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MASS SPECTROMETRY IN CARDENOLIDE CHEMISTRY—I:

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Abstract—The mass spectrum of penta acetyl gitoxin is reported and discussed. This cardenolide derivative has recently been the subject of extensive metabolic study. Fragmentation processes are described which account for all of the major ions. High resolution measurements, metastable peaks and mass shifts, for the propionyl derivative, are reported to establish the validity of these proposals. A brief indication of the potential value of this technique, when combined with deuteroacetylation, to metabolic studies in this field is given, along with comments on the spectra of related compounds.

CARDENOLIDES (cardiac glycosides) have long been of interest because of their pharmaceutical potency.[1,2,3] Recent studies of the metabolites of acetylated cardenolides[4 to 7] have resulted in ambiguous conclusions because there has been no technique for locating acetyl groups within these complex molecules. We wish to illustrate the potential value of mass spectrometry to these studies.

The mass spectrum of penta acetyl gitoxin (IV), Fig. 1, can be rationalised if one considers two major fission processes. The first of these corresponds to a fragmentation already well documented in monosaccharide spectra.[9] The initially formed ion radical, localised on the cyclic ether oxygen of one sugar,[8] initiates cleavage of the adjacent glycosidic bond to generate A type ions. (These are lettered in accordance with Kotechov's nomenclature.[9]) It is of interest that other important fission processes found in monosaccharide spectra, for example C_6—C_5 cleavage (E type ions) or ring cleavage, have been suppressed in this trisaccharide.

Thus the peak at *m/e* 215 corresponds to ion A_3, containing the sugar S_3 (*m/e* 215·0927; $C_{10}H_{15}O_5$ from high resolution measurements). Two other ions *m/e* 155·071 ($C_8H_{11}O_3$; A_3^1) and *m/e* 95·049 (C_6H_7O; A_3^2) are related to A_3 by loss of one and two molecules respectively of acetic acid. Metastable peaks at m* 118·5 (*m/e* 215 → *m/e* 155) and m* 58·3 (*m/e* 155 → *m/e* 95) were found supporting this conclusion.

Location of the ion radical site within the S_2 ring should afford an A_2 ion at *m/e* 387. No ion corresponding to this was found, but its related ion A_2^1 (*m/e* 327·143; $C_{16}H_{23}O_7$) was prominent. In the case of fragmentation of sugar S_1 both anticipated peaks, A_1 at *m/e* 559 and A_1^1 at *m/e* 499, were found. High resolution confirmed the $C_{24}H_{35}O_{11}$ composition (*m/e* 499·2196) of the latter but also revealed an isobaric component presumably arising by breakdown of the aglycone (*m/e* 499·2708; $C_{29}H_{39}O_7$). No further evidence for fragmentation of the aglycone rings was found in the spectrum of compound IV.

The second major fission process again involved cleavage of the saccharide chain, but with charge retention on the aglycone. This, termed T cleavage by us, can be represented by two possible schemes. The first being simple cleavage of the C_3—O bond, with charge retention on the steroid A ring. Alternatively one can consider a tertiary carbonium ion, formed by loss of $OH^{\cdot}$ from C_{14}, undergoing sequential

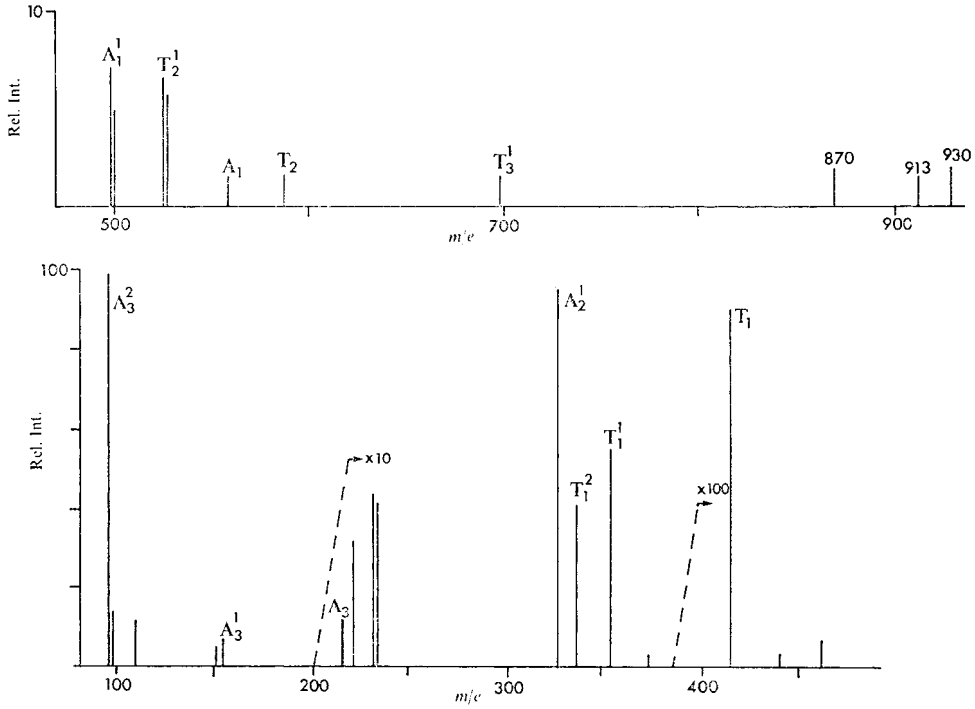
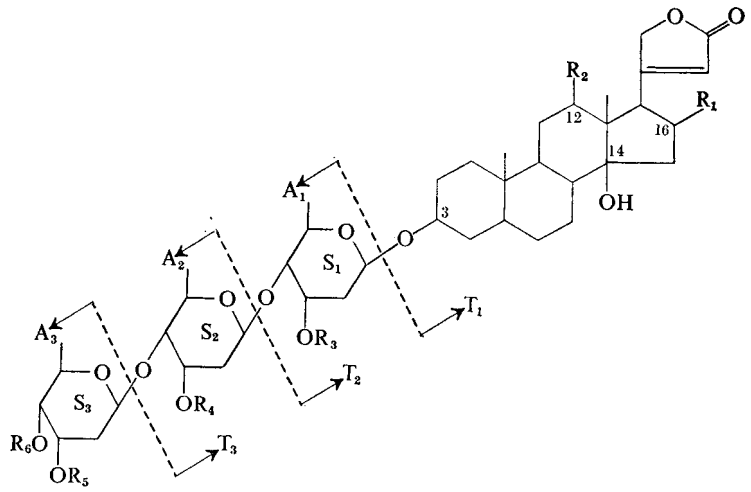
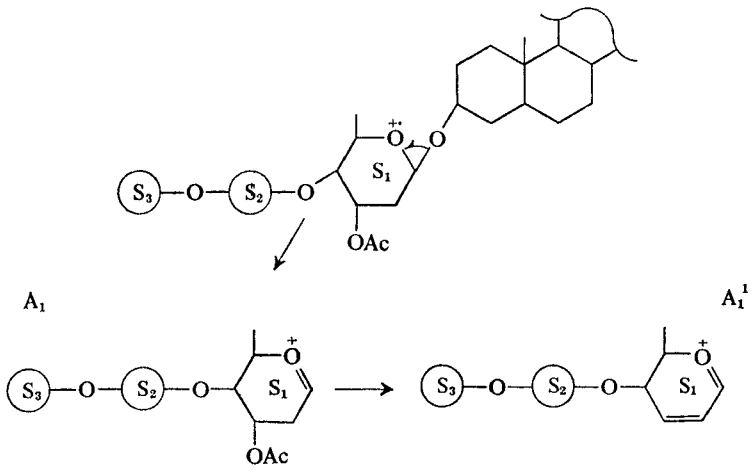


FIG. 1.

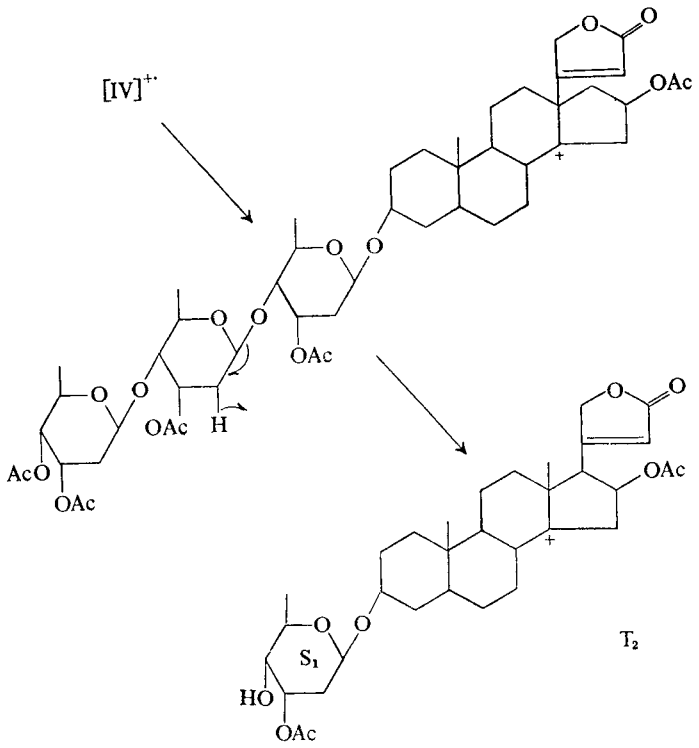


(I) R_2 to R_6 = H; R_1 = OH
(II) R_2 = OH; R_1 = R_3 to R_6 = H
(III) R_1 to R_6 = H
(IV) R_1 = OAc; R_2 = H; R_3 to R_6 = Ac
(V) R_1 = $OCOC_2H_5$; R_2 = H; R_3 to R_6 = COC_2H_5
(VI) R_1 = H; R_2 = OAc; R_3 to R_6 = Ac

SCHEME 1



SCHEME 2 Production of A type fragments.



SCHEME 3 Generation of T type ions.

fragmentation by elimination from the sugar residues. The first member of this series T_1 (*m/e* 415·250; $C_{25}H_{35}O_5$) corresponds to the genin (less $OH^{\cdot}$) formed by elimination from S_1. Further losses of acetic acid, followed by water accounting for T_1^1 [*m/e* 355·227; $C_{23}H_{31}O_3$; m* 304 (*m/e* 415 ← *m/e* 355)] and T_1^2 [*m/e* 337·217; $C_{23}H_{29}O_2$; m* 320 (*m/e* 355 → *m/e* 337)] respectively.

Operation of this fragmentation in the S_2 sugar ring affords ions T_2 (*m/e* 587·323; $C_{33}H_{47}O_9$) and T_2^1 (*m/e* 527·298; $C_{31}H_{43}O_7$). No ion was found corresponding to T_3, i.e. *m/e* 759, but the anticipated T_3^1 ion at *m/e* 699 was observed.

The spectrum of pentapropionyl gitoxin (V) was examined for confirmation of the above assignments. All of the anticipated mass shifts were found (Table 1) but high resolution studies were required to analyse peaks containing isobaric A and T type fragments.

TABLE 1. A AND T TYPE IONS IN THE MASS SPECTRUM OF PENTA PROPIONYL GITOXIN (V)

A_1	*m/e* 615; A_1^1 *m/e* 541·267, $C_{27}H_{41}O_{11}$ (calc. 541·2648)
A_2	*m/e* 429·214, $C_{21}H_{33}O_9$ (calc. 429·2124)
A_2^1	*m/e* 355·176, $C_{18}H_{27}O_7$ (calc. 355·1757)
A_3	*m/e* 243·123, $C_{12}H_{19}O_5$ (calc. 243·1232)
A_3^1	*m/e* 169; A_3^2 *m/e* 95
T_3^1	*m/e* 727; T_2 *m/e* 615
T_2^1	*m/e* 541·318, $C_{32}H_{45}O_7$ (calc. 541·3165)
T_1^1	*m/e* 355·227, $C_{23}H_{31}O_3$ (calc. 355·2273)
T_1^2	*m/e* 337·218, $C_{23}H_{29}O_2$ (calc. 337·2167)

The spectrum of penta acetyl digoxin (VI) was also obtained in order to assess a possible extension of this work. All of the A and T type fragments were found. Additional peaks were also present, presumably reflecting increasing fission of the digoxigenin ring system as compared with gitoxigenin.†

In none of the spectra was a molecular peak observed, but peaks found at *m/e* 930 [M − 60]; *m/e* 913 [M − $OH^{\cdot}$ − 60] and *m/e* 870 [M − 120] in the spectrum of compound IV are typical of acetyl sugars.

From the previous account it is clear that mass spectrometry can provide specific information on the aglycone and each individual sugar in these complex molecules. When used in conjunction with deuteroacetylation this provides a valuable method for characterising partial acetates relevant to metabolic studies in this field.

EXPERIMENTAL

Mass spectra were run, using a direct insertion probe, on an AEI MS-902 instrument (source 250°C; 70 eV ionizing energy; 100 μA emission). High resolution measurements (100 μA or 500 μA as required; apparent resolving power 13,000) using perfluoro tri-n-butylamine as reference were within 5 ppm in all cases.

Commercially available samples of gitoxin (I) and digoxin (II) were used.

Penta acetyl gitoxin (IV)

This compound was prepared according to the method of Hoji.[6] After 2 to 3 days reaction time t.l.c. (silica gel/ethyl acetate solvent) revealed one major component. Excess reagent was removed

† Work is in progress to firmly establish the fragmentation path leading to T type ions and to examine in detail fission of other cardenolides.

and the product was isolated as an amorphous solid. Chromatography on silica gel in (1) pyridine-chloroform (1:4 v/v) and (2) chloroform-acetone (1:1 v/v) revealed one component.

Penta acetyl digoxin (VI)

This compound was prepared in identical manner and its purity was checked by t.l.c. in each of the above solvent systems.

Penta propionyl gitoxin (V)

Gitoxin (50 mg) in dry pyridine (1 ml) was treated with propionic anhydride (1 ml) and set aside for 2 days. Thin layer chromatography revealed several components. After 4 days reaction time only one component was found. Excess reagent was removed and the major component was isolated as a clear gum. After its purity was checked by t.l.c. its mass spectrum was obtained.

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