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MASS SPECTRA OF PUROMYCIN AND SOME DERIVATIVES

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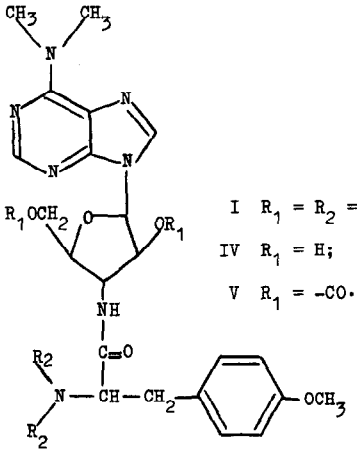
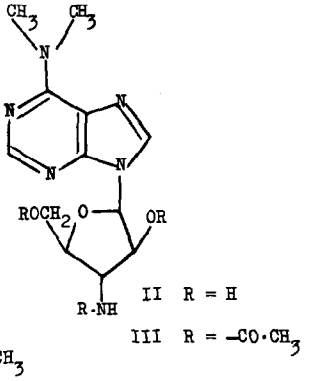
The structure of puromycin (I) has been known for a long time.[1] The promising results obtained by Biemann and McCloskey[2] in their early study of the mass spectrometric fragmentation of a number of nucleosides, prompted us to investigate the behaviour of puromycin and some derivatives, upon electron impact.



I $R_1 = R_2 = H$

IV $R_1 = H$; $R_2 = CH_3$

V $R_1 = -CO\cdot CH_3$; $R_2 = CH_3$



II R = H

III R = $-CO\cdot CH_3$

The base peak in the mass spectrum[3] (Fig. 1) of the puromycin nucleoside (II),[4] occurs at m/e 164, which corresponds to the purine nucleus plus two transferred hydrogen atoms. It has already been shown[2] that the transferred hydrogen atoms originate largely from the hydroxyl groups.

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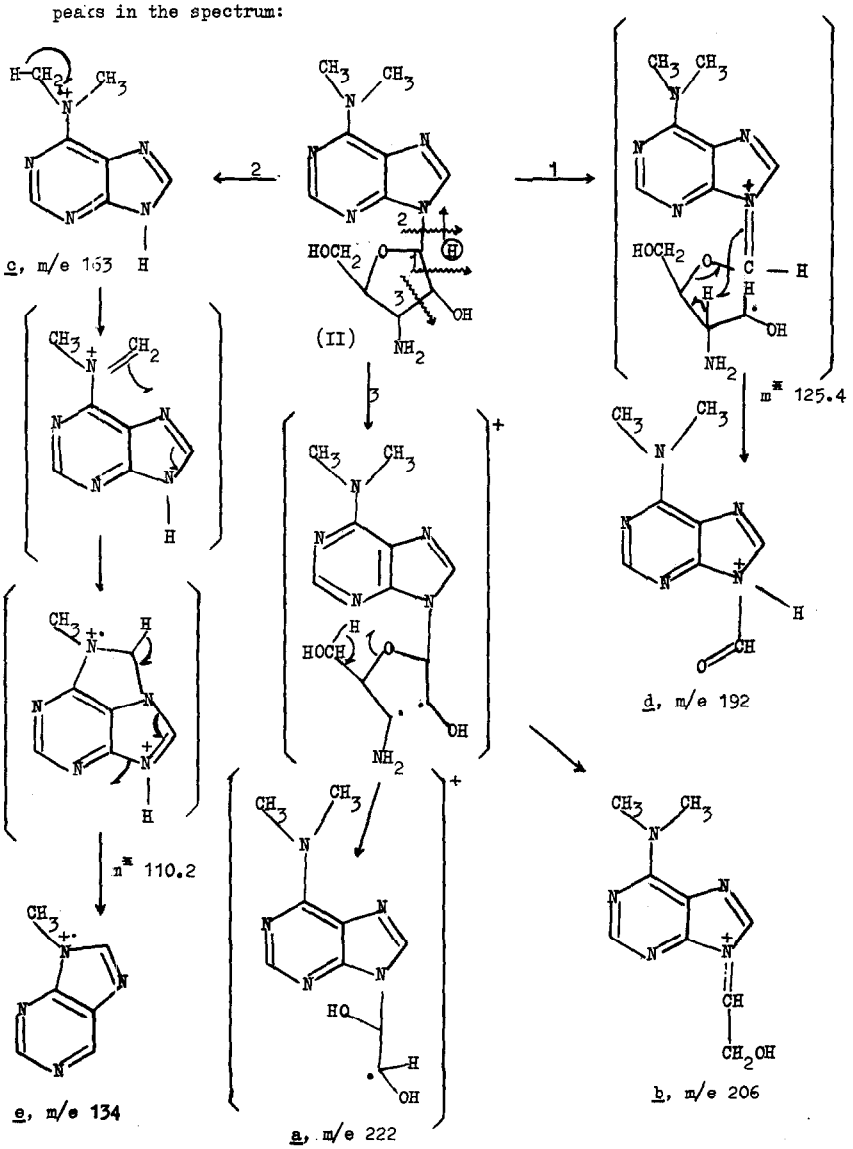
We propose the following scheme to account for the remaining significant peaks in the spectrum:



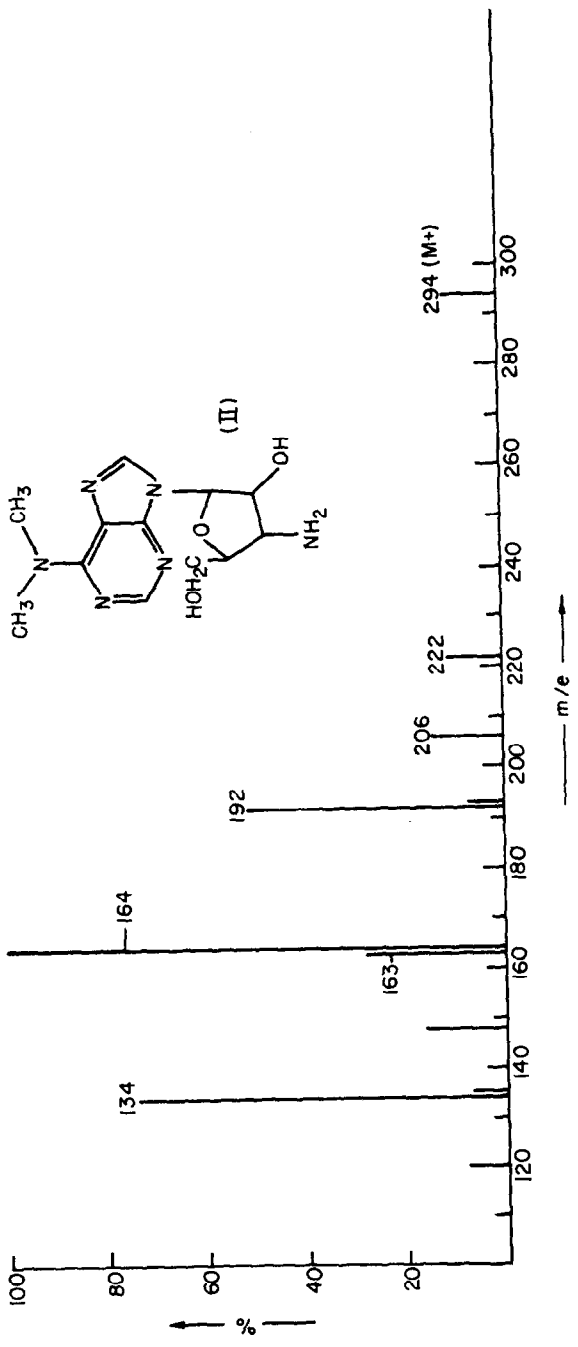


FIG. 1

An accurate mass determination on the peak at m/e 134 indicated that it was mainly due to an ion of composition $C_6H_6N_4$. A metastable peak at 110.2 revealed that the transition m/e 163 ⟶ m/e 134 was concerted. The driving force for this complex rearrangement must be the stability of the end products, protonated hydrogen cyanide and the substituted purine. The fact that a similar rearrangement was not observed in related substances where the amino group was not methylated, such as deoxyadenosine, tends to support the suggested rearrangement.

The tri-acetate of the puromycin nucleoside (III) (Fig. 2) showed a molecular ion at m/e 420, as well as most of the important peaks observed with the free nucleoside. The additional prominent peak at m/e 258 indicated the enhanced tendency of the acetylated sugar residue to accommodate the positive charge. The corresponding peak in the free nucleoside (m/e 132) was negligible. The additional peak at m/e 216 must have arisen from that at m/e 258 through expulsion of ketene. The relative intensity of the peaks at m/e 163 and 164 is of interest. Since Biemann and McCloskey[2] have shown that the hydrogens which are transferred in the formation of equivalent ions originated largely from the hydroxyl groups, there must have been a significant loss of ketene from the acetate groups, to enable the formation of these ions. This loss of ketene was probably thermal, because of the absence of strong peaks at (M-42) and m/e 42. The enhanced intensity of the m/e 163 peak relative to that at m/e 164, when compared with corresponding peaks in the spectrum of the free nucleoside, may indicate that a significant proportion of the m/e 163 peak results from an alternate mechanism, probably through α-cleavage

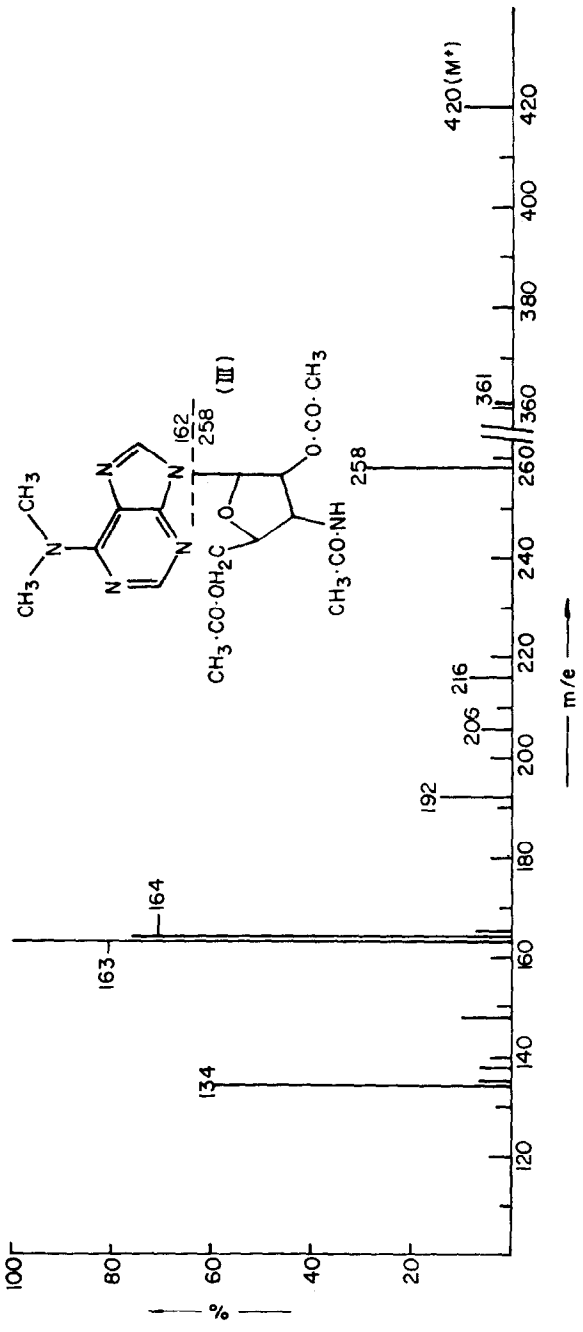
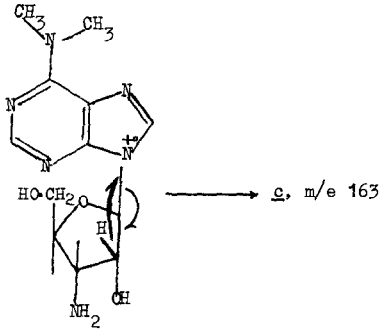

100
80
60
40
20
% —
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
— m/e —
163
164
134
192
205
216
258
361
420 (M+)
(III)
162
258
CH3·CO·OH2C
CH3·CO·NH
O·CO·CH3
CH3
N

FIG. 2

to nitrogen with transfer of the C2' hydrogen atom:[5]



In the spectrum of puromycin (I) (Fig. 3), the base peak occurred at m/e 121 arising from fission of the benzylic bond (m^{*} 243.3, 453 ⟶ 332). Peaks due to the remaining portion of the molecule (m/e 350, M-121 and m/e 332, M-121-18) were also relatively intense. Whereas fragmentation in the first two compounds was dominated by the electron-rich purine residue, this was no longer the case with the amino acid attached to the sugar. Fission on either side of the amide carbonyl led to ions with masses 150 and 178. The peak at m/e 309 was assigned to the sugar residue, after simple cleavage of the purine-sugar linkage without hydrogen rearrangement. Peaks characteristic of the puromycin nucleoside were prominent. Transitions from fragments with masses 350 and 332 to the fragment with m/e 164 were indicated by metastable peaks at 76.8 and 81.0, respectively.

N,N-Dimethylpuromycin (IV) showed the expected molecular ion at m/e 499 (Fig. 4). The base peak appeared at m/e 178 as opposed to m/e 121 in puromycin. This illustrates the enhanced capacity of the dimethylamino group for stabilising a positive charge and directing fragmentation, as opposed to a free amino group. Shifts resulting from the additional substituents are apparent from peaks at m/e 378 (M-121) and m/e 360 (M-121-18), these being 28 mass units higher than the corresponding peaks

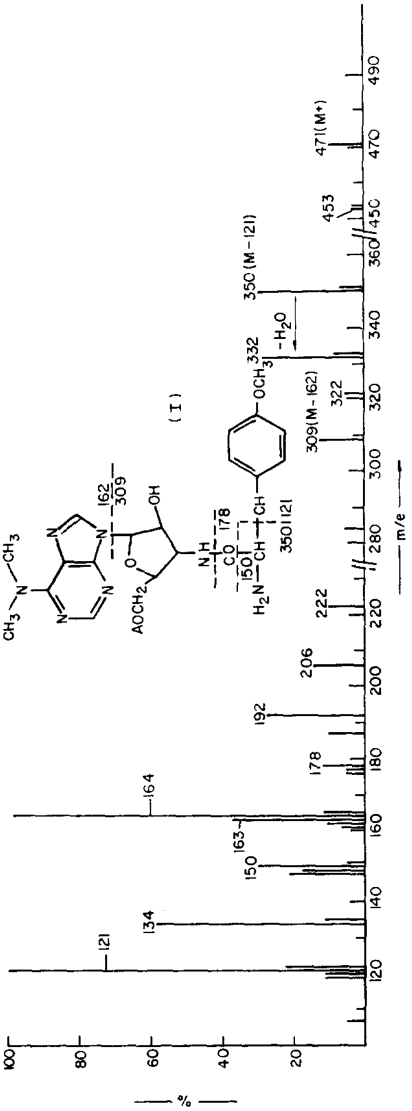

(I)
471(M+)
453
350(M-121)
332
-H2O
322
309(M-162)
222
206
192
178
164
163
150
134
121
m/e
%

FIG. 3

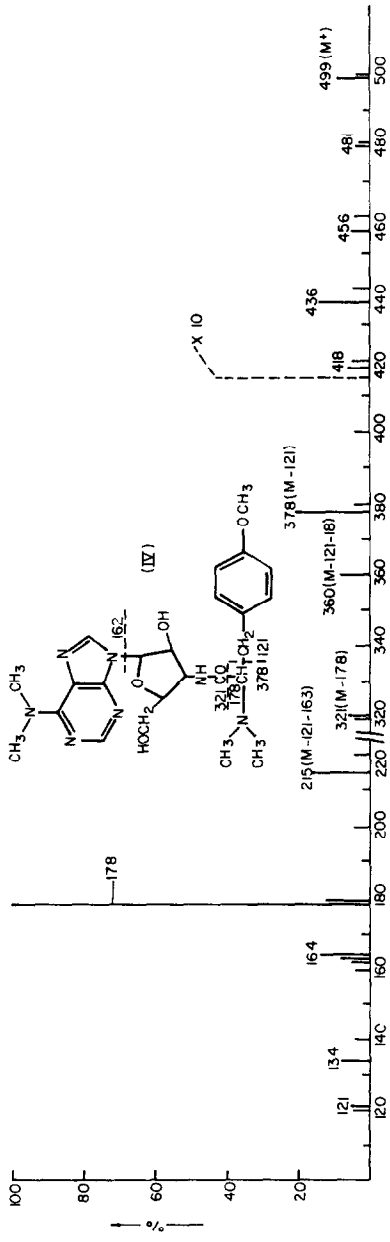

(IV)
X 10
499(M+)
378(M-121)
360(M-121-18)
321(M-178)
215(M-121-163)
178
164
134
121
%
m/e

FIG. 4

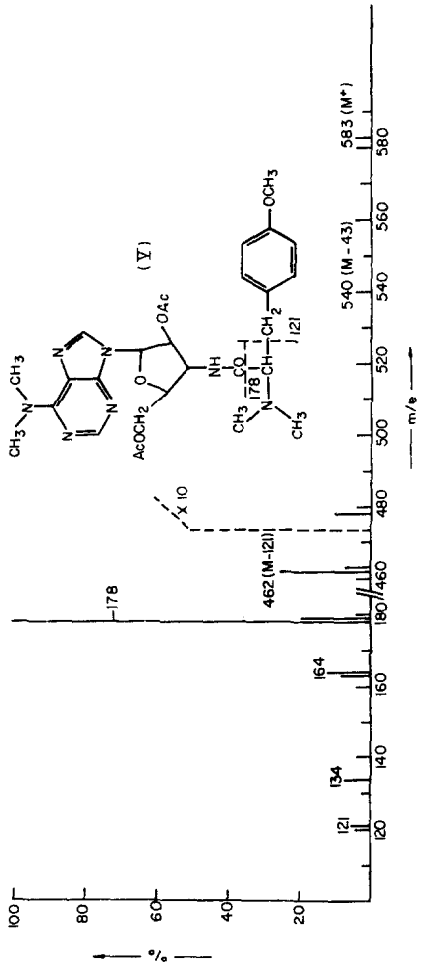

(V)
OAc
NH
CO
CH
CH2
121
OCH3
178
CH3
N
CH3
AcOCH2
O
CH3
N
CH3
N
N
N
N
583 (M+)
540 (M-43)
462 (M-121)
X 10
178
180
164
134
121
100
80
60
40
20
%
120
140
160
460
480
500
520
540
560
580
m/e

FIG. 5

in puromycin. Metastable peaks were observed for transitions m/e 499 ⟶ m/e 378 (m^* 286.3, loss of 121 mass units), and m/e 378 ⟶ m/e 215 (m^* 122.3 loss of 163 mass units). Peaks characteristic of the purine residue (m/e 164 and m/e 134) were present, but of relatively low intensity.

The base peak in the spectrum (Fig. 5) of N,N-Dimethylpuromycin di-acetate V(M^+ 583) was also at m/e 178. However, the spectrum is not as useful as that of the unacetylated material. Judging from the spectra of the two acetylated compounds therefore, it would seem unnecessary and in fact undesirable to attempt to increase the volatility of this type of compound by acetylating hydroxyl or free amino groups.

REFERENCES

1. C.W. Waller, P.W. Fryth, B.L. Hutchings and J.H. Williams, J. Amer. Chem. Soc. 75, 2025 (1953).

2. K. Biemann and J.A. McCloskey, J. Amer. Chem. Soc. 84, 2005 (1962).

3. Spectra were obtained on an Associated Electrical Industries MS-9 mass spectrometer. Compounds I, II and IV were introduced directly into the ion source (temp. ca 150°), in the form of their hydrochloride salts. Only peaks with an intensity ⩾ 5% of the base peak are shown.

4. The origin of some of these compounds will be reported on in a full paper elsewhere.

5. R.S. Gohlke and F.W. McLafferty, Anal. Chem. 34, 1281 (1962).