Tetrahedron 1963, Vol. 19, pp. 2241 to 2246. Pergamon Press Ltd. Printed in Northern Ireland

MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS—XXXVI[1]

ALKALOIDS OF PERIWINKLES—27[2]; THE MASS SPECTRA OF STEREOISOMERS OF THE SARPAGINE-AKUAMMIDINE GROUP[3]

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(*Received* 24 *June* 1963)

Abstract—Mass spectra have been measured of members of the sarpagine-akuammidine group differing in stereochemistry at C-16. Attention is called to possible fallacies in expecting analogous fragmentation behavior of closely related compounds ($N_{(a)}$—H *vs.* $N_{(a)}$—Me) if the mechanism of a specific fragmentation is not known. Attention is drawn to the structural information which can be gained from thermal decomposition processes prior to electron impact. The mutual advantages of an all-glass and a direct inlet system are discussed.

THE mass spectra of several alkaloids containing the skeleton of sarpagine (Ia) have been measured[4–7] in connection with structural work in this class of indole alkaloids and the general fragmentation behaviour seems well established. This knowledge permits ready recognition of unknown bases belonging to this group and a recent example is the observation that an alkaloid isolated from *Vinca difformis* Pourr.[8] possesses the same structure as velosimine (Ib)[9] (no stereochemistry implied), a conclusion reached solely by examination of its mass spectrum and substantiated by comparison with an authentic sample.

Comparison of the spectra of the C-16 stereoisomers polyneuridine (IIa)[5] and akuammidine (IIIa)[10] allowed a further differentiation between these two alkaloids. The most outstanding difference is a very pronounced loss of water (*m/e* 334) in the spectrum (obtained with the use of an all-glass heated inlet system) of polyneuridine

[1] For paper XXXV, see C. Beard, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *J. Amer. Chem. Soc.* in press.

[2] For part 26, see M. Plat, J. LeMen, M.-M. Janot, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *Bull. Soc. Chim. Fr.* 2237 (1962).

[3] The work at Stanford University was supported by grant No. A–4257 from the National Institutes of Health, U.S. Public Health Service.

[4] K. Biemann, *J. Amer. Chem. Soc.* **83**, 4801 (1961); see also M. Gorman, A. L. Burlingame and K, Biemann, *Tetrahedron Letters* 39 (1963).

[5] L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *J. Amer. Chem. Soc.* **84**, 2162 (1962).

[6] E. Clayton, R. I. Reed and J. M. Wilson, *Tetrahedron* **18**, 1449 (1962).

[7] G. Spiteller, C. Brunné, K. Heyns and H. F. Grützmacher, *Z. Naturforsch.*, **17***b*, 856 (1962).

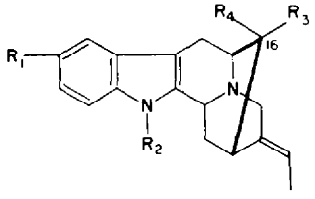
[8] J. Gosset, J. LeMen and M.-M. Janot, unpublished results.

[9] H. Rapaport and R. E. Moore, *J. Org. Chem.* **27**, 2981 (1962).

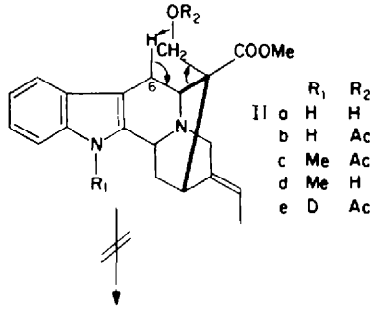
[10] J. Lévy, J. LeMen and M.-M. Janot, *C. R. Acad. Sci., Paris* **253**, 131 (1961).

(IIa, Fig. 2) which is absent in that of akuammidine (IIIa, Fig. 1). An explanation for the ready loss of water from polyneuridine (IIa) was given[5] by assuming the operation of a six membered transition state as indicated by the arrows in II to give IV, which would be sterically impossible in III because of the inverted stereochemistry at C-16.

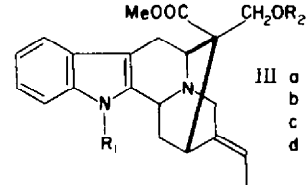
The resulting configuration of polyneuridine (IIa) was consistent with the one established by chemical interrelation with vincamajine[11] and macusine A,[11] the relative configuration of which had been established by X-ray studies.[12] Furthermore, the stereochemistry of akuammidine (IIIa) has been secured by another X-ray analysis.[13] The mass spectroscopic arguments found some support in the observation that both polyneuridine acetate (IIb; Fig. 6) and its d_2-analog containing a CD_2OAc group readily lost the elements of acetic acid (M-60), while akuammidine acetate (IIIb; Fig. 5) did not show this type of fragmentation. This striking difference in the fragmentation behavior of compounds differing solely in the stereochemistry at C-16 seemed to offer a simple means for determining the configuration at this center.



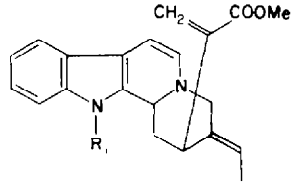
	R_1	R_2	R_3	R_4
I a	OH	H	CH_2OH	H
b	H	H	CHO	H
c	H	Me	CH_2OAc	H
d	H	Me	H	CH_2OAc
e	H	H	CH_2OH	H



	R_1	R_2
II a	H	H
b	H	Ac
c	Me	Ac
d	Me	H
e	D	Ac



	R_1	R_2
III a	H	H
b	H	Ac
c	Me	Ac
d	Me	H



IV

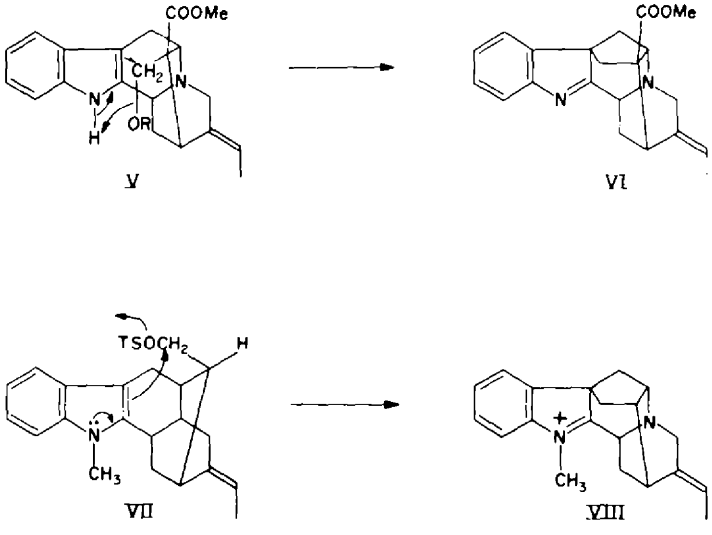
[11] M.-M. Janot, J. LeMen, J. Gosset and J. Lévy, *Bull. Soc. Chim. Fr.* 1079 (1962).

[12] A. T. McPhail, J. M. Robertson, G. A. Sim, A. R. Battersby, H. F. Hodson and D. A. Yeowell, *Proc. Chem. Soc.* 223 (1961).

[13] S. Silvers and A. Tulinsky, *Tetrahedron Letters* 839 (1962).

In order to test the validity of this method, we decided to measure the spectra of several additional representatives of this group. One of these was vincamajine alcohol ester acetate, prepared from vincamajine[11] and therefore presumed to possess structure IIc. The mass spectrum (Fig. 9) of this compound—measured with the use of a heated inlet system—clearly exhibited an M-59, *but no* M-60 *peak*. This finding suggested that vincamajine alcohol ester acetate should be represented by IIIc rather than by IIc. Similarly, Reed *et al.*[6] concluded that voachalotine[14] (which has been demonstrated[11] to be identical with vincamajine alcohol ester) should be N-methyl-akuammidine (IIId) rather than N-methylpolyneuridine (IId). Neither one of these conclusions is compatible with the chemical[11] and X-ray[12,13] results.

An explanation for this inconsistency could consist in a different fragmentation behavior of compounds bearing an $N_{(a)}$-Me group compared with those possessing a hydrogen atom on the indole nitrogen. In order to check this possibility, the mass spectra of deoxyajmalol A and B acetate (Ic and Id)[15] were measured, and turned out to be essentially identical, both exhibiting a very pronounced loss of 59 rather than 60 mass units. Since the C-16 isomeric alkaloids possessing an $N_{(a)}$-hydrogen atom exhibit different fragmentation patterns (using a heated inlet system) as compared to their $N_{(a)}$-methyl homologs, it can be deduced that the presence of the $N_{(a)}$-hydrogen atom is essential for the loss of either acetic acid or water. The mechanism[5] implied by the arrows in II and involving the hydrogen on C-6 can therefore not be operative. This was demonstrated further by measuring the spectrum of $N_{(a)}$-deuterated polyneuridine acetate (IIe) which exhibited a loss of 61 (CH_3CO_2D) rather than 60 mass units (CH_3CO_2H). For this reason the following mechanism (V → VI) for the loss of acetic acid (or water) from alkaloids of the polyneuridine type seems more adequate.



[14] N. Defay, M. Kaisin, J. Pecher and R. H. Martin, *Bull. Soc. Chim. Belg.* **70**, 475 (1961).

[15] We wish to thank Drs. W. I. Taylor and M. F. Bartlett for samples of the two alcohols (cf. M. F. Bartlett, R. Sklar, W. I. Taylor, E. Schlittler, R. L. S. Amai, P. Beak, N. V. Bringi and E. Wenkert, *J. Amer. Chem. Soc.* **84**, 622 (1962) and Dr. Y. Nakagawa of this laboratory for the preparation of the two acetates.

This reaction is reminiscent of the cyclization effected by heating deoxyajmalol-A tosylate (VII) to yield VIII,[16] and thus aroused the suspicion that the loss of water from polyneuridine (IIa) and of acetic acid from polyneuridine acetate (IIb) occurred by heating prior to electron impact. This assumption could be substantiated by measuring the spectra of polyneuridine (IIa; Fig. 4) and akuammidine (IIIa; Fig. 3) as well as those of their acetates (IIb and IIIb; Figs. 8 and 7) using a direct inlet system[17] which is known to cause minimal thermal decomposition. Under these conditions, neither the loss of water from IIa nor the loss of acetic acid from IIb could be observed.

These observations clearly indicate that the conclusions derived from free NH-compounds concerning the loss of water or acetic acid cannot be extended to their N-methylated analogs and therefore that the above stated deductions concerning the stereochemistry at C-16 of vincamajine alcohol ester acetate (IIc) and voachalotine (IId)[6] (= vincamajine alcohol ester[11]) are not valid. The apparent inconsistencies of the mass spectral conclusions with chemical and X-ray evidence are thus resolved.[18]

These examples demonstrate how much caution has to be exercised in explaining a certain fragmentation process by an "obvious" though unproved mechanism, especially if it is applied to related compounds. Futhermore, our results show that both the direct[17] and the heated all-glass inlet system can have their particular advantages. The former minimizes the catalytic and thermal decomposition and in the present case furnishes very similar spectra of stereoisomers and N-methyl homologs, thus allowing a direct comparison. The heated glass inlet system, besides offering more reproducible spectra,[19] may furnish additional structural information[20] specifically because of the thermal decomposition which in this instance was shown to be subject to stereochemical features.

All spectra were measured with a CEC 21–103C mass spectrometer using either a heated all-glass inlet system (200°) or a direct inlet system.[17] The ionizing current was maintained at 50 μA and the ionizing energy at 70 eV.

[16] M. F. Bartlett, B. F. Lambert, H. M. Werblood and W. I. Taylor, *J. Amer. Chem. Soc.* **85**, 475 (1963).

[17] J. F. Lynch, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Experientia* **19**, 211 (1963).

[18] On the other hand, spectra obtained with a normal (heated) glass inlet system of compounds with an unsubstituted indole nitrogen may well be used for stereochemical assignments. As anticipated, vincamajine alcohol ester acetate (IIc)[11] gives the same fragmentation pattern using either a heated glass (Fig. 9) or a direct (Fig. 10) inlet system.

[19] The glass inlet system allows more even heating and a steadier gas flow than the direct one, the latter giving rise to comparatively minor, but recognizable, changes in the relative intensities of the peaks in different runs of the same substance.

[20] It should be mentioned that R. I. Reed in one of his early papers on electron impact phenomena (*J. Chem. Soc.* 3432 (1958)) pointed out this possibility.

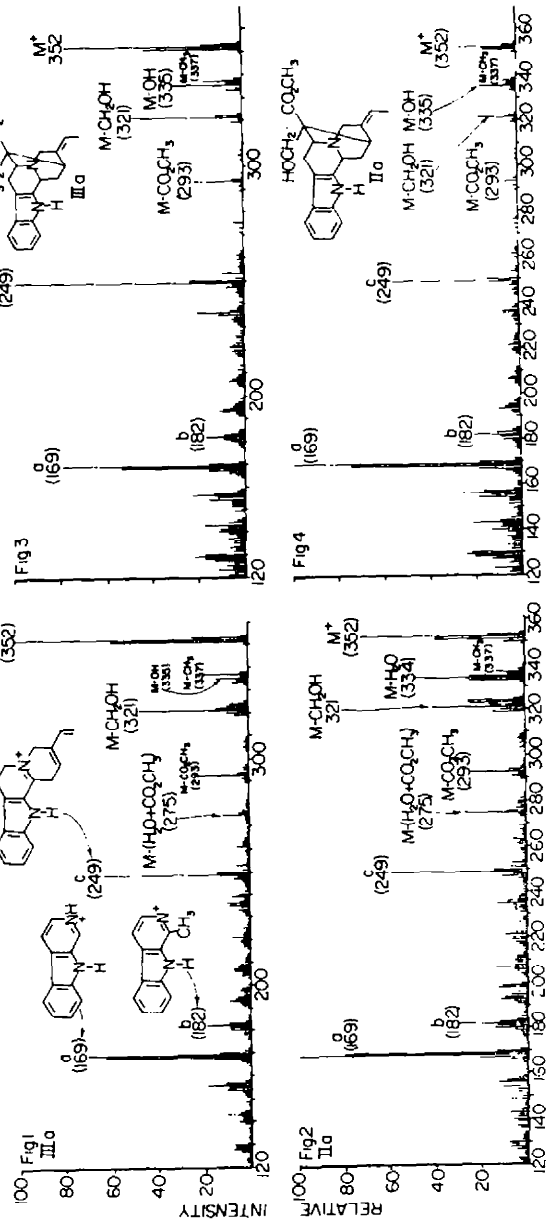


FIG. 1. Mass spectrum (200° glass inlet system) of akuammidine (IIIa).

FIG. 2. Mass spectrum (200° glass inlet system) of polyneuridine (II_2).

FIG. 3. Mass spectrum (direct inlet system) of akuammidine (IIIa).

FIG. 4. Mass spectrum (direct inlet system) of polyneuridine (IIa).

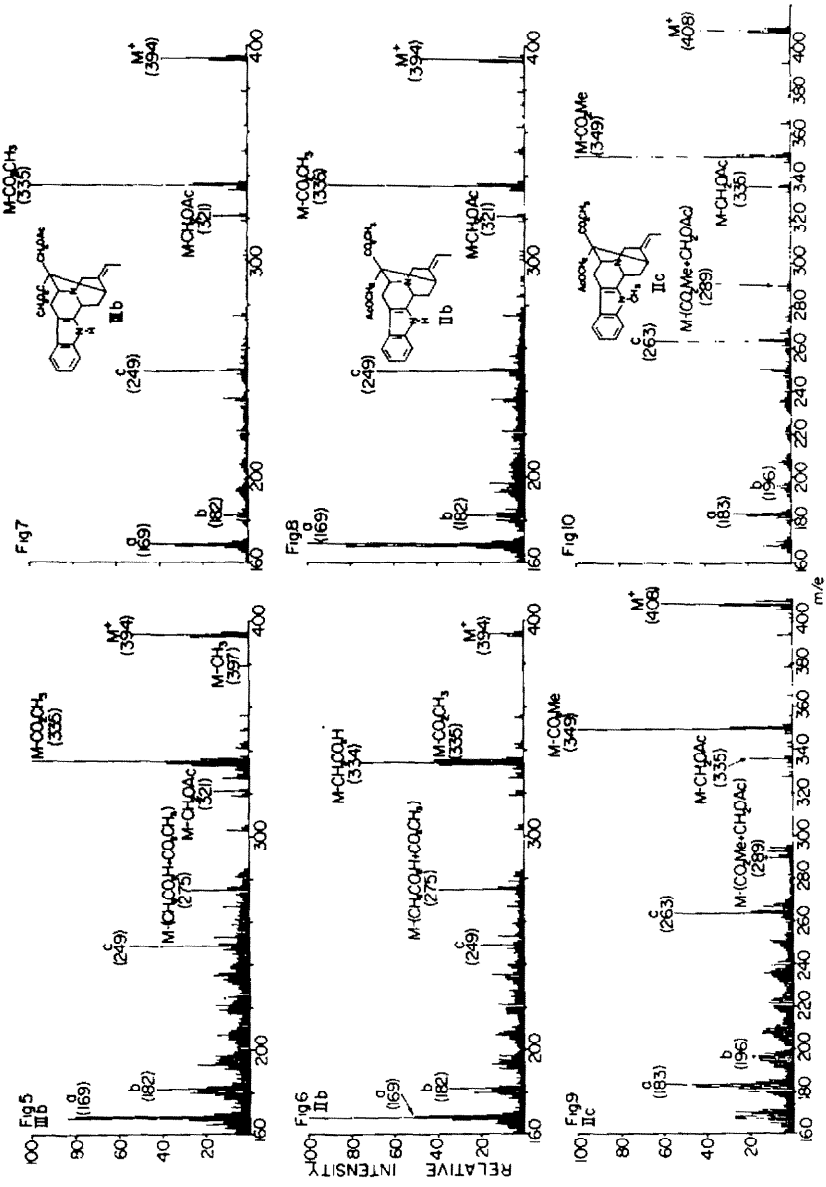


FIG. 5. Mass spectrum (200° glass inlet system) of akuammidine acetate (IIIb).

FIG. 6. Mass spectrum (200° glass inlet system) of polyneuridine acetate (IIb).

FIG. 7. Mass spectrum (direct inlet system) of akuammidine acetate (IIIb).

FIG. 8. Mass spectrum (direct inlet system) of polyneuridine acetate (IIb).

FIG. 9. Mass spectrum (200° glass inlet system) of vincamajine alcohol ester acetate (IIc).

FIG. 10. Mass spectrum (direct inlet system) of vincamajine alcohol ester acetate (IIc).