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MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS.[1]

ECHITAMIDINE

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Echitamidine[2] is a minor alkaloid accompanying echitamine in a number of *Alstonia* species. While the structure of echitamine--a subject of extensive chemical investigations[3]--has been settled by x-ray analysis,[4] very little information has been accumulated on its companion, echitamidine. Its discoverer, Goodson,[2] ascribed to it the empirical formula $C_{20}H_{26}N_2O_3$ and Raymond-Hamet[5] noted that its ultraviolet absorption spectrum was nearly identical with that of akuammicine. Since the latter's

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[1] Paper XIII. For paper XII see M. Plat, J. LeMen, M.-M. Janot, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *Bull. Soc. Chim. France* in press. The work at Stanford University was supported by grants 2G-682 and A-4257 from the National Institutes of Health of the U.S. Public Health Service.

[2] J. A. Goodson, *J. Chem. Soc.* 2626 (1932).

[3] Leading references are listed by T. R. Govindachari and S. Rajappa, *Tetrahedron* **15**, 132 (1961).

[4] J. A. Hamilton, T. A. Hamor, J. M. Robertson and G. A. Sim, *Proc. Chem. Soc.* 63 (1961); H. Manohar and S. Ramaseshan, *Tetrahedron Letters* 814 (1961).

[5] Raymond-Hamet, *Compt. rend.* **233**, 560 (1951).

constitution has since been established[6] in terms of structure Ia, partial formula II can most likely be attributed to echitamidine, a supposition which is also supported by the extremely high negative rotations of the two alkaloids. Quite recently, Chatterjee and Ghosal,[7] without listing actual analytical figures, modified Goodson's[2] empirical formula from $C_{20}H_{26}N_2O_3$ to $C_{20}H_{22}N_2O_3$ and assigned structure Ib to echitamidine on the basis of biogenetic likeliness. We should now like to report mass spectrometric and n.m.r. evidence which makes the Indian structure proposal Ib untenable, but which suggests structure IIIa as the most likely representation for echitamidine.

Our analytical results with echitamidine (found for material dried 6 hrs. at 20°/0.01 mm : C, 67.11; H, 7.34; N, 8.17; dried 8 hr at 100°/0.01 mm : C, 67.14, 67.42; H, 7.18, 7.25; N, 7.87, 7.84) do not differentiate between the empirical formulae $C_{20}H_{24}N_2O_3 \cdot H_2O$ (calcd.: C, 67.02; H, 7.31; N, 7.82) or $C_{20}H_{22}N_2O_3 \cdot H_2O$ (calcd.: C, 67.39; H, 6.79; N, 7.86) or in fact $C_{21}H_{26}N_2O_3 \cdot H_2O$ (calcd.: C, 67.72; H, 7.58; N, 7.52), the latter being a distinct possibility in view of our recent observations that the chromophore II can also exist in C_{21} alkaloids such as vincadifformine (IVa)[8] and tabersonine (IVb).[9] The elementary analysis of

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[6] [a] K. Aghoramurthy and R. Robinson, Tetrahedron 1, 172 (1958); [b] P. N. Edwards and G. F. Smith, J. Chem. Soc. 152 (1961); [c] J. Levy, J. LeMen and M.-M. Janot, Bull. Soc. Chim. France 979 (1960); [d] K. Bernauer, W. Arnold, C. Weissmann, H. Schmid and P. Karrer, Helv. Chim. Acta 43, 717 (1960).

[7] A. Chatterjee and S. Ghosal, Naturwiss. 48, 219 (1961).

[8] C. Djerassi, H. Budzikiewicz, J. M. Wilson, J. Gosset, J. LeMen and M.-M. Janot, Tetrahedron Letters 235 (1962).

[9] M. Plat, J. LeMen, M.-M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa and C. Djerassi, Tetrahedron Letters 271 (1962).

echitamidine picrate (m.p. 218-219°; found C, 55.19; H, 4.95) is equally compatible with a $C_{20}H_{24}N_2O_3 \cdot C_6H_3N_3O_7$ (calcd.: C, 54.83; H, 4.78) or a $C_{21}H_{26}N_2O_3 \cdot C_6H_3N_3O_7$ (calcd.: C, 55.57; H, 5.01) formulation. Functional group analysis demonstrated the presence of one C-methyl group (found: 4.09; calcd.: 4.19), at least one active hydrogen atom (found: 0.34; calcd.: 0.28), one methoxyl function (found: 6.21; calcd.: 8.60) and possibly one N-methyl group (found: 4.46; calcd.: 4.19). The dubious nature of this latter result has already been noted by Goodson[2] and the n.m.r. results cited below definitely show that the N-methyl value originates from the methoxyl substituent.

A definite decision about the correct empirical composition could be reached by mass spectrometry since the molecular ion peak at m/e 340 requires a $C_{20}H_{24}N_2O_3$ (mol. wt. 340) formulation rather than the earlier attributed[2,7] $C_{20}H_{26}N_2O_3$ or $C_{20}H_{22}N_2O_3$ alternatives. The alkaloid did not consume any hydrogen in ethanolic solution in the presence of platinum oxide and the absence of olefinic protons was established by the n.m.r. spectrum.[10] Structure Ib, suggested by Chatterjee and Ghosal,[7] therefore cannot represent echitamidine. The n.m.r. spectrum exhibited a peak at 3.89 δ, due to the carbomethoxyl group, but did not contain any signals attributable to a N-methyl function. The aromatic region (6.7 - 7.4 δ) resembled that of akuammicine (Ia) and the proton on the indoline nitrogen was responsible for a characteristic low-field peak at 8.68 δ. Most importantly, there was observed a doublet (J = 6 cps.) centered at 1.16 δ, corresponding in area to three protons and, hence, attributable to a methyl group located on a carbon atom bearing a single hydrogen.

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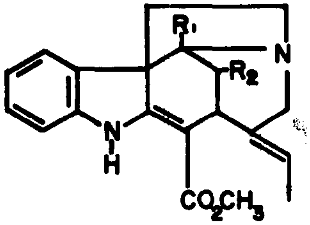
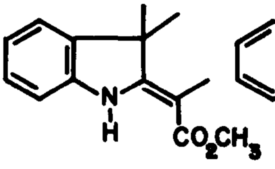
[10] N.M.R. spectra were measured in $CDCl_3$ solution, by Dr. Lois J. Durham, using a Varian A-60 spectrometer. All signals are reported in ppm as δ values (cps/60), the internal standard tetramethylsilane being taken as δ = 0.0.



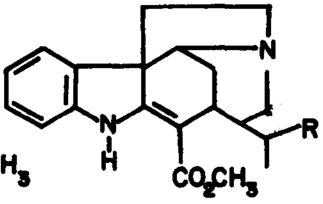
I a $R_1 = R_2 = H$

b $R_1 = OH$; $R_2 = H$

c $R_1 = H$; $R_2 = OH$



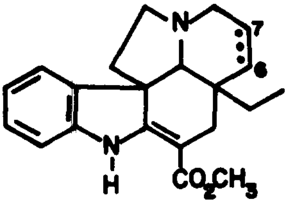
II



III a R = OH

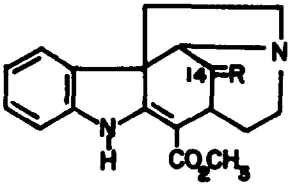
b R = OAc

c R = H



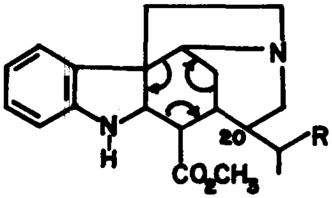
IV a 6,7 - saturated

b 6,7 - double bond



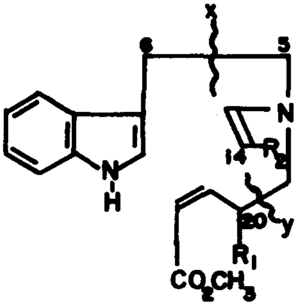
V a $R = \overset{H}{\llcorner}CHOHCH_3$

b $R = {=}CHCH_3$



VI a R = OH

b R = H



VII a $R_1 = CHOHCH_3$; $R_2 = H$

b $R_1 = H$; $R_2 = CHOHCH_3$

c $R_1 = C_2H_5$; $R_2 = H$

The peak of highest mass in the mass spectrum of echitamidine (after the m/e 340 molecular ion peak) occurs at m/e 322 and is thus indicative of the loss of water. It can be assumed, therefore, that the third oxygen function in echitamidine is a hydroxyl group and this is confirmed by the formation of an acetate IIIb (m.p. 220°, λ_{max}^{mujol} 5.78 µ), the empirical formula $C_{22}H_{26}N_2O_4$ (mol. wt. 382) of which was established by mass spectrometry (molecular ion at m/e 382). The n.m.r. spectrum of echitamidine acetate showed the same characteristic features as mentioned above for that of echitamidine, except that there appeared now a sharp peak at 2.09 δ due to the three acetate protons.

The mass spectrometrically determined empirical formula, and the lack of additional double bonds other than that incorporated in partial structure II, requires a pentacyclic skeleton for echitamidine. A C_{20} empirical formula together with partial structure II and the $CH(OH)CH_3$ functionality (indicated by the n.m.r. spectrum) is best accommodated in structure IIIa, based on an akuammicine skeleton (Ia), or the alternative Va, which is related to condylocarpine (Vb).[11] In view of the extremely limited amount of echitamidine at our disposal, an experiment had to be selected which would hopefully decide between these two alternatives and at the same time support our assumption of a strychnine-like framework.

For this purpose, a 5 mg sample of echitamidine acetate (IIIb) was heated under reflux for 80 min in 10% methanolic sulfuric acid with 1 g of zinc dust to afford dihydroechitamidine (also obtained in a similar reduction experiment directly from echitamidine), which was purified by thin-layer chromatography (methanol) on silica gel. The amorphous

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[11] A. Sandoval, F. Walls, J. N. Shoolery, J. M. Wilson, H. Budzikiewicz and C. Djerassi, Tetrahedron Letters No. 11 (1962); K. Biemann, A. L. Burlingame and D. Stauffacher, ibid. in press.

dihydroechitamidine (VIa) now exhibited a typical dihydroindole ultraviolet absorption spectrum (λ_{max}^{EtOH} 235 and 290 mμ, log ε 3.71 and 3.36) and its mass spectrum showed a molecular ion peak at m/e 342, thus establishing the $C_{20}H_{26}N_2O_3$ empirical formula (mol. wt. 342). As indicated in our earlier paper[12] dealing with the structure of mossambine (Ic), the most intense peak in the mass spectrum of tetrahydroakuammicine (VIb)[6b,c] occurs at m/e 196 and can be rationalized by fragmentation of ring C[13] (see arrows in VI) to the ion VIIc, followed by cleavage of the activated 5-6 bond (wavy line "x" in VII). A less intense, but nevertheless characteristic peak is found at m/e 199 and can be attributed[13] to alternate fission of the activated 20-21 bond of VII (wavy line "y"). Two other significant peaks are noted at m/e 130 and 144; these are due to the indole fragment with one and two attached CH_2 groups.

Turning now to the mass spectrum of dihydroechitamidine (VIa), it exhibits the same m/e 130, 144 and 199 peaks as noted in the tetrahydroakuammicine (VIb) spectrum, but the latter's most intense m/e 196 peak is now shifted to m/e 212 (16 mass unit difference due to extra oxygen atom) as would be predicted on the basis of structure VIa. If the hydroxyethyl side chain were attached at C-14 (Va), then the identical m/e 130, 144 and 212 peaks would be derived from the ion VIIb. The m/e 199 peak (wavy line "y" in VIIc) of the tetrahydroakuammicine (VIb) spectrum, however, would be shifted by 44 mass units (wavy line "y" in VIIb) to m/e 243 and no such peak is observed in the mass spectrum of dihydroechitamidine. Further support is provided by comparing the mass

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[12] X. Monseur, R. Goutarel, J. LeMen, J. M. Wilson, H. Budzikiewicz and C. Djerassi, Bull. Soc. Chim. France in press.

[13] See K. Biemann, M. Friedmann-Spiteller and G. Spiteller, Tetrahedron Letters 485 (1961).

spectra of 19,20-dihydroakuammicine (IIIc) and echitamidine acetate (IIIb), which show remarkable similarities in the range below m/e 220. The base peak of the 19,20-dihydroakuammicine (IIIc) spectrum at m/e 225 is now found at m/e 283 (base peak) in the mass spectrum of echitamidine acetate (IIIb), which is in accord with the molecular weight increment of 58 mass units due to the additional acetate function.

We conclude, therefore, that dihydroechitamidine is represented by structure VIa and echitamidine by expression IIIa. In this connection it is pertinent to recall that Robinson in his akuammicine paper[6a] made the following brief comment on echitamidine: "If the base were $C_{20}H_{24}O_3N_2$ (C analysis 1% low), it could be a hydroxy-dihydroakuammicine." Our present studies confirm both these predictions.