# SOME QUESTIONS OF THE MASS SPECTROMETRY OF THE CARDENOLIDES

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In spite of the fact that fragmentation mass spectrometry has been used fairly widely to prove the structures of cardenolides [1-3], the mechanism of the fragmentation of their steroid skeleton has been studied by only a few authors [4, 5], and this without the use of isotopic substitution. This is perhaps due to the fact that the main fragmentary ions in the spectra of the majority of the cardenolides arise by the splitting out of the functional groups, and products of the deeper degradation of the molecules have a low abundance. In those cases where the number of oxygen-containing substituents is small (digitoxigenin) it is possible to characterize the more intensive fragmentary ions formed by the cleavage of bonds of the basic skeleton [5]. Subsequently, all workers referred to this paper of Spiteller, and only some years later did Fayez [6] attempt to generalize the result obtained up to this time.

Having to deal mainly with aglycones isolated from Central-Asian species of plants, and also with some other cardenolides, we have attempted to establish additional characteristics distinguishing the mass spectra of the compounds considered. We have studied the spectra of 19 cardenolides and their derivatives and also their deutero analogs (MKh-1303 instrument, direct introduction of the substance, temperature 100-170°C, ionizing voltage 40 V).

## Splitting off of Functional Groups

With an increase in the number of oxygen-containing substituents in cardenolide molecules, the proportion of skeletal cleavage falls. The successive splitting off of OH and C = O groups gives rise to the most intensive peaks of the mass spectra. We decided to check whether this fact is a consequence of the thermal degradation of the cardenolides in the inlet system of the mass spectrometer. For this purpose the internal surface of the tube used for the introduction of the sample was coated with a thin layer of the sample (strophanthidin) and was kept in the inlet system under mass-spectrometry conditions (~ 150°C, pressure ~ $3 \cdot 10^{-7}$ torr) for about 1 h. Then the sample was extracted from the tube and was chromatographed in the presence of markers: pure strophanthidin and anhydrostrophanthidins (pachygenin and difugenin). The chromatogram revealed only traces of foreign impurities not coinciding with the anhydrocardenolides, and the main spot of the extracted sample was found at the same level as that of strophanthidin. Consequently, the dehydration process took place under the action of electron impact.

It may be considered that the first act of the decomposition of $M^+$ is the cleavage of the $C_1-C_{10}$ bond with the localization of the charge on $C_{10}$ [7, 8], after which hydroxy groups are eliminated in the form of water and also as C = O or $CH_2O$ if CHO or $CH_2OH$ is present on $C_{10}$. The results of a comparison of the spectra show that the main contribution to the cleavage processes is made by the substituents in ring A, and the 14β-OH is split off with considerably more difficulty.

Table 1 gives, together with the number and nature of the substituent groups of the compounds (I-XVIII) studied, the total intensities of all the processes connected with the splitting off of two, three, and four ROH groups (where R = H or Ac) in relation to the total intensity of the processes involving the split-

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TABLE 1. Functional Groups and Characteristics of Their Splitting Off

| Compound | Substituent | | | | | No. of OR groups in the molecule | Relative intensities of the processes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3β | 5 | 10 β | 14 β | 17 | | $M^+$ | ΣM−−ROH | ΣM−−2ROH | ΣM−−3ROH | ΣM−−4ROH |
| Digitoxigenin (I) | OH | β−H | $CH_3$ | OH | B | 2 | 0,15 | 1 | 0,65 | — | — |
| Digitoxigenone (II) | C=O | β−H | $CH_3$ | OH | B | 1 | 30 r | 1 | — | — | — |
| Digitoxigenin acetate (III) | AcO | β−H | $CH_3$ | OH | B | 2 | 0,07 | 1 | 0,25 | — | — |
| Uzarigenin acetate (IV) | AcO | α−H | $CH_3$ | OH | B | 2 | 0,33 | 1 | 0,45 | — | — |
| Pachygenin (V) | OH | $\Delta^5$ | CHO | OH | B | 2 | 0 | 1 | 0,51 | — | — |
| Pachygenin acetate (VI) | AcO | $\Delta^5$ | CHO | OH | B | 2 | 0 | 1 | 0,47 | — | — |
| Diffugenin (VII) | OH | β−OH | CHO | $\Delta^{14}$ | B | 2 | 0,04 | 1 | 3,0 | — | — |
| Diffugenin acetate (VIII) | AcO | β−OH | CHO | $\Delta^{14}$ | B | 2 | 0,03 | 1 | 6,8 | — | — |
| Periplogenin (IX) | OH | β OH | $CH_3$ | OH | B | 3 | 0,01 | 1 | 0,34 | 0,17 | — |
| Strophanthidin (X) | OH | β−OH | CHO | OH | B | 3 | 0 | 1 | 2,2 | 0,57 | — |
| Strophanthidin acetate (XI) | AcO | β−OH | CHO | OH | B | 3 | 0 | 1 | 120 | 55 | — |
| Strophanthidol (XII) | OH | β−OH | $CH_2OH$ | OH | B | 4 | 0,1 | 1 | 2,4 | 3,0 | — |
| 17α-Hydroxystrophanthidin (XIII) | OH | β−OH | CHO | OH | α−OH, B | 4 | 0 | 1 | 3,1 | 1,2 | 0,15 |
| 17α-Hydroxystrophanthidin acetate (XIV) | AcO | β−OH | CHO | OH | α−OH, B | 4 | 0 | 1 | 2,15 | 3,3 | 1,7 |
| Isostrophanthidin (XV) | OH | β−OH | CHO | $C_{14}$ O $C_{21}$ | | 2 | 0 | 1 | 2,2 | 0,13 | — |
| 20,22-Dihydrodiffugenin (XVI) | OH | β−OH | CHO | $\Delta^{14}$ | Dihydro-B | 2 | 0 | 1 | 0,62 | — | — |
| 20,22-Dihydrostrophanthidin (XVII) | OH | β−OH | CHO | OH | Dihydro-B | 3 | 0 | 1 | 6,7 | 4,0 | — |
| 21-Hydroxy-20,22-dihydrodiffugenin (XVIII) | OH | β−OH | CHO | $\Delta^{14}$ | 21-Hydroxy-dihydro-B | 3 | 0 | 1 | 4,7 | 4,6 | — |

Note. B − butenolide ring.

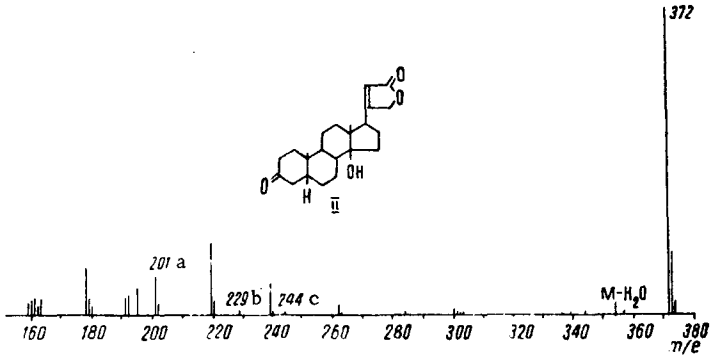



Fig. 1. Mass spectrum of digitoxigenone (II).

ting off of one ROH group, taken as unity. As an example we may state that in the case of digitoxigenin (I) the intensity of the $(M-H_2O) + (M-H_2O-CH_3)$ peaks is taken as unity, and the intensity of $(M-2H_2O) + (M-2H_2O-CH_3)$ amounts to 0.65 of this value. The intensity of $M^+$ was also considered with respect to its ratio to M−ROH. For a molecule containing a 14β-OH group, the total intensity of the acts of splitting out of the latter R−OH group averaged almost three times less than the magnitude characterizing the splitting out of the preceding group (without taking the figures for digitoxigenin into account). At the same time, in the spectrum of digitoxigenone (II), the molecule of which contains a hydroxyl only in the 14β position, the $M^+$ ion was the maximum ion, and the intensity of the M−18 ion amounted to only one thirtieth of it. It is possible that the splitting out of the 14β-OH is made difficult by skeletal cleavage taking place close to this center; however, the spectrum of digitoxigenone (Fig. 1), in contrast to that of digitoxigenin (I) [5], contains no strong peaks indicating a fragmentation of the skeleton. In addition to this, in the spectra of diffugenin (VII) and its acetate (VIII) with a double bond in the 14,15 position, the intensities of the later acts of cleavage are higher than those of the earlier ones. However, the ratio between the intensities of the two stages of cleavage are approximately the same as in the corresponding stages of the aglycones (X, XII, XIII). The features observed (see Table 1) are connected in some degree with the presence of a butenolide ring in each of the molecules (I-XIV). In the spectra of substances (XVI-XVIII), which lack the $\Delta^{20,22}$ double bond, these characteristics are disturbed. This shows the possible interaction of the 14β-OH with the butenolide ring under the action of electron impact. If the ratios of the intensities of strophanthidin (X) and isostrophanthidin (XV), in which the 14β-OH is linked to the butenolide ring, are compared, we see that in the first two stages the ratios coincide and in the subsequent stage the intensity in the case of (XV) is only several times lower than in the case of (X), although in this case the third molecule of water is eliminated by the closure of the tetrahydropyran ring.

The intensity of the $M-H_2O$ peak in the spectrum of digitoxigenone (II) makes up approximately the same fraction of the intensity of $M^+$ as the intensity of the $M-3H_2O$ peak in (XV) with respect to the sum of the intensities of the preceding stages.

## Cleavages of the Bonds of the Main Skeleton

For the fragments known from the literature, we shall retain the classification proposed by Fayez [6]. The methylcyclopentanooctahydronaphthalene cation is denoted by *a*. When one O−R group is present in ring A or B, this ion is the maximum ion (I, III, IV). The spectra of the deutero analogs show that the ion *a* contains none of the isotopic label.

In the spectra of the aglycones with a large number of oxygen-containing substituents, the peaks corresponding to the ion *a* are of medium or low intensity. Let us give the mass numbers of the ions *a* in the various spectra in m/e: for (I, III, and IV) 203; for (V, VI, X, XI, XIII, and XIV) 215; for (IX) 201; and for (XII) 217.

Also characteristic for substances (V, VI, X-XIV) with an oxygen-containing substituent at $C_{10}$ are peaks with m/e 187, corresponding to the splitting out of the substituent (in the form of CO or $CH_2O$) from the ions given above.

In the spectrum of isostrophanthidin (XV), the ion with m/e 187 is too weak, so that it is impossible to state that the ions with m/e 215 and 216 have structures corresponding to the ions *a*.

Out of all the spectra considered, only in that of periplogenin (IX) is there a hydroxyl-containing ion with m/e 219 which, by losing a molecule of water, is converted into an ion with m/e 201 (m* = 184.5). Another route for the formation of the latter from the ion with m/e 354 ($M-2H_2O$) can be traced by means of the metastable peak at 114.2 amu.

The ion of type b in the spectra of (I, III, and IV) is characterized by a low intensity, while it does not appear in the other spectra.

The greatest interest is provided by a study of ions of the third type, to which Fayez [6] ascribed structure c, while Spiteller [5] assigned the structure c'. In both variants of the ions there is a 14β-OH group and, consequently, in the spectra of the deutero analogs the corresponding peak should be shifted by one mass unit. In actual fact, in the spectra of digitoxigenin (I) and its acetate (III) in which the peak with m/e 246 is one of the strongest, no displacement at all is observed. In both cases, the following course of fragmentation is found:

$$M^+ \xrightarrow[-AcOH\ III]{-H_2O\ \ I} 356 \xrightarrow{-110} 246.$$

Although a small M−110 peak due to the splitting off of the lactone ring together with $C_{17}-C_{16}$ directly from $M^+$ can be seen, it does not form the ion with m/e 246 by the ejection of water as Fayez [6] considers. In actual fact, in the spectrum of the D analog of (I) the peak with m/e 264 is shifted by two units. If the ion with m/e 246 were formed from this molecule, then molecules containing two deuterium atoms should lose water only in the form of $D_2O$, which is unlikely. Consequently, the existence of two independent mechanisms of the loss of 110 amu is possible. This hypothesis is confirmed by the spectrum of digitoxigenone (II) (see Fig. 1). The M−110 ion (m/e 262) retains the same intensity as the peak with m/e 264 in the spectrum of (I). At the same time, the ion with m/e 244, corresponding to m/e 246 in (I) has a very low intensity. Thus, we come to the conclusion that there is a specific mechanism for the formation of the ion with m/e 246 as the result of two factors: the splitting off of the substituent from the 3β position and the subsequent splitting off of 110 amu with the necessary participation of the hydrogen of the 14β-OH group. The realization of this mechanism may take place when this hydroxyl undergoes closure with the lactone ring in the manner of the iso compounds and with the decomposition of the ions obtained in accordance with the following scheme:

Scheme 1

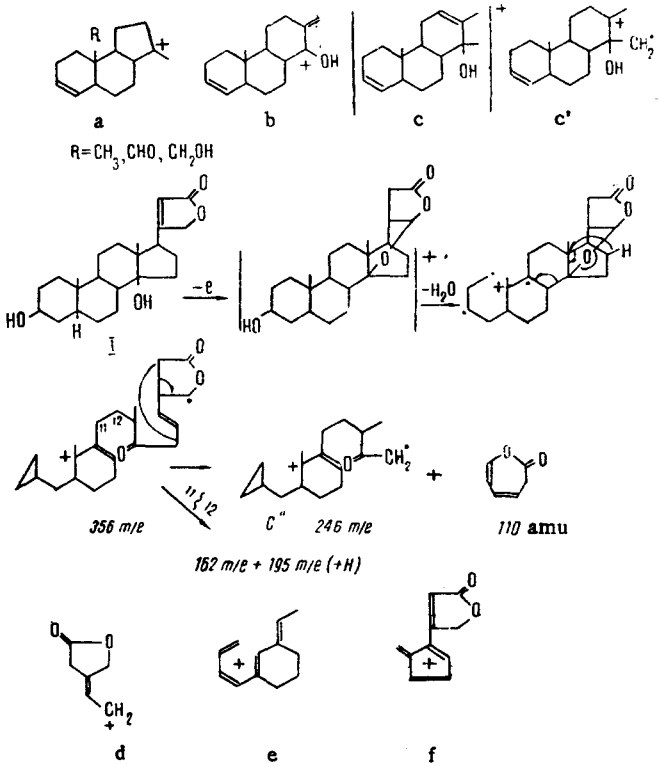


TABLE 2. Mass Numbers of the Ions c and Their Precursors

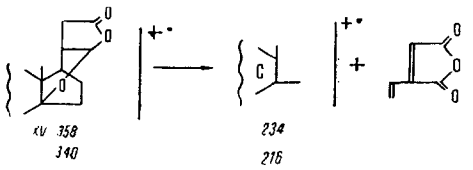
| Compound | Initial ion | Ion c |
|---|---|---|
| Digitoxigenin (I) | 374 ($M^+$) | 264 |
| | 356 ($M-H_2O$) | 246 |
| Digitoxigenone (II) | 372 ($M^+$) | 262 |
| Digitoxigenin acetate (III) | 356 ($M-AcOH$) | 246 |
| Uzarigenin acetate (IV) | 416 ($M^+$) | 306 |
| Pachygenin (V) | 350 ($M-2H_2O$) | 240 |
| | 322, 321 ($350-CO$, HCO) | 212, 211 |
| Diffugenin (VII) | 322 ($M-2H_2O-CO$) | 212 |
| Diffugenin acetate (VIII) | 350 ($M-2H_2O$) | 240 |
| | 322, 321 ($350-CO$, HCO) | 212, 211 |
| Periplogenin (IX) | 372 ($M-H_2O$) | 262 |
| Strophanthidin (X) | 322 ($M-3H_2O-CO$) | 212 |
| Strophanthidol (XII) | 322, 321 ($M-3H_2O-CH_2O$) | 212, 211 |
| Diethyl acetal of 5,14-dianhydrostrophanthidin (XIX) | 322 ($M-HCOOC_2H_5$) | 212 |

Aglycones with a large number of oxygen-containing substituents, apart from periplogenin, form anhydro variants of ions of type c after the splitting out of all the OH groups present in the molecule (Table 2). The intensities of these peaks are low and of approximately the same order as the intensities of the analogous peaks in the spectra of the 14-anhydro compounds (VII, VIII).

The mechanism of the formation of the c ion of compounds of the strophanthidin series is apparently similar to that described (Shannon, cited in [4]), but it must be observed that 110 amu is always split off, and not 111 as this author states. At the same time, the mass number of the type d ion (m/e 111), with the localization of the charge in the lactone ring, agrees in both investigations.

Assuming the existence of a common mechanism for the formation of the ion with m/e 246 in the spectra of (I) and (III), it could of course be expected that the molecule of isostrophanthidin (XV) would behave in a similar fashion to some extent. Nevertheless, the spectrum of the latter did not show peaks corresponding to the splitting out of 110 mass units from the strongest ions with m/e 358 ($M-H_2O-CO$) and 340 ($M-2H_2O-CO$). We explain this difference by the influence of the substituent at $C_5$ and $C_{10}$, favoring fragmentation by a mode more characteristic of compounds of the strophanthidin series. The spectrum of isostrophanthidin exhibits peaks with mass numbers of 234, 233; 216, 215, which correspond to the cleavage of the $C_{16}-C_{17}$ chain and of the lactone ring together with the ethereal oxygen bridge from the ions with m/e 358 and 340, respectively (Scheme 2).

Scheme 2




Very important from the point of view of the structural characterization of compounds are the differences observed in the spectra of digitoxigenin acetate (III) (cis-A/B) and uzarigenin acetate (IV) (trans-A/B). According to Egger [9], the ejection of the substituent from the 3β position in the case of the cis linkage of rings A and B takes place with an intensity several times greater than for the trans linkage. This is confirmed by the spectra of (III) and (IV) (Table 3).

Thus, for digitoxigenin acetate (III) the route shown in Scheme 1 is the main one. For uzarigenin acetate (IV) one of the main processes is the alternative ejection of a molecule of water at the expense of the 14β-OH and of 110 amu, after which a molecule of acetic acid is now split off. The greater sensitivity of the molecule of (IV) to electron impact [4% for (IV) as compared with 1.5% for (III)] agrees well with the relative conformational energies of the isomers calculated by Caillet and Pullman [10].

TABLE 3. Intensities of the Peaks in the Spectra of Digitoxigenin and Uzarigenin Acetates (III and IV, respectively) as Proportions of the Total Ion Current, %

| Compound | $M^+$ (416) | M−AcOH (356) | M−110 (306) | M−110−$H_2O$ (288) | 246 (c) | 288−AcOH 228) | 203 (a) |
|---|---|---|---|---|---|---|---|
| III | 1,5 | 28 | — | — | 12 | 2 | 20 |
| IV | 4 | 8,5 | 2 | 11 | 2 | 5 | 21 |

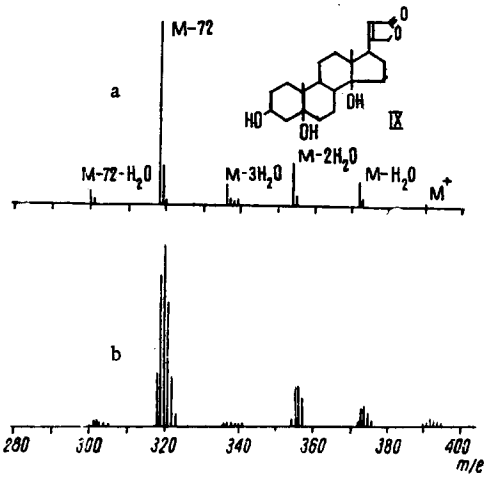



Fig. 2. Mass spectra of periplogenin (a) and [D]periplogenin (b).

Let us consider cases of the occurrence of a retrodiene reaction of ring A for the compounds that we have studied. This process consists in the ejection of the substituent from position 3 and the subsequent elimination of butadiene at the expense of the $C_1-C_4$ chain. The mechanism of this direction of fragmentation has been studied in detail by Budzikiewicz [11], and in his monograph [4] it is given as an example of the decomposition of cholestanol acetate. Fayez [6] considers that such a cleavage of ring A is uncharacteristic for compounds with an OH group in position 5. However, our experiments show differently: in the spectra of compounds without an OH group at $C_5$ (I, III, IV) no indications of the occurrence of the reaction under consideration are found. On the other hand, the spectrum of periplogenin (IX) (Fig. 2) indicates that the retrodiene decomposition is the main route of fragmentation of this molecular ion. In a consideration of the spectrum of strophanthidol (XII), attention was directed to the M−72 peak, which could be taken as M−$4H_2O$. However, an investigation of the spectrum of the D analog (XII) has shown that this ion was formed as the result of the retrodiene decomposition. We have found no significant peaks corresponding to such a process in the other spectra. Thus, it may be concluded that the retrodiene decomposition is suppressed by the other fragmentation proeesses of the skeleton (I, III, IV) or it is suppressed by the aldehyde group at $C_{10}$ in substances of the strophanthidin series.

In the region of mass numbers below 200 m/e, we have detected a number of peaks of ions that may have analytical value. The spectra of (I, III, and IV) contain ions with m/e 162 and 195. Both ions apparently arise from an ion with m/e 356 by the cleavage of the $C_{11}-C_{12}$ bond. Ring B, with the substituents, gives the ion with m/e 162, and charge transfer to ring D with the migration of one hydrogen atom forms the ion with m/e 195. In the case of compounds with an oxygen function at $C_{19}$, a group of peaks of variable intensity is found; the most considerable of them has m/e 160. The strongest of these peaks in the spectra of strophanthidin (X) and strophanthidol (XII), where a metastable peak is observed at 75.3 amu, indicates that the ion considered arises from an ion with m/e 340.

In the spectra of substances with a 14,15 double bond, the ions with m/e 160 are absent. This suggests that a condition for the appearance of these ions is the cleavage of the $C_8-C_{14}$ bond, which is prevented by the double bond in the case of the 14-anhydro compounds. It was shown above that the substituent at $C_{14}$ is split off subsequently, and, therefore, $C_8-C_{14}$ cleavage is energetically favorable in the ion with m/e 340. So far as concerns the structure of the ion with m/e 160, this most probably corresponds to the hydrocarbon composition $C_{12}H_{16}$. One of the possible structures of such an ion is e (Scheme 1). The possibility of the formation of the ion with m/e 160 as the result of the aromatization of ring D (f) is not excluded. This hypothesis is confirmed by the partial shift of the peak of the ion with m/e 160 to m/e 162 in the spectrum of 20,22-dihydrostrophanthidin (XVII). Consequently, the peak under discussion may be composite. In view of the fact that the elementary compositions of e and f differ, the answer to this equation could be obtained by means of high-resolution mass spectrometry.

## Features of the Spectra of the Deutero Analogs

The samples were deuterated by short-time treatment in $CD_3OD$, with brief heating for complete dissolution. The use of deuteromethanol is due to the comparatively good solvent properties for the materials studied and also to the fact that it is a good source of labile deuterium.

The analysis of the mass spectra of the deuterated cardenolides showed an additional shift (AS) of 1-2 mass units more than the number of deuterium atoms calculated for the particular fragment. An additional shift was observed in those cases where it was possible to record the peaks of the molecular ions. Thus, the shift of the fragmentary peaks cannot be ascribed solely to a mechanism of the splitting out of fragments in which the isotopic label remains in the molecule. Since it was previously assumed that under these conditions only the hydrogens of the hydroxyls are replaced, it was to be expected that the peaks of the fragments corresponding to the ejection of a number of water molecules equal to the number of hydroxyls would not (apart from some corrections) be shifted. However, as already mentioned, these peaks were shifted by 1-2 units.

On comparing a large number of spectra, we came to the conclusion that the additional shifts arise as the result of the replacement of the hydrogens at $C_{21}$. It is known [12] that cardenolides and model cyclohexyllactones liberate an additional mole of hydrogen in the Zerewitinoff reaction. Consequently, the additional shift is a specific feature of an $\alpha,\beta$-unsaturated lactone. There are no additional shifts in the mass spectra of the deutero analogs of the 20,22-dihydro derivatives of strophanthidin or in the spectra of the products of the alkaline isomerization of diffugenin [8]. Furthermore, we have observed an isotopic shift in the spectrum of the diethyl acetal of dianhydrostrophanthidin (XIX), the molecule of which does not contain hydroxy groups. According to Shannon (cited in [4]) the peak with m/e 111 formed at the expense of the lactone ring together with the $C_{16}-C_{17}$ chain is shifted by one and, to a smaller extent, by two mass units in the spectra of the deuterocardenolides. Conversely, the charged fragments obtained by the splitting out of the same chain (110 amu) do not undergo an additional shift. The peaks with m/e 212 in the spectrum of the diethyl acetal (XIX) (322−110) and with m/e 246 in the spectrum of digitoxigenin (I) (356−110) may serve as examples.

Recently, Haberland [13] has reported that the heating of acetyldigoxin in $D_2O$ in dimethylformamide in the presence of certain amines forms 21,21,22-trideutero derivatives. Our experiments have shown that the replacement of the hydrogens at $C_{21}$ also takes place in a neutral medium. In order to exclude the possible influence of the glass walls of the tube in which deuteration was performed, some of the experiments were performed in a quartz vessel; nevertheless similar results were obtained. Then an attempt was made to deuterate periplogenin (IX) with heavy water; in spite of the very poor solubility of this aglycone in water, a spectrum was obtained which differed little from the spectra obtained by the use of $CD_3OD$.

Under these conditions considered, isotopic exchange takes place in 50-95% of the molecules. The smallest percentage of unsubstituted molecules is found for polyhydroxy compounds (strophanthidin, strophanthidol). A large number of active hydrogens increases the possibility of exchange, but simultaneously decreases the probability of the presence of molecules in which all the active hydrogens have been replaced. We obtained the distribution of the intensities of the deutero derivatives as a result of the action of both these factors (see Fig. 2). Without a special treatment of such spectrograms, it is fairly difficult to judge the participation or nonparticipation of the isotopic label in any particular act of fragmentation. This relates to the greatest extent to the bands of low intensity which, after redistribution, become even less intense.

We have developed the following procedure for treating the mass spectra of the deutero analogs: first the relative proportions (mole %) of molecules with different numbers of heavy atoms relating to ions of one type were calculated (according to Biemann). Then these values were compared successively for ions of two types of which it was known that one is obtained from the other.

Let us give an example of the comparison of the proportions in the $M-H_2O$ and $M-2H_2O$ ions in the spectrum of [D]digitoxigenin (I) (Table 4).

In view of the fact that the most probable composition of the molecules ejected in this process is HDO and that this leads to a fall in the number of deuterons in the molecule by one, we may consider the matter as follows: as can be seen from Table 4, among the $M-H_2O$ ions there are 2% with four deuterons, while the $M-2H_2O$ ions do not contain analogous ions. Consequently, 2% of the $D_4$-containing ions were converted into $D_3$-containing ions. Among the $M-2H_2O$ ions $4.0-2.0 = 2.0\%$ of $D_3$ ions unconverted into $D_2$-

TABLE 4. Comparison of the Percentage Contents of Ions with Different Numbers of D Atoms for the $M-H_2O$ and $M-2H_2O$ Peaks in the Spectrum of Digitoxigenin (I)

| Groups of peaks | Relative proportions of the ions, mole % | | | | |
|---|---|---|---|---|---|
| | D=0 | D=1 | D=2 | D=3 | D=4 |
| $M-H_2O$ | 27,5 | 40,4 | 24,5 | 5,6 | 2,0 |
| $M-2H_2O$ | 34,5 | 42,3 | 19,2 | 4,0 | 0 |
| Corrected proportion | 27,5 | 33,4 | 15,6 | 2,0 | — |
| Percentage transition with the loss of a D atom | 0,0 | 7,0 | 8,9 | 3,6 | 2,0 |

TABLE 5. Values of A and B in the Mass Spectra of Some Compounds

| Compound | Transition | A | B |
|---|---|---|---|
| [D] Digitoxigenin (I) | $M/M-H_2O$ | 45,1 | 0,282 |
| | $M-H_2O/M-2H_2O$ | 48,6 | 0,292 |
| [D] Periplogenin (IX) | $M/M-H_2O$ | 61,5 | 0,587 |
| | $M-H_2O/M-^9H_2O$ | 21,0 | 0,110 |
| | $M-72/M-^{90}$ | 47,9 | 0,410 |
| [D] Strophanthidol (XII) | $M/M-H_2O$ | 46,6 | 0,425 |
| | $M-H_2O/M-2H_2O$ | 33,5 | 0,271 |
| | $M-2H_2O/M-3H_2O$ | 38,6 | 0,324 |
| | $M-H_2O-CH_2O/M-2H_2O-CH_2O$ | 32,6 | 0,267 |
| [D] Dihydrostrophanthidin (XVII) | $M/M-H_2O$ | 79,4 | 0,645 |
| | $M-H_2O/M-2H_2O$ | 89,5 | 0,725 |
| [D]-5α,14β-Pregnane-3β, 14β, 20α-triol (XX) | $M/M-H_2O$ | 93,5 | 0,930 |
| | $M-H_2O/M-2H_2O$ | 54,7 | 0,414 |
| [D]-5α-Pregn-14-ene-3β-20α-diol (XXI) | $M/M-H_2O$ | 86,5 | 0,660 |
| | $M/M-45$ | 67,0 | 0,520 |
| | $M-45/M-45-H_2O$ | 87,5 | 0,740 |

containing ions remain. Thus, 5.6−2.0 = 3.6% of $D_3$ ions have been converted into $D_2$-containing ions. Then, in a similar manner, we calculate the percentage of molecules losing HDO down to those containing one D atom, 7% of which are converted into ions without deuterons.

The sum of the figures in the last column of Table 4 plus 27.5% of the first column gives the percentage of deuterated molecules losing water in the form of HDO, plus the percentage of nondeuterated molecules losing $H_2O$. Let us denote this sum by A. In the case considered, A = 49%. Thus, 51% of deuterated molecules lose water in the form of $H_2O$. These are the molecules in which: 1) deuterons are present only at $C_{21}$; 2) H has been replaced by D in only part of the hydroxyls; and 3) the water molecule is eliminated by a mechanism in which the deuterium migrates into the charged fragment.

Even more striking for the mass spectra of the [D]cardenolides is the magnitude B, characterizing the ratio of the percentage of molecules losing HDO to the percentage of deuterated molecules. In the example given, $B = \frac{7.0 + 8.9 + 3.6 + 2.0}{100 - 27.5} = 0.296$ . Table 5 shows the values of A and B for a series of transitions of a number of [D]cardenolides the spectra of which contain the $M^+$ peak. For comparison, the lower part of the table gives information on steroid compounds of the noncardenolide types. The absence of an additional shift in the spectra of the latter causes a marked increase in A and B (averaging twofold). This shows that among the three factors mentioned above that affect the magnitude 100−A, the first is the most significant. Consequently, the replacement of the hydrogens at $C_{21}$ takes place so readily that the percentage of molecules containing deuterium in this position alone is fairly high. As already mentioned, there is no additional shift in the spectrum of [D]-20,22-dihydrostrophanthidin and the values of A and B are comparable with the values calculated for pregnane compounds.

It is difficult to explain the fact that the values of A and B for the successive processes of fragmentation of a single substance differ markedly. This may be due to a number of factors and, in the first place, the dissimilar capacities of different hydroxyls for deuterium exchange. A comparison of the spectra of

the deuterium analogs of the cardenolides permits the conclusion that tertiary OH groups in positions 5 and 14 exchange to a considerably smaller extent than the secondary 3β-OH group. For this reason the values of A and B for the ejection of the first molecule of water in the spectrum of periplogenin (IX) may be greatly increased. It is a fact that for this aglycone the main direction of decomposition is the ejection of a butadiene molecule from the $M-H_2O$ ion formed by the elimination of the 3β-OH group. The $M-H_2O/M-2H_2O$ transition, in which the tertiary hydroxyls mainly participate, gives low values of A and B. No such predominant direction of decomposition is observed in the mass spectrum of strophanthidol (XII), but in this case, because of the participation of the retrodiene reaction of ring A, the value of B for the ejection of the first water molecule is high.

The molecule of 5α,14β-pregnan-3β,14β,20α-triol (XX) [14] has two secondary and one tertiary hydroxyls. The participation of the 3β-OH group in reactions splitting out water is insignificant, since the A/B ring linkage is trans [9]. In actual fact, the mass spectrum shows the successive splitting out of only two $H_2O$ molecules. From certain considerations, the OH group in position 20 is split out in the first stage and the OH group in position 14β in the second. As can be seen from Table 5, the values of A and B for the second stage are considerably smaller than for the first.

The increase in A and B in the first stage may be due to the ejection of water in the form of $D_2O$ if the mutual positions of the deuterium atoms favor this. However, the contribution of such a mechanism is not susceptible of evaluation.

The results of a comparison of the deuterium contents can be applied not only to the ejection of water but also to the determination of the nature of other fragmentation processes. Thus, on comparing the isotopic abundances of the groups of peaks M and M−45 in the spectrum of [D]-5α-pregn-14-ene-3β,20α-diol (XXI) it is found that A amounts to 67% and B to 0.52. This means that some part of the molecular ions of (XXI) (~ 20%) forms the M−45 ions by an exchange of hydrogen between the OH in position 20 and the charged part of the molecule.

The amounts of the isotope in the $M-H_2O$ and M−72 groups of peaks in the spectrum of periplogenin (IX) are almost the same. This permits the conclusion that the M−72 ion arises only by the ejection of butadiene from $M-H_2O$ and that the fragment split out does not bear a label.

## CONCLUSIONS

1. The mass spectra of 19 cardenolides and related compounds have been studied. The characteristics of the splitting out of the substituents have been determined.

2. The directions of fragmentation of the steroid skeleton has been considered. The dependence of the nature of the cleavage on the linkage of rings A/B and on the presence of oxygen-containing substituents has been shown.

3. The additional shift of the peaks in the spectra of the deuterium analogs of the cardenolides has been explained. A new method of treating these spectra has been proposed.

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