THE STABILITY OF THE MOLECULAR AND SOME FRAGMENTARY

IONS IN THE MASS SPECTRA OF CARDENOLIDES

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Many workers have reported that in the mass spectra of the cardenolides the molecular ion has a low intensity or is absent [1-6]. A comparison of spectra has shown that a decrease in the size of this peak is observed with an increase in the number of oxygen-containing substituents in the steroid nucleus of the aglycone. There is no doubt that because of the low volatility of the compounds under consideration a certain role may be played by the splitting off of substituents before electron impact. The absence of traces of dehydration products on the chromatogram of a sample of strophanthidin kept under the conditions of the inlet system of a mass spectrometer [6] is not a definitive proof of the absence of thermal reactions. According to Marshall and Williams [7], the decomposition of the molecules of the sample may take place at the walls of the ion source after the evaporation of the molecules from the inlet system. Such a possibility is shown by a redistribution of intensities in the spectra of aglycones of the strophanthidin series with an increase in the duration of the experiment.

We had available experimental results showing that the stability of the M^+ ion of the aglycones depends not only on the total number of substituents but also on the nature of some of them. In order to study the influence of various substituting groups on the stability of the molecular ions, we have compared the spectra of a number of cardenolides. To reduce to a minimum the differences caused by the dissimilar volatilities of the substances selected, the mass spectra were taken under comparable conditions (MKh-1303 instrument; temperature of the inlet tube $\approx 160°C$ and of the ionization chamber $\approx 125°C$; ionizing voltage 40 V).

Table 1 gives for the compounds (I-XI) studied the mass numbers and intensities of the molecular ions in relation to the total ion current in the sequence of a decrease in the latter magnitude.
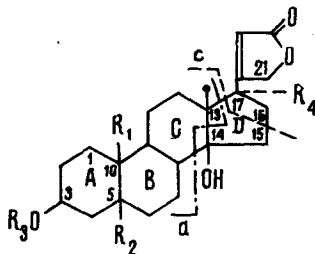
Let us first compare the stabilities of the molecular ions of substances differing by some one substituent. On passing from digitoxigenin (III) to periplogenin (VI) and from the methyl ester of cannogenic acid (IV) to the analogous ester of strophanthidinic acid (V), i.e., on the replacement of 5-H by 5-OH, the intensity of M^+ decreases four- to fivefold. A decrease of this magnitude also takes place with an increase in the degree of oxidation of the angular substituent R_1. In a series of compounds where all the other substituents are the same, periplogenin (VI, $R_1 = CH_3$) has the greatest value of M^+ (0.2%); in strophanthidol (VII, $R_1 = CH_2OH$) it is 0.1%, and in the spectra of strophanthidin (VIII, $R_1 = CHO$) and strophanthidinic acid (XI, $R_1 = COOH$), the molecular ion is absent.
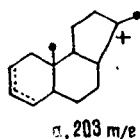
The 10β-hydroxy-19-norsteroids (I) and (II) [8], the molecular ions of which are the most stable, occupy a special position. While in the spectrum of 17α-hydroxystrophanthidin 3-acetate (IX) M^+ is absent,

TABLE 1. Mass Numbers and Intensities of the Molecular Ions and the Ions of Series a and c in the Spectra of the Cardenolides (I–XI)

Compound	Substituents				M^+	Ionic series a							Ionic series c					
	R_1	R_2	R_3	R_4		$a_{1,2,3}$	$a_{1,2}$	$a_{2,3}$	a_1	a_2	a_0	Σa %	$c_{1,2,3}$	$c_{1,2}$	c_1	c_2	c_0	Σc %
10β-Hydroxy-19-norperiplogenin (I)	OH	OH	H	H	392 (1,5)	239 (0,5)	221 (11,5)		203 (13,4)		185 (5,3)	30,7		264 (0,6)	246 (2,3)		228 (0,5)	3,4
10β,17α-Dihydroxy-19-norperiplogenin 3β-acetate (II)	OH	OH	Ac	OH	450 (1,2)				203 (5,0)		185 (7,5)	12,5		264 (0,4)	246 (1,5)		228 (1,5)	3,4
Digitoxigenin (III)	CH₃	H	H	H	374 (1,0)		203 (18,8)					18,8	264 (0,9)	246 (2,3)				3,2
Methyl cannogenate (IV)	COOCH₃	H	H	H	418 (0,8)	265 (3,0)	247 (6,7)	233 (1,3); 205 (5,6)		215 (1,3); 187 (6,0)		23,9	308 (0,7)	290 (0,9)		231/230 (1,5); 213/212 (1,8)		5,1
Methyl strophanthidinate (V)	COOCH₃	OH	H	H	434 (1,2)	281 (0,2)	263 (3,6)	249 (0,3)	245 (3,2)	231 (2,5); 203 (2,4)	213 (1,5); 185 (3,8)	17,5		306 (0,5); 272 (0,2)	288 (0,3)	247/246 (1,0); 229/228 (2,1)	211/210 (3,5)	7,4
Periplogenin (VI)	CH₃	OH	H	H	390 (0,2)		219 (4,5)		201 (7,9)			12,4		262 (0,8)	244 (0,6)			1,4
Strophanthidol (VII)	CH₂OH	OH	H	H	406 (0,1)				217 (0,9)		187 (0,9)	1,8					212/211 (1,3)	1,3
Strophanthidin (VIII)	CHO	OH	H	H	404 ( )				215 (0,8)		187 (2,4)	3,2					212/211 (1,0)	1,0
17α-Hydroxystrophanthidin 3β-acetate (IX)	CHO	OH	Ac	OH	462 ( )				215 (0,4)	189 (0,4)	187 (0,5)	0,9					212/211 (1,0)	1,0
Cannogenic acid (X)	COOH	H	H	H	404 ( )		233 (0,2)	207 (0,2)				0,8		276 (0,2); 258 (2,9)		232/231 (0,5); 214/213 (1,9)		5,5
Strophanthidinic acid (XI)	COOH	OH	H	H	420 (0)				231 (0,3)		187 (0,9)	1,2			256 (0,1)	230/229 (0,9)	212/211 (4,2)	5,2

174

in the spectrum of the acetate (II), which differs from the preceding compound only with respect to R_1, the intensity of the molecular ion amounts to 1.2%. Thus, when the other structural elements of rings A, B, and C are constant the substituent at C_{10} has the greatest influence on the stability of the M^+ ions of the cardenolides.

According to the literature [9], one of the primary acts of the decomposition of M^+ of steroids is the cleavage of the C_1-C_{10} bond. This leads to a rapid elimination of the oxygen-containing substituents of ring A, and also to an increase in the stability of the ions obtained by the elimination of these substituents [6].

10β-Hydroxy-19-nor compounds differ from the other compounds by the fact that the C_{10} atom here is tertiary and not quaternary. With this fact we connect the decrease in the probability of the initial rupture of the C_1-C_{10} bond as a consequence of which the stability of the M^+ ion of compounds (I) and (II) increases.

Apparently, a definite competition exists between the processes of the splitting off of the side chains and the far-reaching decomposition of the steroid nucleus under electron impact. In the preceding paper [6], we did not give any concrete characterization of the latter process, limiting ourselves to some qualitative ideas. Consequently, in Table 1 we give the intensities of the peaks forming the ions of series a and c. These ions are the most important of all those formed in the fragmentation of the cardenolide skeleton [2, 6, 10]. They include the part of the steroid nucleus shown in the general formula.

The numerical indices attached to the letters a and c (Table 1) show the numbers of the substituents of ring A retained by this ion. For example, $a_{1,2,3}$ means that this ion contains the substituents R_1, R_2, and R_3O. The ions a_0 and c_0 do not include any of these substituents. Thus, the index attached to the ion a or c with the greatest mass shows the maximum number of substituents capable of being retained in the fragment of this series. It can be seen from the Table that, with some exceptions, the more stable molecular ions correspond to the greater number of substituents retained by fragments a and c.

There is no apparent relationship between the intensities of M^+ and the sizes of the ions a and c greatest in mass. At the same time, on summing the intensities of all the ions of series a and c (Table 1) it is not difficult to observe a definite regularity. The level of the values of Σa of compounds with stable molecular ions (I-VI) is, as a rule, an order of magnitude higher than the corresponding values in spectra where M^+ is absent (VIII-XI) or is very small (VII).

In the values of Σc, excluding the acids (X) and (XI) and their esters (IV) and (V) a symbatic relationship with the stability M^+ appears.

Thus, with an increase in the stability of M^+ to electron impact a general tendency is noted to a rise in the total contribution of the ions formed by the cleavage of the bonds of the main skeleton.

Before beginning a discussion of the causes of the anomalies in compounds (IV, V, X, and XI) we must dwell on the pathways of the origin of the individual ions of series a and c. Two independent decomposition pathways of M^+ have been considered in the literature [2-5, 11], i.e.

$$
\begin{aligned}
1 \quad &\longrightarrow (M-H_2O)^+ \rightarrow (M-2H_2O)^+ \rightarrow \cdots \rightarrow (M-nH_2O)^+, \\
&M^+ \\
2 \quad &\longrightarrow a^+ \rightarrow (a-H_2O)^+ \rightarrow (a-2H_2O)^+ \rightarrow \cdots \rightarrow (a-nH_2O)^+.
\end{aligned}
$$

Analysis of the spectra of the cardenolides has shown, however, that routes 1 and 2 are interdependent, i.e., the ions $(a-nH_2O)^+$ can be formed not only from a^+ but also from $(M-nH_2O)^+$. For this reason, the individual ions of series a (just like c) can differ not only by the number of double bonds, as has been considered [1-5], but also by the structure of the whole skeleton.

Let us consider the spectrum of the simplest of the aglycones — digitoxigenin (III). Although its M^+ ion is one of the most stable (1.0%), we do not observe in it the peak of the ion $a_{1,2,3}$ with m/e 221.

The intensity of the single ion of this series, $a_{1,2}$, with m/e 203 is of the same order as Σa of compounds the spectra of which contain all the ions of this series, including $a_{1,2,3}$ (I, IV, V). Consequently, the ion with m/e 203 was formed as the result of an extremely energetically favorable process. The structure a with the charge on C_{13} and the double bond at $C_3=C_4$ [10] or $C_2=C_3$ [2] usually ascribed to this ion contradicts such a characteristic. From these points of view, it is not clear why the hydrated form of this ion with m/e 221 is unstable.

$a, 203\ m/e$

At the same time, the metastable peak with 115.7 a.m.u in the spectrum of (III) shows the presence of the transition 356^+ $(M - H_2O) \rightarrow 203^+$, i.e., the formation of the ion $a_{1,2}$ is preceded by the ejection of a molecule of water at the expense of the 3β-OH group (the elimination of H_2O with the participation of the 14β-OH group would greatly reduce the probability of the appearance of the ions a). This assumes the transfer of the active center from one point of the cardenolide ion to another.

We have shown previously [6] that the ions $c_{1,2,3}$ and $c_{1,2}$ in the same spectrum of (III) are formed by different pathways and are not connected with the transition $c_{1,2,3} \xrightarrow{-H_2O} c_{1,2}$. Then the metastable transition $c_{1,2} \rightarrow a_{1,2}$ (m* = 167.5 a.m.u) was observed.

What has been said above permits the following scheme for the decomposition of M^+ of digitoxigenin to be put forward:
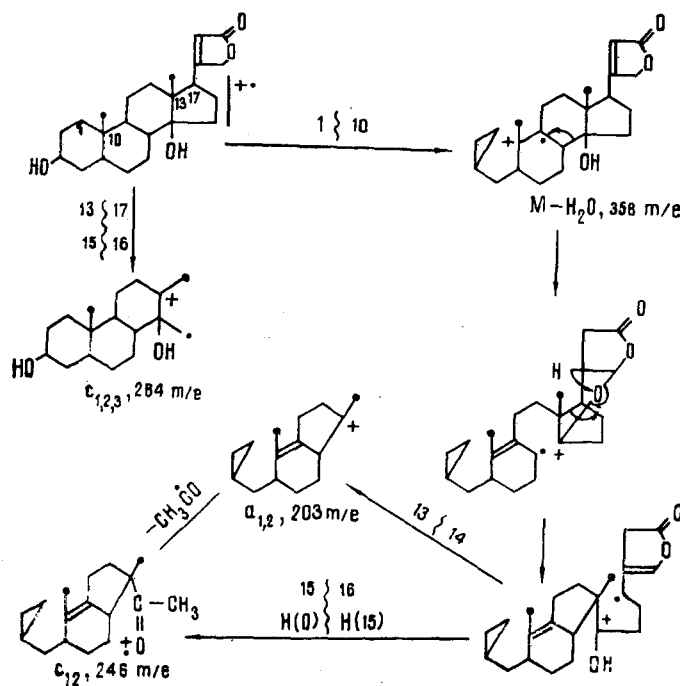
After the cleavage of the $C_{13}-C_{17}$ bond, the cleavage of the $C_{15}-C_{16}$ bond takes place, which immediately leads to the ion $c_{1,2,3}$ with m/e 264. The formation of the ion $c_{1,2}$ with m/e 246 is preceded by the cleavage of the C_1-C_{10} bond, the elimination of water at the expense of the 3β-OH group, the transfer of the active center ring D, and decomposition through the intermediate state of the iso compound [6]. Apparently, the formation of the ions c of the majority of the compounds considered takes place similarly. Only in the spectrum of the methyl ester (IV) is there an ion $c_{1,2,3}$ arising in the same way as the ion 264 m/e (Scheme).

In general, the localization of the charge on the C_{10} atom is probably less characteristic of compounds with a $COOCH_3$ group (IV and V); the main decomposition pathways begin with the cleavage of the $C_{13}-C_{17}$ bond, and the ions $a_{1,2,3}$ are present in their spectra.

Now we must explain the anomalously high value of Σc in the spectra of the acids (X) and (XI) and the esters (IV) and (V). The most acceptable explanation may be the lactonization of these compounds by the elimination of water (or methanol) through the 19-COOR group and one of the hydroxyls. This process may lead to the stabilization of the ions. Here there are analogies with the 8,19'-lactone of pregnane-19-carboxylic acid (XIII) [12]. In the spectrum of this compound taken recently, the molecular ion has a considerable intensity, while in the spectrum of the corresponding acid (XII) obtained by the oxidation of strophanthidin (VIII), the M^+ ion is absent.

176

Ion	Substituents retained in the fragmentary ions				d
	19-COOCH₃	3β-OD	14β-OD	bute-nolide ring	
M⁺	+	+	+	+	1,00
M—H₂O	+	−	+	+	0,70
M—2H₂O	+	−	−	+	0,52
M—CH₃OH	−	+	+	+	0,82
M—CH₃OH—H₂O	−	−	+	+	0,65
M—CH₃OH—2H₂O	−	−	−	+	0,40
265($a_{1,2,3}$)	+	+	−	−	0,25
247($a_{1,2}$)	+	−	−	−	0,00
233($a_{2,3}$)	−	+	−	−	0,15
215(a_2)	−	−	−	−	0,00
205($a_{2,3}$)	−	+	−	−	0,18
187(a_2)	−	−	−	−	0,00
308($c_{1,2,3}$)	+	+	+	−	0,44
290($c_{1,2}$)	+	−	+	−	0,23
231/230(c_2)	−	−	+	−	0,00
213/212(c_2)	−	−	−	−	0,00
111 m/e	−	−	−	+	0,47

If the elimination of water at the expense of the 19-COOR and C_{14}-OH groups under electron impact will take place without the transfer of the oxygen function to C_3, the formation of a CO—O—C_{14} bond will prevent the appearance of the ions of series a. At the same time, this process does not prevent the formation of ions c, since cleavage of the C_{15}—C_{16} bond is necessary for this.

For a proof of lactonization it was necessary to determine whether the splitting off of the OH (or OCH₃) group from C_{19} takes place simultaneously with that of the hydrogen from any of the hydroxyls. It was necessary to study the spectra of the OD analogs, since in this process of lactonization a molecule of D_2O (or CH₃OD) and not of HDO (CH₃OH) as usual, must be eliminated. However, the possibility is not excluded of the elimination of D_2O at the expense of two hydroxy groups. Consequently, we dwelt on the products of the deuterium-exchange of the methyl esters (IV) and (V), considering that although their lactonization is less preferred than that of the acids, its detection may be more reliable.

XII   XIII
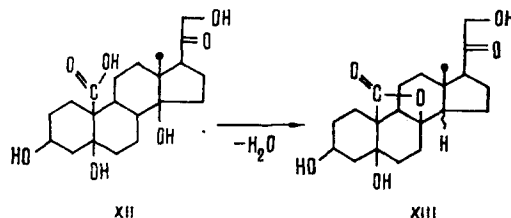
For a quantitative evaluation of the distribution of the isotopic label in the various fragments of the spectra of the deuteroanalogs, we had recourse to the following method of calculation. By Biemann's method [13] we calculated the precentage contribution of the D_n ions containing 0, 1, 2. . .n deuterium atoms for each fragment. Then the magnitudes obtained were multiplied by the number of deuterons corresponding to them and the products were added:

$$D_0 \cdot 0 + D_1 \cdot 1 + D_2 \cdot 2 + \cdots + D_n \cdot n = D_1 .$$ (1)

The value of D_1 for the molecular ion (D_1^M) calculated by means of Eq. (1) is the greatest among the values of D_1 for this spectrum. The values of D_1 of the fragmentary ions (D_1^F), referred to D_1^M, showed the relative contents of deuterium in each fragment:

$$d = D_1^F / D_1^M .$$ (2)

Thus, d for the molecular ion is equal to unity. The differences between the values of d of the ions passing into one another numerically characterize that fraction of the total amount of isotope that is lost during the given transition.

The distribution of the values of d between the individual ions of the spectra of the deuterated esters (IV) and (V) is shown in Tables 2 and 3. Table 2 also shows the substituents retained in the given ions with the greatest probability.

TABLE 3. Relative Deuterium Contents (d) in Various Ions of the Spectrum of Deuterated Methyl Strophanthidinate (V)

Ion	d	Ion	d
M^+	1,00	—	—
$M-H_2O$	0,78	$M-H_2O-CH_3OH$	0,66
$M-2H_2O$	0,69	$M-2H_2O-CH_3OH$	0,52
$M-3H_2O$	0,55	$M-3H_2O-CH_3OH$	0,40
		$111\ m/e$	0,46

The elimination of two molecules of water and a molecule of methanol from M^+ of (IV) can take place by six alternative routes, the elimination of a molecule of water decreasing the value of d by an average of 0.24, and the ejection of methanol by 0.12.

On the elimination of water, the mean decrease in d in the spectrum of the deuterated ester (V) is about 0.15. Almost the same change in this value takes place on the ejection of methanol (Table 3). The latter fact, just like the analogous phenomenon in the case of the ester (IV) is evidence in favor of the absence of lactonization.

Some of the fragments of series c of the esters (IV) and (V) are doublets, which is connected with the possibility of the elimination of R_1 either in the form of $COOCH_3$ or in the form of $HCOOCH_3$. (In the case of the acids (X) and (XI), either $COOH$ or CO_2 is split off.)

The ions 231-230 m/e (c_2) in the spectrum of (IV) do not contain an isotopic label. This confirms the fact that in the formation of the ions c the mobile hydrogen of the 14β-OH group can migrate into the fragments split out in the same way as has been shown in the scheme for the ion with m/e 246 of digitoxigenin. The possibility of the isomerization of the lactone ring with the participation of the 14β-OH is not excluded, either [6].

From this point of view, it is useful to consider the magnitude of d for the ion with m/e 111 including the lactone ring and the $C_{16}-C_{17}$ chain [2, 6]. In the spectra of both esters it occupies an intermediate position [0.47 (IV) and 0.46 (V)] between the values of d of the ions obtained in the successive elimination of all the water molecules [0.52 (IV) and 0.55 (V)] and the magnitudes calculated for the ions which have lost, in addition, a molecule of methanol [0.40 (IV) and 0.40 (V)].

This circumstance apparently reflects the competition between the processes of isomerization and lactonization, since in the first case the 14β-OD deuterium atoms are transferred into the lactone ring [14], thus increasing the value of d of the ion with m/e 111, and in the second case almost all the deuterons of this group are split off in the form of CH_3OD. Furthermore, it must be borne in mind that the usual mechanism of the elimination of a molecule of water at the expense of the 14β-OD group is superposed on the processes described above.

SUMMARY

The stability of the molecular ions of the cardenolides depends to the greatest extent on the nature of the substituent at C_{10}. A tendency is observed to a decrease in the intensities of the peaks of the main ions of the series formed by the cleavage of the bonds of the steroid skeleton with a decrease in the stability of the M^+ ion. The anomalous increase in the intensity of some fragmentary ions in the spectra of the 19—COOR compounds is due to the lactonization of the latter under the conditions of mass spectrometry.

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