A COMPARISON OF THE MASS-SPECTROMETRIC PROPERTIES OF COMPOUNDS OF THE PREGNANE SERIES WITH THE PROPERTIES OF SUBSTANCES SIMILAR TO CARDENOLIDES

Ya. V. Rashkes, M. B. Gorovits,
F. S. Khristulas, and N. K. Abubakirov

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The mass spectra of pregnanes of the 14α series have been studied fairly fully [1-3]. In recent years, the mass-spectrometric method has been widely used for interpreting the structure of highly hydroxylated compounds relating to the group of phytopregnanes [4-6]. Interest in the investigation of the behavior of another class of plant steroids – cardenolides – under the action of electron impact has become traditional [7-10].

The spectra of the 14α-pregnanes and of the cardenolides possess several features enabling them to be distinguished from one another. The reason for these differences may be the C/D-cis linkage of the rings with a hydroxyl in position 14β which is characteristic for natural materials. At the same time, a general property is the idea, extended to many types of steroids, of the predominant localization of the positive charge close to the angular substituents – at C_{10} and C_{13}.

We have had the possibility of tracing the gradual transformation of the mass-spectrometric properties with, as example, the spectra of the intermediates in the synthesis of the cardenolide uzarigenin from 5α-pregn-16-enolone [11].

The spectra of 5α, 14α-pregnane-3β, 20α-diol (I) (mass spectrum I, see [3]) and of its diacetate (II) were compared with the spectra of 5α,14β-pregnane-3β,14β,20α-triol (III) and its monoacetate (IV) and diacetate (V), and also with the spectra of the corresponding 14β,15β-epoxypregnanes with various substituents in positions 3β and 20α (VI-IX). In addition, the spectrum of the product of one of the last stages of the synthesis of uzarigenin with a double bond in the 20(22) position – ethyl 5α-pregnanecarboxylate (X) – was studied.

The spectra were recorded on an MKh-1303 instrument (direct introduction of the sample) at 80-100°C with an ionizing voltage of 40 eV. The OD analogs of substances (I, III, VI, VII, and X) were prepared by the brief immersion of samples in CD_3OD with substance elimination of the solvent in the lock system of the mass spectrometer.

According to chemical results, recently confirmed by quantum-mechanical calculations [12], the trans-anti-trans-anti-trans steroid ring system (substances I and II) is the most stable. Mass spectrometrically, this fact is confirmed by any one predominant direction in which the fragmentation of the molecular ion takes place. For the diol (I) such a process is the cleavage of ring D, leading to the formation of the ions $M-86$ and $M-87$ (Scheme 1 on the following page).

Secondary pathways of fragmentation are likewise connected with the elements of this ring. The contribution of ring A is apparently slight. Even such a readily eliminated substituent as hydroxyl in position 3β with a trans A/B linkage is of low mobility [3, 10]. It may be considered that the ejection of the first molecule of water from the ion M^+ (I) takes place almost solely at the expense of $C_{20}-OH$. The subsequent process of the elimination of the second molecule of water is of insignificant intensity. At the same time, the ejection of water from the ions $M-86$ and $M-87$ takes place to a considerable extent (ions with 216 and 215 m/e). Since the transition $M^+ \rightarrow 234^+$, 233^+ is confirmed by a metastable peak, and the transition

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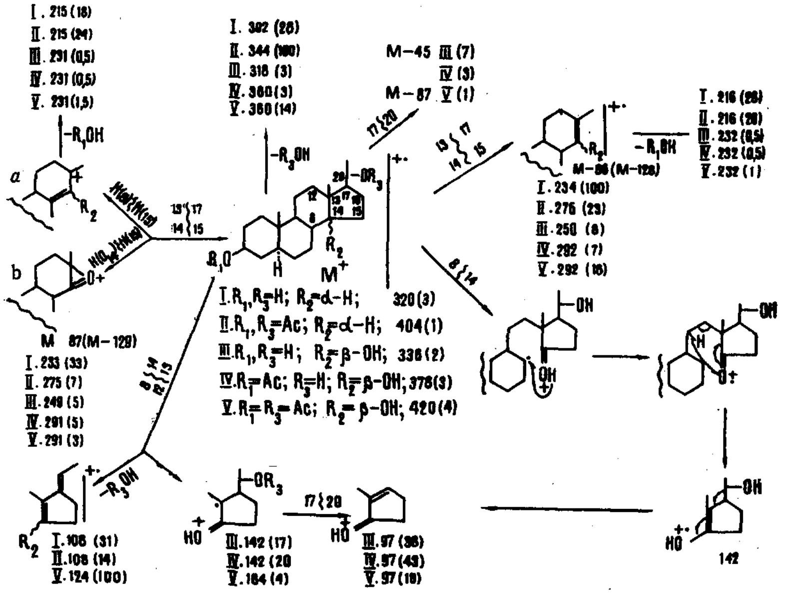
Scheme 1



$M-H_2O \rightarrow 216^+$, 215^+ is not confirmed, this is in favor of the assumption of the ejection of the OH at C_{20} in the first stage. In the spectrum of the diacetate (II), the splitting off of the first molecule of CH_3COOH is characterized by the 100% peak, and although the ejection of the second molecule takes place with a fairly high intensity (21%), nevertheless the transition $M-CH_3COOOH \rightarrow 216^+$, 215^+ is not observed.

In the mass spectra of compounds (III-V), the molecules of which contain the less stable trans-anti-trans-syn-cis system, the proportion of processes taking place with the participation of the fragments and substituents of ring D increases still further. In this case, the splitting off of the ring itself, which figures in the spectra of (I) and (II) as the main process [the ions M−86 (M−128) and M−87 (M−129)] is of secondary importance. The intensities of the two peaks in the spectra of (III) and (IV) are similar. The relative increase in the M−87 ion is, in our opinion, caused by the fact that, in contrast to 14α-H compounds, which are capable of being stabilized in the *a* form [1], the 14-hydroxy compounds may also be fixed in form b.

In the spectrum of the OD analog of the triol (III), the peak of the ion M−87 is only partially shifted by 2 amu. This means that, on the cleavage of the $C_{14}-C_{15}$ bond, part of the hydrogens of the 14β-OH groups migrate to the uncharged fragment, which leads to the production of form b. The increase in the intensity of the peak of the M−128 ion in the spectrum of the diacetate (V) with a simultaneous decrease in M−129 is obviously connected with an increase in the volume of the fragment being split out. The fragmentation of the 3β substituent in the spectra of (III-V) takes place to an insignificant degree. Most indicative in this respect is the spectrum of the monoacetate (IV) in which the peak of the M−60 ion is absent and there are no peaks in the high-mass region indicating the fragmentation of the 3β acetoxy group. If we compare the intensitities of the ejection of a second molecule of acetic acid in the diacetates (II) and (V), we see that for the latter this magnitude (the sum of the intensities of the M−2AcOH and $M-2AcOH-H_2O$ peaks) is approximately three times smaller than for M−2AcOH in the spectrum of (II). Furthermore, marked differences between the spectra of the 14α-H and 14β-OH compounds are observed in the region of low mass numbers. This is connected with the localization of the positive charge on the oxygen of the tertiary OH group. As a result of such a primary act and the subsequent cleavage of the C_8-C_{14} and $C_{12}-C_{13}$ bonds, ions arise with m/e 142 (III, IV) and 184 (V). The further stabilization of these fragments takes place after the splitting out of the substituent at C_{17}, which leads to the formation of one of the most prominent ions in the spectra of (III-V), with m/e 97. According to Teschesche et al. [13], an analogous ion is obtained in the decomposition of the molecular ion of 14β-hydroxy-14β-pregnane-3,11,12,20-tetrone. The same spectrum contains the peak of an ion with m/e 141 corresponding to the substituent present at C_{17}; however, these authors do not show whether there is a transition between the ions with m/e 141 and 97, while the conversion of the ion with m/e 142 into the 97 ion has been confirmed for compounds (III) and (IV) by a metastable peak at 66.3 amu. According to the same authors, when the sample was deuterated, the peak with m/e 97 was displaced by one unit only to the extent of 30%. Since in our experiments with substance (III) the shift was of approximately the same magnitude, this induced us to seek other possible methods for the formation of the ion with m/e 142, the most probable of which is shown in Scheme 1. After the cleavage of the C_8-C_{14} bond and the migration of the hydrogen atom from the hydroxyl at C_8, there is a general rearrangement of the McLafferty type [18] which likewise favors the production of an ion with m/e 142. The latter, by losing a $CH_3\dot{C}HOH$ radical, is converted into an ion with m/e 97 which should not contain the isotopic label (if the spectrum of the OD analog is analyzed). In addition to this, the directions of the processes taking place close to ring D largely depend on the size of the substituent at C_{20}. The presence of an acetoxy group in this position leads to its predominant expulsion in the first stage, and the subsequent cleavage of the bonds of ring C lead to the formation of a maximum peak with m/e 124 in the spectrum of the diacetate (V). Here the role of the C/D-cis linkage is fairly evident, since in the case of the diacetate (II) (C/D-trans) the corresponding peak with m/e 108 amounts to only 14% of the maximum peak. In view of the low stability of the substituent at C_{20}, in the spectrum of (V) the intensities of the peaks with m/e 184 and 97 have decreased.

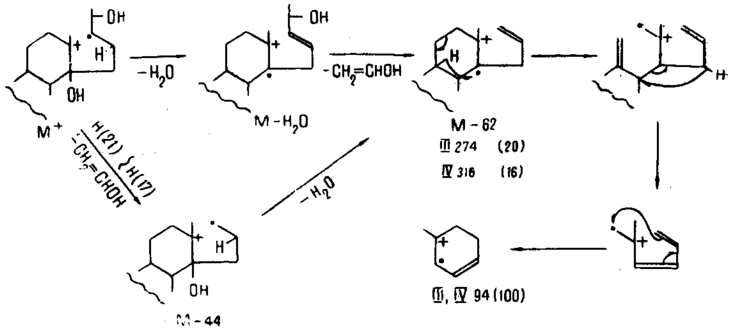
TABLE 1. Mass Numbers and Intensities of the Peaks (%) in the Spectra of the Epoxy Compounds

Substance	Substituent		M^+	M−18	M−43	M−44	M−45 M−87	M−62 M−104	M−84 M−126	M−101 M−143	M−102 M−144	M−129 M−171	298	271	253	215	187	M−60	M−74
	R_1	R_2																	
VI	H	H	334 (100)	316 (4)	—	290 (43)	289 (54)	272 (18)	250 (54)	233 (14)	232 (8)	205 (8)	(2)	(4)	—	(12)	(11)	—	—
VII	H	$COCH_3$	376 (60)	358 (5)	333 (42)	—	289 (84)	272 (60)	250 (32)	233 (44)	232 (56)	205 (23)	(22)	(25)	(11)	(53)	(32)	316 (100)	—
VIII	$COCH_3$	$COCH_3$	418 (48)	400 (5)	375 (43)	—	331 (76)	314 (50)	292 (29)	275 (26)	274 (53)	247 (14)	(5)	(17)	(20)	(62)	(40)	358 (100)	—
IX	COC_2H_5	$COCH_3$	432 (51)	414 (4)	389 (44)	—	345 (72)	328 (49)	306 (36)	289 (25)	288 (51)	261 (13)	(6)	(19)	(26)	(74)	(51)	372 (100)	358 (9)

Characteristic for the mass spectra of (III) and (IV) is a maximum peak with m/e 94, the intensity of which exceeds that of the peak with m/e 97 approximately 2.5-fold. According to the tables of Unruh and Spiteller [14], the peak with m/e 94 can be formed either through ring A with a retrodiene reaction in ring B or through ring D as a result of the decomposition of 17-acetoxy steroids or Δ^{16}-steroids. In all the examples given, the ion considered has the composition C_7H_{10}.

By a careful analysis of metastable transitions we have established that in the spectra of (III) and (IV) the ion with m/e 94 is formed directly from the ion M−62, which is the most intense in the region of high masses. Metastable peaks appear at 32.2 amu (III) and 28.0 amu (IV). The fragment with 62 amu must contain not less than two oxygen atoms and correspond to $C_2H_6O_2$ (the composition CH_2O_3 is unlikely). Thus, the appearance of the M−62 ions can be explained by the successive ejection of the substituents at C_{14} (17 units) and at C_{17} (45 units). In neither spectrum is the peak of an M−17 ion observed. However, the M−45 ion is accompanied by an M−44 ion. Consequently, the formation of the M−62 ion can be represented in the form of the alternative ejection of 18 units (H_2O) and 44 units (C_2H_4O). However, the ejection of 44 units (in the form of an acetaldehyde molecule) is not shown by metastable peaks either in the first or in the second stage. At the same time, there is a strong m* peak showing the direct transition M → M−62. Since cases of the description of two-stage processes by a one-stage metastable transition are known [15, 16], we assume that the decomposition of M^+ leading to the formation of the ion with m/e 94 apparently takes place in the way shown in Scheme 2.

Scheme 2

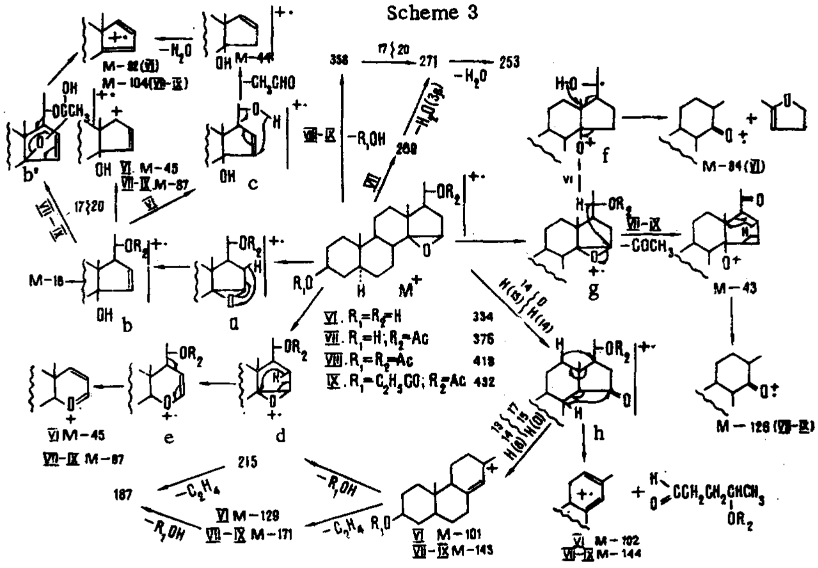


The mass spectrum of the OD analog of (III) confirms this scheme. The M^+ peak is shifted by three units [the number of active hydrogens in the molecule of (III)]. The M−44 peak is shifted by two units, which shows the absence of the migration of deuterium from the 20α-OD group into the charged fragment on the ejection of a molecule of vinyl alcohol (acetaldehyde). The peak of the M−62 ion is shifted by one unit (through the 3β-OD group). And, finally, the peak of the ion with m/e 94 undergoes no isotopic shift whatever. This peak will possibly play the role of analytical peak in the discovery of 20α-hydroxy-14β-pregnanes, since the mass spectra of, for example, pregnane compounds in the molecules of which these OH groups exist but which also have a number of hydroxyls in ring C [4-6] do not contain any appreciable peaks whatever with m/e 94. This type of decomposition becomes secondary when the 20-OH group is replaced by an acetoxy group, as can be seen from the spectrum of the diacetate (V), where the intensity of the peak with m/e 94 is 8% of the maximum.

Let us further consider the mass spectra of the 14β,15β-epoxy compounds (VI-IX). On the one hand, the introduction of the oxido ring simplifies the system mentioned and makes the molecular ion more stable, this being the 100% ion in the case of the epoxy diol (VI) (Table 1), and on the other hand we observe a greater diversity of fragmentation pathways, obviously due to the localization of the charge on the oxygen of the epoxide.

To explain the fragmentation of compounds (VI-IX) the basic concepts of the work of Strong, Brown, and Djerassi [17] on the decomposition of the molecule of cyclohexane oxide and its various deutero analogs are applicable. Two main points emerge from this work: 1) the decomposition of the molecular ion of an epoxy compound is preceded by a number of intramolecular rearrangements, and 2) in cyclic epoxides, in contrast to aliphatic epoxides, the C−O bonds or the bridge C−C bonds, and not the C−C bonds of the ring, are cleaved first. It has also been possible to extend these ideas to the spectra of epoxides in which the epoxide ring is attached to the five-membered ring D.

Scheme 3 shows the main fragmentation pathways of the epoxy compounds (VI-IX), and Table 1 gives the corresponding mass numbers and intensities. Attention is attracted first of all by the high intensities of the M−45 (M−87) peaks, which distinguish these spectra from those of the 14β-hydroxy compounds. Here at least two routes for the formation of these ions are possible. The first is connected with the cleavage of the C_{15}−O bond and the migration of hydrogen from C_{16} (*a*), which leads to the formation of the ion b, from which the ejection of the substituent at C_{17} is extremely probable. The fragment b, through a transitional cyclic form b', is converted into an M−104 ion (VII-IX). In the case of the diol (VI), the ion b may give rise through a six-membered state c to an M−44 ion which, by eliminating water, is converted into the analogous M−62 ion. The mechanism given for the elimination of a molecule of acetaldehyde is confirmed by the fact that in the spectrum of the OD analog of the diol (VI) the M−44 peak is shifted by two units. Analysis of the shifts of the M−18 peak in the same spectrum shows that only about half the molecules lose water in the form of HDO. The remainder of the M−18 ions are apparently formed by the ejection of H_2O from the ion b.


Scheme 3

The second method for the formation of the M−45 (M−87) ions is the cleavage of the bridge $C_{14}-C_{15}$ bond (d), leading to the production of the dihydrogenated ion e, which is stabilized in the oxonium form.

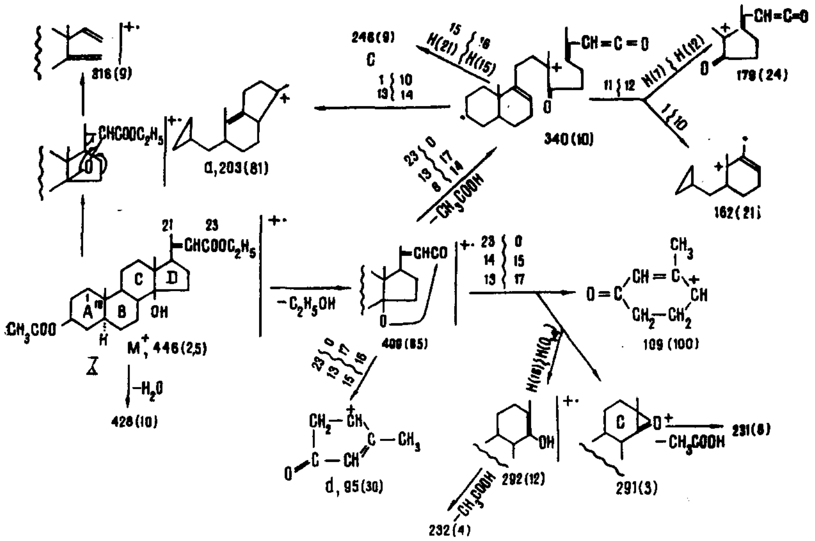
Epoxy compounds with acetoxy groups in the 20α positions (VII-IX) form the M−43 radical ion through the cleavage of the $C_{15}-O$ bond and the migration of hydrogen from C_{20} (g). Subsequent decomposition of the M−43 ion leads to the ion M−126. The latter can also be produced from the form g without going through the stage of formation of the M−43 ion. Like the M−126 ion, the M−84 ion in the spectrum of the diol (VI) is obtained by the cleavage of the bonds of ring D (f) and the migration of the hydrogen of the $C_{20}-OH$ group. This is shown by an isotopic shift of the M−84 ion by two units.

Another variety of intramolecular rearrangements of epoxides leads to the appearance of the oxo form h, which eliminates the elements of ring D. The ions M−102 (M−144) so formed are fairly stable, while the ions with masses greater by one unit (M−101, M−143) undergo further decomposition with the alternative ejection of a molecule of ethylene and R_1OH. The result of this process is intense peaks of ions with m/e 215 and 187. All the transitions are confirmed by metastable peaks.

Let us further attempt to evaluate the contribution of ring A in the fragmentation of the compounds considered. For this purpose it is necessary to compare the mass spectra of the diacetate (VIII) and of the 20α-acetate 3β-propionate (IX). In both spectra, just as in the spectrum of the monoacetate (VII) the peak of the M−60 ion is the maximum peak. The elimination of a molecule of propionic acid from M^+ (IX) takes place with a far lower intensity, and the size of the peak of the M−74 ion is only 9%. It can be seen from Scheme 3 that all the main processes of the decomposition of the ions begin in ring D, and only in the later stages of decomposition does the elimination of the 3β substituent in the form of R_1OH take place. In accordance with this, all the main peaks in the region of high masses in the spectra of (IX) are shifted by 14 units as compared with the corresponding peaks of the spectrum of (VIII) (see Table 1). The right side of the table gives the ions having the same values of m/e in all the spectra and, consequently, reflecting the contribution of ring A or, rather, its substituent in position 3β. The cause of the appearance of the ion with m/e 298 may be the successive expulsion of the R_2OH and R_1OH molecules from M^+. Nevertheless, in the pure form this property appears only in the spectra of the completely esterified compounds (VIII) and (IX), since the ion with m/e 298 in the spectra of (VI) and (VII) can arise as the result of the ejection of the H_2O formed at the expense of the epoxide oxygen from the $M-R_2OH$ ion. A study of the spectrum of the 3β-OD analog of compound (VII) has shown that the ratio of the intensities of the peaks with m/e 298 and 299 is the same as that of the peaks of M^+ and M+1. Consequently, the 3β-OH group does not take part in the process under consideration. In addition to this, in the spectra of (VI) and (VII) the ion with m/e 298 does not eliminate a molecule of water, giving an ion with m/e 280, while in the spectra of (VIII) and (IX) the intensity of the latter is 5-6%. Thus, the expulsion of the 3β substituent begins to take place to an appreciable extent only after the cleavage of the bonds of the main skeleton (ions with m/e 215 and 187), and its participation in the first stages of decomposition depends strongly on the corresponding volume. In this connection, it was interesting to consider the routes of appearance of the ions with m/e 271 and 253. Characteristic for ethers (VIII) and (IX) is the successive alternative ejection of 87 amu substituent at C_{17}) and of R_1OH, leading to the formation of an ion with m/e 271, which, by eliminating water, is converted into an ion with m/e 253. Metastable peaks confirm both sequences of the formation of the ion with m/e 271. For the monoacetate (VII), only one of the directions of decomposition is confirmed: $M^+\xrightarrow[-87]{}289\xrightarrow[-H_2O]{}271\xrightarrow[-H_2O]{}253$. Since, as has been shown above, R_1OH is not eliminated from (VII) in the first stage and the ion with m/e 271 undergoes no shift in the spectrum of the OD analog of (VII), it follows from this that in the transition 289 → 271 a molecule of water is eliminated through the R_1OH group, and in the latter transition it is as the result of the cleavage of the epoxide bonds. In the spectrum of the diol (VI) the peak of the ion with m/e 271 is of low intensity, and the ion with m/e 253 is absent, which also shows the stability of ring A to electron impact in this molecule.

The mass spectrum of ethyl pregnanecarboxylate (X) simultaneously contains elements characteristic of the 14β-hydroxy compounds (III-V) and, in addition, has features approximating it to the spectra of the cardenolides. But it is first necessary to observe those features of the spectrum of (X) which are due to the presence of an ester group at C_{22}. The detachment of a molecule of ethanol from M^+ taking place in the first stage (Scheme 4) and possessing a low intensity forms an ion with m/e 400 which is the source of all the main ions in the spectrum of (X). The deuteration of the compound at the 14-OH group has shown that the molecule of ethanol is formed with the participation of the hydrogen of this group, since the peak with m/e 400 undergoes no shift whatever in the spectrum of the OD analog. The high intensity of this peak is apparently due to the fact that some of the ions are stabilized in the form of lactone ions. The further transformation of the remainder of the ions depends on the possibility of the cleavage of the bonds of ring C and, especially, ring D. By analogy with compounds (I-IX), the spectrum of (X) contains the peaks of ions with m/e 291 and 292, of medium intensity, giving a doublet with m/e 231 and 232 after the ejection of a CH_3COOH molecule. A characteristic feature of the mass spectrometry of (X) is the localization of the charge at C_{17}, leading to the appearance of intense peaks in the low-mass region. Thanks to this circumstance, in the cleavage of the same bonds the maximum ion with m/e 109 is formed. The transition 400 → 109 is well described by a metastable peak at 29.8 amu. The other ion of this series with m/e 95 arises as the result of the cleavage of the $C_{15}-C_{16}$ bond. Essentially there is a parallel here with the spectra of the cardenolides, in which ions of type d appear [8, 10], formed through the lactone ring with the $C_{17}-C_{16}$ chain. The cleavage of the C_8-C_{14} bond initiates the expulsion of hydrogen from C_9, which, as is well known [3, 10] may depart with the substituent at C_3. This gives rise to a radical ion with m/e 340 which decomposes in several directions. It is possible that the large number of daughter ions is explained by the fact that some of the ions with m/e 340 exist in a doubly charged form. The ion under consideration corresponds to the 356 (M−60) ion in the spectra of the acetates of uzarigenin and of digitoxigenin [10] and, like the latter ion, on cleavage of the $C_{15}-C_{16}$ bond, gives an ion with m/e 246 (c [8, 10]) not containing the isotopic label in the spectrum of the OD analog. In the spectrum of (X) there is also an intense ion with m/e 203 (*a*) which is characteristic of cardenolides. An analogy with the spectra of uzarigenin and digitoxigenin also appears in the presence of peaks with m/e 179 and 162 obtained by the cleavage of the $C_{11}-C_{12}$ bond and the localization of the charge in one or other part of the molecule. We must also remember the presence in the spectrum of (X) of an ion with m/e 316 apparently arising through the decomposition of one of the forms of the molecular ion in which the 14β-hydroxyl undergoes ring closure with the 20(22) double bond.

Scheme 4



the cardenolides, in which ions of type d appear [8, 10], formed through the lactone ring with the $C_{17}-C_{16}$ chain.

SUMMARY

1. The mass spectra of several intermediates in the synthesis of uzarigenin from 5α-pregn-16-enolone have been characterized. Their general features and the differences between the spectra of 14α-H and 14β-OH compounds have been discussed.

2. It has been established that all the main processes of the decomposition of the molecular ions of the compounds studied take place close to ring D, and the contribution of ring A is small. The sequences of ejection of the 3β and 20α substituents and also the characteristics of their fragmentation in dependence on their volume have been investigated.

3. Schemes are proposed which explain the fragmentation pathways of 14β,15β-epoxy compounds.

4. It has been shown that the introduction of a double bond into the 20(22) position of 14β-hydroxy steroids leads to the appearance in the mass spectra of fragments due to the decomposition that is typical for the cardenolides.

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