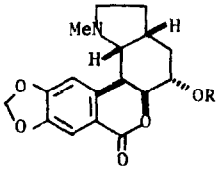
[++] NASA Fellow 1965-1966.



I

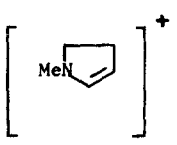


II

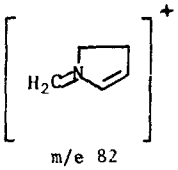


III

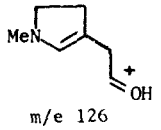
The most intense peak in the spectrum of clivonine occurs at m/e 83. The structure ascribed to this ion and to the other prominent ions at m/e 82, m/e 96, m/e 126, m/e 162 and m/e 190 are given below, and with the exception of the ions at mass 162 and 190, which vary depending on the aromatic substitution pattern, they are characteristic of the fragment ions observed in the mass spectra of 3a, 4-dihydro-5-hydroxylactones of the benzo[3,4g]indole series (6). In summary, the mass spectral data is entirely consistent with the structure of clivonine as represented in II, but does not provide any information on the stereochemistry.



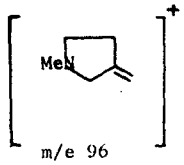
m/e 83



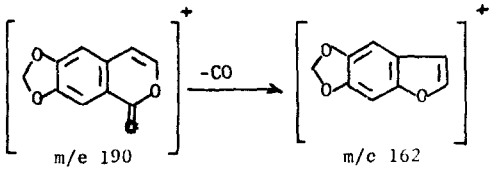
m/e 82



m/e 126



m/e 96



m/e 190 m/e 162

A detailed study of the NMR spectra of clivonine and its O-acetate was therefore undertaken. The 60 Mc.s spectrum of clivonine shows one-proton singlets at δ 7.72 (fine splitting) and δ 7.40 compatible with the aromatic pattern in II. A two-proton singlet

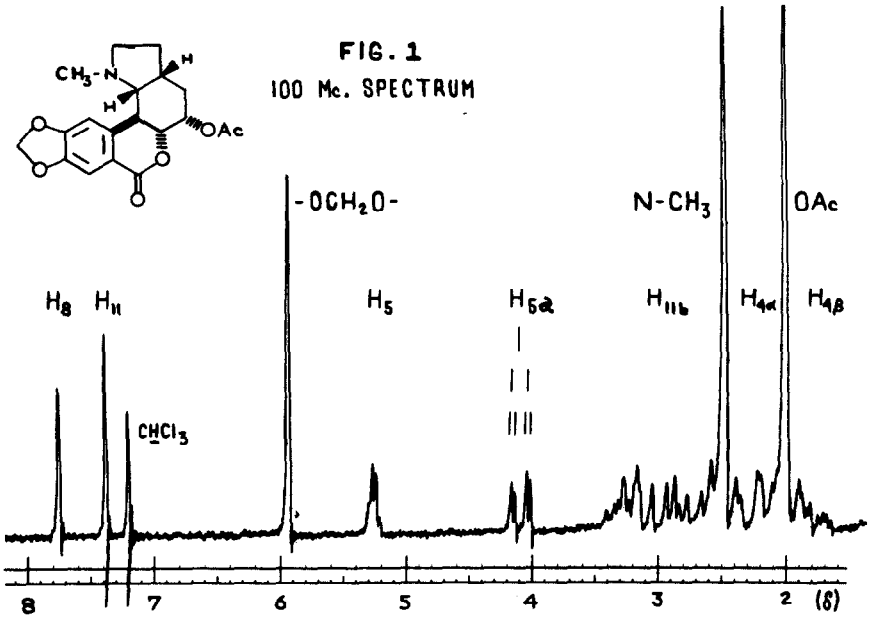
at δ 5.97 arises from the methylenedioxy group and an overlapping one-proton multiplet and one-proton doublet of doublets which appear at δ 4.18 and δ 4.06 are attributed to the signals from the C(5)- and C(5a)-hydrogens respectively. Support for the assignment of the signal at δ 4.18 to the C(5)-hydrogen resonance is obtained from the observation of the characteristic downfield shift of this signal on acetylation of clivonine (further evidence for the correctness of this assignment is given in the sequel). The hydrogens at C(11b) and C(11c) give rise to overlapping multiplets centered at δ 3.08 and δ 3.32 respectively. A three-proton singlet appears at δ 2.51 for the N-methyl signal and the signals of the remaining seven hydrogens occur as a series of unresolved peaks between δ 3.52-1.68.

The 100 Mc.s NMR spectrum* of O-acetylclivonine (Fig. 1), in which the C(5)- and C(5a)-hydrogen resonances are well separated and some clarification of the signals in the region δ 2.5-1.5 occurs, was analyzed in detail. Assignment of the double doublet at δ 4.16 to the C(5a)-hydrogen resonance on the basis of its chemical shift was confirmed by spin-decoupling experiments. Saturation at the frequency of the C(5)-hydrogen resonance (δ 5.35) removed the weak coupling in causing the collapse of the C(5a)-hydrogen signal to a doublet (see Figure 2). This experiment permitted the evaluation of the coupling constants $J_{5,5a}$ = 3.0 c/s and $J_{5a,11b}$ = 12.5 c/s. Whereas the weak coupling between the C(5) and C(5a) hydrogens is consistent with a cis relationship of the oxygen functions as assigned previously (2) the large magnitude of $J_{5a,11b}$ implies that the hydrogens at C(5a) and C(11b) are trans-diaxial. Clivonine, therefore, must possess a trans B:C ring fusion, which is in contrast to the other members of this series of lactone alkaloids whose stereochemistry at this junction is known to be cis-fused (5).

Irradiation at the C(5)-hydrogen resonance frequency also caused some simplification of a group of signals between δ 2.42-1.60, which consequently allowed them to be assigned to the C(5)-methylene hydrogens. The appearance of the decoupled spectrum is that of a pair of quartets typical of the AB part of an ABX pattern (see Fig. 2). Analysis (7) gives $J_{4\alpha,4\beta}$ = 15.5 c/s and $J_{4\alpha,3a}$ = 3.5 c/s and $J_{4\beta,3a}$ = 6.5 c/s. The magnitudes of the coupling of the C(3a)-hydrogen are best accommodated by this hydrogen being equatorial from which it follows that the C(3a)-C(2) bond is axial with respect to ring C. Consequently the C:D-ring

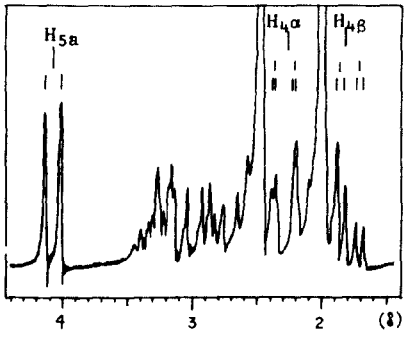
*The 100 Mc.s spectra were obtained on a Varian HA100 by Mrs. M. G. Miller through the courtesy of Professor C. Moreland, University of North Carolina, Raleigh.

juncture is therefore cis-fused, and the stereochemistry of clivonine is defined as portrayed in structure IV.



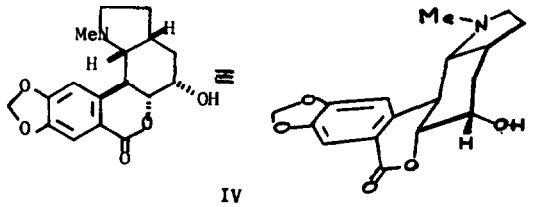
Saturation of the C(5a)-hydrogen signal indicated the location of the C(11b)-hydrogen resonance at δ 3.18 and also permitted the assignment of $J_{11b,11c}$ = 11.0 c/s, which is in full accord with the trans-diaxial relationship of the C(11b)- and C(11c)-hydrogens as depicted in structure IV.

FIG. 2

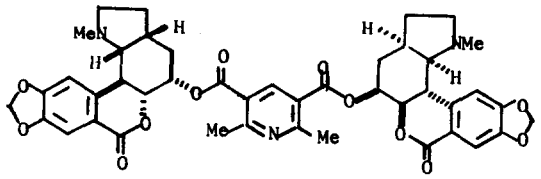


Partial spectrum showing signals of the C(5a)- and C(4)-hydrogens during irradiation at the C(5)-hydrogen resonance frequency.

Further support for the trans-B:C stereochemistry in clivonine was obtained from ORD and CD measurements. The ORD spectra of all Amaryllidaceae lactone alkaloids in which the stereochemistry of the B:C ring juncture is known to be cis (5) exhibit a negative Cotton effect of large amplitude in the region of 320mμ (8). In contrast, the ORD spectrum of clivonine showed only a very weak negative Cotton effect (a = 3.2) in the same region.



IV



V

Corroboration of the ORD results was obtained from a comparison of the CD spectrum[+] of clivonine with that of α-dihydrohippeastrine (III, R=H). The spectrum of clivonine shows a weak negative maximum $[\theta]_{300}$-407° whereas the CD curve of α-dihydrohippeastrine exhibits a much stronger negative maximum $[\theta]_{305}$-1221°.

The modification of the stereochemistry of clivonine necessitates the structure I proposed for clivimine be revised to V. High resolution mass spectral measurements* demonstrated the composition of the molecular ion of clivimine as $C_{43}H_{43}O_{12}N_3$ (obs. 793.288. req. 793.285.), which is in agreement with the molecular formula proposed by Mehlis (3). Prominent peaks occurred at m/e 494, m/e 299, m/e 96, and m/e 83. Cleavage of the C(5)-O bond with hydrogen transfer from C(5a) with retention of the charge on the major fragment (VI) accounts for the peak at m/e 494, and the same process with retention of the charge by the other fragment provides a reasonable explanation of the origin of the ion VII (m/e 299). A retro-Diels-Alder type fragmentation of VII can lead to the fragment VIII (m/e 216) which, in turn, can

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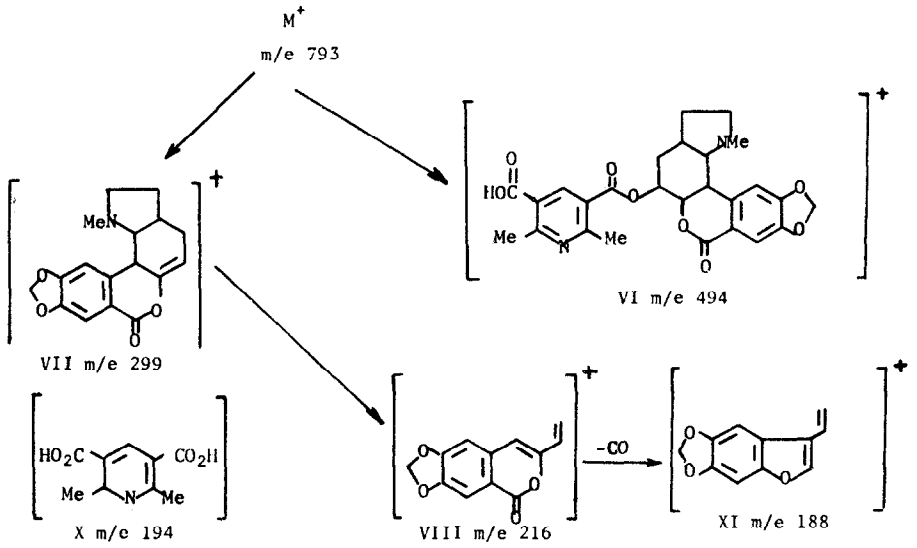
[+]We are indebted to Dr. Stanley Duke of Durrum Instruments, Palo Alto, for these spectra.

*We are grateful to Dr. R. J. Highet, National Institutes of Health, Bethesda, Md., for carrying out this determination.

lose carbon monoxide to form the ion IX (m/e 188). The peaks at m/e 96 and m/e 83 are also present in the mass spectrum of clivonine and their structures are assigned accordingly. A fragment of m/e 194 occurs and is attributed to the 2,6-lutidine-3,5-dicarboxylic acid ion (X). The loss of water, carbon dioxide, and OH from X is evident from the appearance of ions at m/e 176, m/e 150 and m/e 177 respectively.



The 60 Mc.s NMR spectrum of clivimine showed a one-proton singlet at low field δ 8.53, which is ascribed to the signal from the C(4)-hydrogen on the 2,6-lutidine-3,5-diester residue. Aside from the presence of a six-proton singlet at δ 2.84, attributed to the methyl resonances of the lutidine system, the remaining features of the spectrum bore a close resemblence to the spectrum of O-acetyl clivonine. The doublet of doublets of the C(5a)-hydrogens ($J_{5,5a}$ = 3.0, $J_{5a,11b}$ = 11.5) at δ 4.18 showed the characteristically large coupling associated with the trans B:C ring junction present in each of the two clivonyl groups.

Acknowledgements: Two of us (P.W.J. and D.S.F.) are grateful to the Eli Lilly Company for a grant-in-aid used in partial support of this work. The work at Berkeley was supported by National Aeronautics and Space Administration Grant NsG 101 to the University of California.

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