Steroid Mass Spectral Correlations. I. Hydroxyprogesterones

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Mass spectra of eight monohydroxyprogesterones have been studied. These spectra frequently exhibit fragmentations directed by the hydroxyl groups rather than the enone function.

Hydroxy steroids frequently occur as products of biological oxidation (microbiological or metabolic), and mass spectrometry, with its small sample size requirement, provides a potentially attractive analytical method for these compounds, since it might be anticipated that the polar hydroxyl groups would direct fragmentation along distinctive paths. In the present paper we discuss the mass spectra of hydroxyprogesterones; in the succeeding paper[1] we treat the spectra of hydroxydeoxycorticosterones.

Simple Progesterones

As an aid to interpretation of the hydroxyprogesterones' spectra, we determined under the same conditions the spectra of progesterone (I) itself and of the monomethylprogesterones (II–VIII) (Table I, p 1744). These spectra are similar to those of progesterone itself,[2,3] of IV,[3] and of 6α,16α- and 6α,16β-dimethylprogesterones,[2] which were reported, though not discussed in detail, earlier.

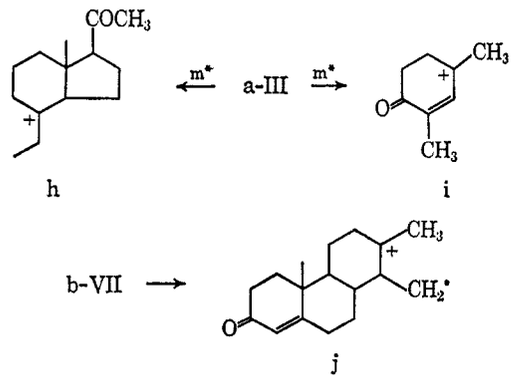
The first point of interest to the present study was the relative extent to which the two carbonyl groups in substituted progesterones direct the fragmentation of the molecule *via* ions a and b (Figures 1 and 2). Most of the major peaks in the mass spectra of I–VIII (see Table I) can be attributed to fragmentations (ion a, Figure 1) described for Δ^4-androsten-3-one[4,5] and found in other Δ^4-3-ones[2,3] which lack the 20-oxo group. Metastable ion peaks (Table II) for most of the present compounds suggest that ions c, d, and e arise from one-step losses of mass from the molecular ion. Ion b plays a more limited role in directing fragmentation, leading principally (Figure 2) to the acetylium ion at *m/e* 43 and to ion f. The acetylium ion g was not strong in the spectrum of Δ^4-androsten-3-one, which lacks the acetyl side chain,[5] but is one of the two or three most intense in the spectra of progesterone and all its methyl derivatives and is, thus, undoubtedly due principally to the simple loss of acetylium ion from the progesterone side chain (Figure 2).

The ion at M − 85 in the progesterone spectrum could be from ion f (Figure 1), ring-A fragmentation,[5] or from ion f′ (Figure 2), ring-D fragmentation, as suggested by Peterson.[3] One metastable ion was found, in the spectrum of II, which indicates that transition e → f occurs, but other metastable ions relating to ions f or f′ for I–VIII are absent. The results in Table I indicate that both fragmentations (to f and f′) occur in some methylprogesterones, hence probably in all. In those compounds (II, VII, VIII) where the two proposed fragmentations would result in ions of different masses, the A ring loss to give ion f predominates by a ratio of 3 or 4 to 1.

A second point of interest is the effect of the 6- and 7-methyl groups on cleavage of the C-6–C-7 bond since if the methyl substituent were in a position to stabilize the ion resulting from bond cleavage it should favor the ion bearing the methyl group. This was indeed observed. Table I shows that in most cases the rupture in ring B leads mainly to ion c rather than to ion d. The usual ratio is 4 or 6 to 1, favoring charge retention on the A-ring fragment with its unsaturated ketone. The principal exceptions to this usual ratio are the 6α- and 6β-methylprogesterones (IV and V) and 7α-methylprogesterone (VI). In the spectra of IV and V ion c is favored by 10 or 20 to 1, whereas in that of VI the two ions c and d are produced in nearly equal amounts.

A third point of interest is the effect of stereochemistry in spectra of the two epimeric pairs of methylprogesterones IV–V and VII–VIII. The relative intensities of the peaks c–f show no qualitative difference between the spectra for the two pairs if the relative intensities of the peaks are referred to that of the molecular ion,[6] although, on the same basis,[6] the postulate that the thermodynamically more stable epimer "will exhibit the more intense molecular ion (when expressed in terms of total ionization)"[7] is true for the 6-methyl pair where IV (equatorial α-methyl) shows a relatively more intense molecular ion than V (axial β-methyl).

Only a few ions are formed by specific fragmentations near the methyl groups. These include ions h and i from III, formed from the molecular ion ($328 \xrightarrow{m^* 129.3} 206$, $328 \xrightarrow{m^* 46.2} 123$, respectively; calcd m* 129.6, 46.1), and ion j (*m/e* 244) from VII and VIII.



(1) M. F. Grostic, T. H. Kinstle, J. E. Wilson, and K. L. Rinehart, Jr., manuscript in preparation.

(2) L. Peterson, *Anal. Chem.*, **34**, 1781 (1962).

(3) H. Audier, M. Fetizon, and W. Vetter, *Bull. Soc. Chim. Fr.*, 415 (1964).

(4) R. H. Shapiro, J. M. Wilson, and C. Djerassi, *Steroids*, **1**, 1 (1963).

(5) R. H. Shapiro and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 2825 (1964).

(6) A peak of markedly differing intensity is the acetylium ion g. Since in spectra of V and VII this provides the base peak, the other intensities appear different although their ratios are nearly the same.

(7) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964.

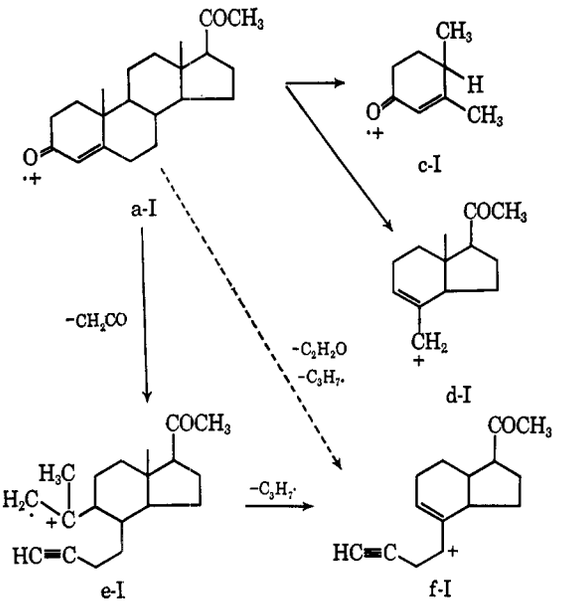


Figure 1.—Ions derived from ion a.

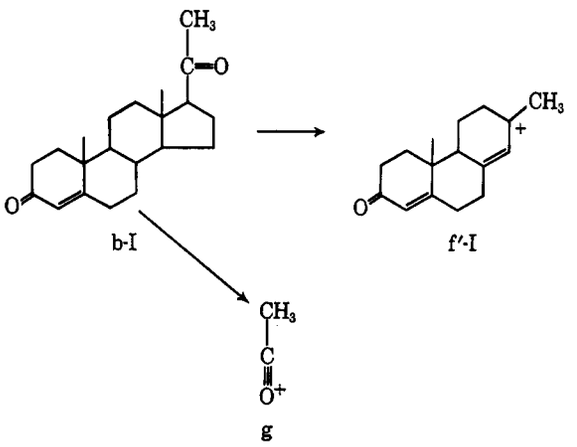


Figure 2.—Ions derived from ion b.

Monohydroxyprogesterones

Pertinent peaks from the mass spectra of the monohydroxyprogesterones studied are shown in Table I and the spectra themselves in Figure 3.

The molecular ion, tho ugh generally less intense than for compounds I–VIII, is found in all the spectra; in the spectra of three of the compounds studied it is the base peak.[3–10]

Loss of water is a major mode of fragmentation in the compounds. The ion at M − 18 is found in nearly all the spectra and is very strong in some; in the spectrum of 11β-hydroxyprogesterone (XI) it is the base peak. The difference between the large M − 18 peak in the spectrum of XI, where the hydroxyl is axial, and the moderate M − 18 peak in that of X, where the hydroxyl is equatorial, is striking. This difference has led Zaretskii, *et al.*,[11] to predict that the spectra of all monoaxial hydroxy steroids will have relative ion intensities M − 18 > M, and all monoequatorial hydroxy steroids will have relative ion intensities M > M − 18. Their conclusion was derived from one epimeric pair (11α, 11β) and they deduced from this that the 15β- and 16α-hydroxyprogesterones and their 15α and 16β epimers had quasi-axial and quasi-equatorial configurations, respectively.

In the present compounds, four of the same compounds were studied (X, XI, XIII, XIV) and the relative intensities of their M and M − 18 ions were approximately as described by the Russian group. However, all the other alcohols (not described by Zaretskii, *et al.*) had much more intense molecular ions than M − 18 ions. These alcohols included two with tertiary axial hydroxyl groups (9α and 14α), another with a tertiary hydroxyl group on a cyclopentane ring (17α),[12] and a primary alcohol (21). Accordingly, the suggested rule[11] is of more limited utility than claimed, although it probably has some validity in distinguishing between secondary alcohols on rigid six-membered rings, provided that both isomers are available.

The proclivity of the hydroxy progesterones toward dehydration is exaggerated in the spectrum of the one acetate studied (XVII), whose molecular ion is much less intense than the ion at M − 60, corresponding to the loss of acetic acid, even though the acetoxyl group is equatorial. Indeed, peaks due to loss of acetic acid dominate the spectrum and most of the typical ions (c, d, e, f) either are of much reduced intensity or are replaced by ions 60 mass units lower.

12β,15α-Dihydroxyprogesterone (XVIII) was also studied. This compound readily lost 2 mol of water and gave ions at M (21% of base peak), M − H_2O (26%), and M − $2H_2O$ (41%). Here again the proposed rule of M *vs.* M − 18 relative intensities[11] does not hold, since the 12β-hydroxyl is equatorial and the 15α-hydroxyl was the isomer reported to be less subject to dehydration.[11]

Whereas it is not certain that thermal dehydration of the neutral molecule is not contributing to the intensity of the peak at M − 18 in the spectra of these compounds, the presence of appropriate metastable ion peaks (Table II) in all of the spectra indicates much of the dehydration, at least, is due to electron impact.

Not only is water lost from the parent ion but dehydation peaks are found as satellites below many of the other peaks in the spectra. In Table I the major peaks are followed individually, where found, by the peaks corresponding to the loss of water from them. This is not meant to imply that the ion is formed, then loses water; the reverse process could obtain. Actually, only one ion is certain in this respect; in the spectrum of 15α-hydroxyprogesterone (XIII) a metastable ion peak was observed for the loss of water (to give a peak at M − 60) from ion e (at M − 42). In the following discussion we shall first treat the peaks already discussed (c–g), then turn to those restricted to the hydroxyprogesterones.

Ions c–g.—These characteristic ions from the spectrum of progesterone are rather prominent in the spectra of all the hydroxyprogesterones except 21-hydroxyprogesterone, which follows an individualistic fragmentation path.[1] Where appropriate, the ions are accompanied by dehydration peaks 18 mμ below them

(8) The ratio of the intensity of the molecular ion of cholesterol to that of its peak at M − 18 has been employed as a measure of the effectiveness of an inlet system in avoiding thermal reactions.[9,10]

(9) C. Brunnee, presented at the 14th Annual Conference on Mass Spectrometry, ASTM Committee E-14, Dallas, Texas, 1966.

(10) R. Ryhage and E. Stenhagen, *J. Lipid Res.*, **1**, 361 (1960).

(11) V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, L. M. Kogan, N. E. Voishvillo, and I. V. Torgov, *Tetrahedron*, **22**, 1399 (1966).

(12) The Russian workers ascribed[11] equatorial character to the 17β-hydroxyl group, citing the relatively abundant molecular ion. However, as we see here, 17α-hydroxyprogesterone has a very abundant molecular ion.

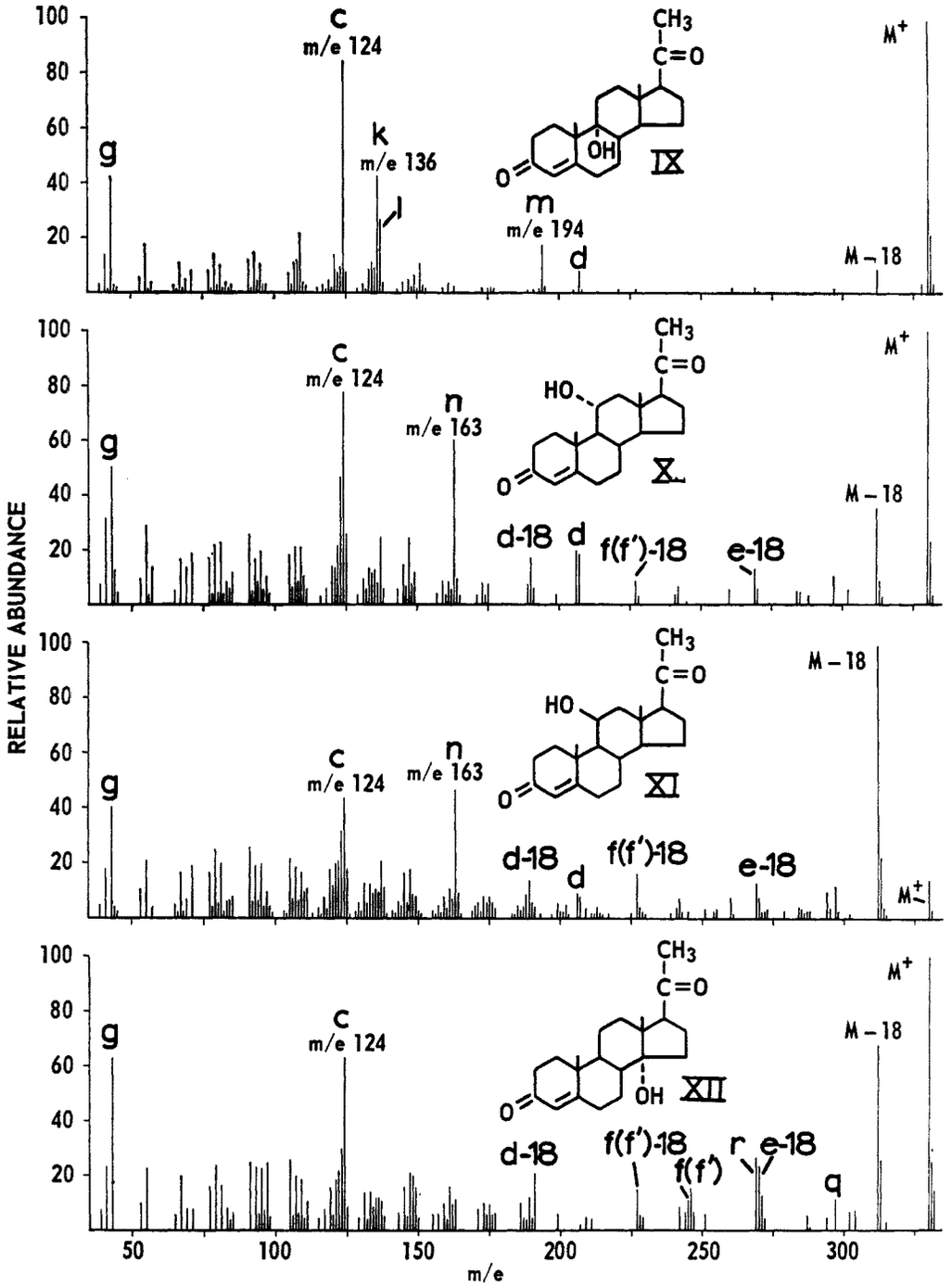


Figure 3a.—Mass spectra of substituted progesterones: IX, 9α-hydroxyprogesterone; X, 11α-hydroxyprogesterone; XI, 11β-hydroxyprogesterone; XII, 14α-hydroxyprogesterone.

(d − 18, e − 18, f − 18, f′ − 18). Ions c, f, and f′ deserve additional comment.

Ion c, at *m/e* 124, which provides one of the three strongest peaks in spectra of the hydroxyprogesterones (except XVI) is especially strong in spectra of IX and X. In the former (84%) the hydroxyl group renders the C-9–C-10 bond especially fragile, whereas in the latter (78%) the 11α-hydroxyl group facilitates cleavage of the C-11–H-11β bond. In view of the earlier demonstration of transfer of H-11 to form c,[5] it is significant that 11β-hydroxyprogesterone (XI) gives much less fragmentation to c. Apparently the axial 11β-hydrogen is the one preferentially transferred.

The results for ions f and f′ (Table I), as with the methylprogesterones, generally favor the Shapiro and Djerassi fragmentation mechanism[4,5] over that of Peterson.[2] In three compounds where appropriate substitution allows a choice between the two proposed mechanisms, *i.e.*, 15α-, 16α-, and 17α-hydroxyprogesterones (XIII, XIV, and XV), the ions at M − 85 and M − 103 (f and f − 18), taken together, are much more abundant than that of M − 101 (f′).

9α-Hydroxyprogestrone (IX).—The hydroxyl group can trigger fragmentation pathways not found with the methyl progesterones. For example, the mass spectrum of IX shows important peaks at *m/e* 194 (18%), 137 (26%), and 136 (43%) which are not accounted for by the mechanisms discussed above, but can be rationalized in terms of the destabilization of the C-9–C-10 bond by the C-9 hydroxyl group.

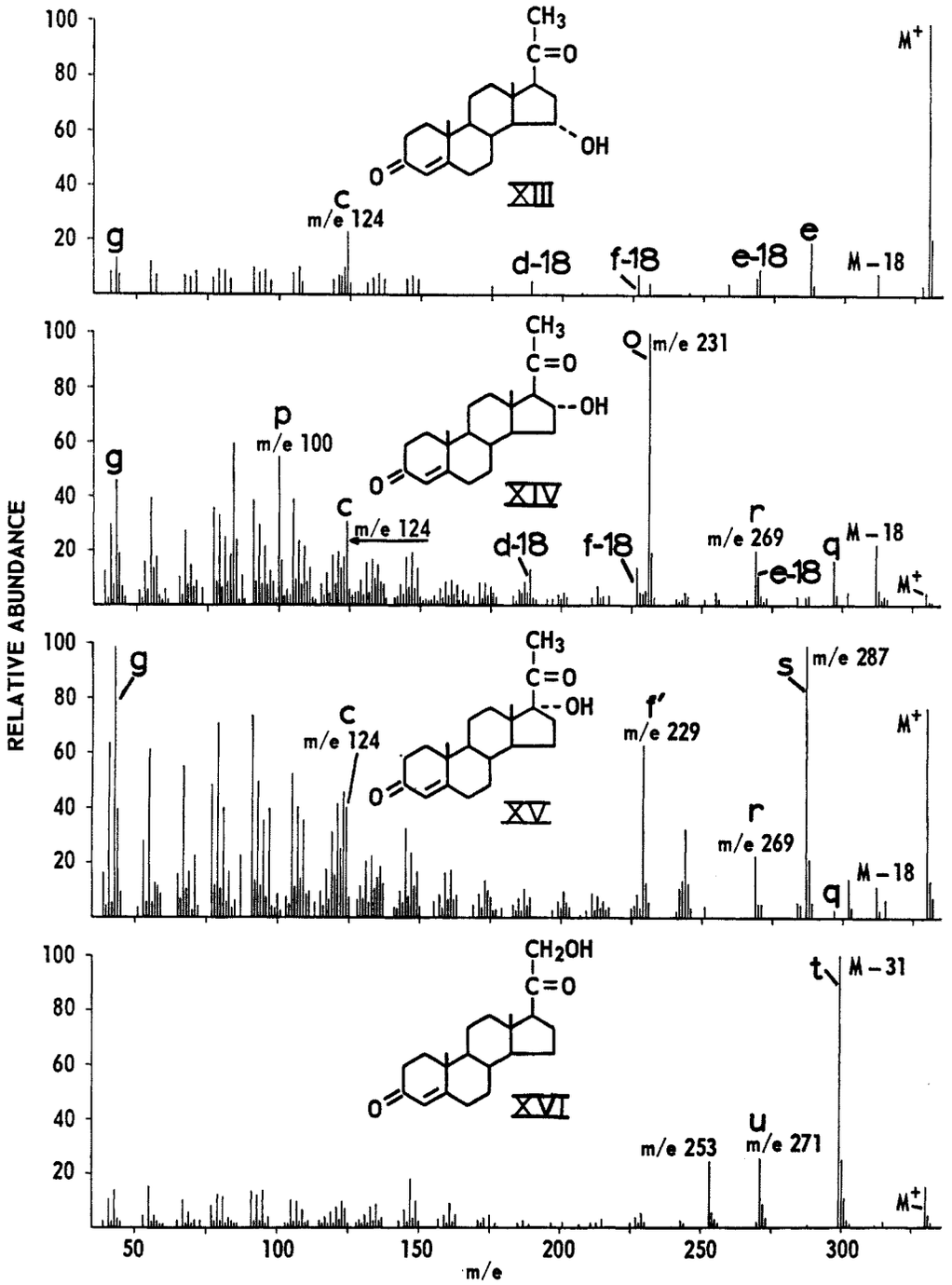
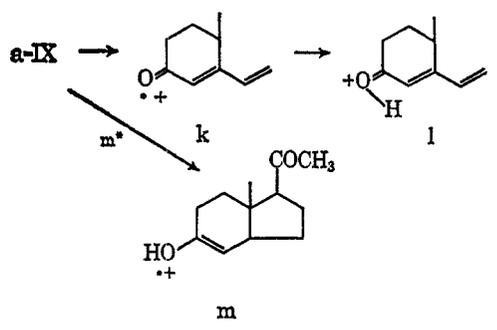


Figure 3b.—Mass spectra of substituted progesterones: XIII, 15α-hydroxyprogesterone; XIV, 16α-hydroxyprogesterone; XV, 17-α-hydroxyprogesterone; XVI, 21-hydroxyprogesterone

Cleavage between C-9 and C-10 (much like that which leads to ions c and d) followed by C-7–C-8 cleavage (instead of C-6–C-7 cleavage) without hydrogen transfer gives ion k (*m/e* 136), or with hydrogen transfer gives ion l (*m/e* 137). Charge retention on the fragment containing rings C and D gives ion m (*m/e* 194). That ion m is formed from the molecular ion is indicated by a metastable ion at *m/e* 113.0 (calcd 113.1).



11α- and 11β-Hydroxyprogesterones (X and XI).—These epimeric compounds, which differ from one another considerably in the relative intensities of the usual hydroxyprogesterone peaks, give one peak not found in spectra of the other compounds, a relatively intense peak at *m/e* 163 (60% in X, 47% in XI). This peak, which was noted but not assigned by Zaretskii, *et al.*,[11] is reasonably ascribed to the ion n, whose formation is tiggered by the labilizing effect of the C-11 hydroxyl on the C-9–C-11 bond. The ion

TABLE I

CORRESPONDING PEAKS IN MASS SPECTRA OF PROGESTERONES

Ion	Positions, *m/e* (and peak intensities, % of base peak)[a]																	
	Parent compound I	2α-Methyl II	4-Methyl III	6α-Methyl IV	6β-Methyl V	7α-Methyl VI	16α-Methyl VII	16β-Methyl VIII	9α-Hydroxy IX	11α-Hydroxy X	11β-Hydroxy XI	14α-Hydroxy XII	15α-Hydroxy XIII	16α-Hydroxy XIV	17α-Hydroxy XV	21-Hydroxy XVI	11α-Acetoxy XVII	12β,15α-Dihydroxy XVIII
M, a, b	314 (100)	328 (72)	328 (100)	328 (78)	328 (30)	328 (100)	328 (87)	328 (100)	330 (100)	330 (99)	330 (14)	330 (100)	330 (100)	330 (5)	330 (78)	330 (15)	372 (14)	346 (21)
M − 18									312 (9)	312 (35)	312 (100)	312 (67)	312 (8)	312 (23)	312 (11)	312 (0.4)	312 (100)[c]	328 (26)[d]
c	124 (72)	138 (46)	138 (31)	138 (100)	138 (37)	124 (21)	124 (52)	124 (43)	124 (84)	124 (78)	124 (44)	124 (63)	124 (23)	124 (31)	124 (40)	124 (7)	124 (19)	124 (43)
d	191 (19)	191 (9)	191 (6)	191 (10)	191 (2)	205 (16)	205 (13)	205 (10)	207 (8)	207 (18)	207 (8)	207 (2)	207 (0.9)	207 (0.3)	207 (0.8)	207 (1.0)	249 (0.0)	223 (4)
d − 18									189 (1)	189 (7)	189 (14)	189 (12)	189 (5)	189 (13)	189 (7)	189 (1)	189 (8)[c]	205 (4)[d]
e	272 (47)	272 (45)	286 (5)	286 (30)	286 (12)	286 (17)	286 (30)	286 (35)	288 (0.1)	288 (3)	288 (3)	288 (1)	288 (20)	288 (3)	288 (22)	288 (0.8)	330 (9)	304 (1)
e − 18									270 (0.5)	270 (6)	270 (8)	270 (23)	270 (9)	270 (11)	270 (5)	270 (2)	270 (6)[c]	286 (9)[d]
f	229 (29)[b]	229 (22)	243 (8)[b]	243 (22)[b]	243 (7)[b]	243 (12)[b]	243 (17)	243 (18)	245 (0.4)[b]	245 (1)[b]	245 (2)[b]	245 (11)[b]	245 (1)	245 (3)	245 (12)	245 (0.0)	287 (0.3)[b]	261 (0.0)
f − 18									227 (1)[b]	227 (9)[b]	227 (16)	227 (15)[b]	227 (7)	227 (14)	227 (9)	227 (3)	227 (16)[b,c]	243 (4)[d]
f′	229 (29)[b]	243 (5)	243 (8)[b]	243 (22)[b]	243 (7)[b]	243 (12)	229 (5)	229 (5)	245 (0.4)[b]	245 (1)[b]	245 (2)[b]	245 (11)[b]	229 (1)	229 (4)	229 (63)	229 (5)	287 (0.3)[b]	245 (4)
f′ − 18									227 (1)[b]	227 (9)[b]	227 (16)[b]	227 (15)[b]					227 (16)[b,c]	227 (5)
g	43 (65)	43 (100)	43 (85)	43 (50)	43 (100)	43 (38)	43 (100)	43 (55)	43 (42)	43 (100)	43 (40)	43 (63)	43 (13)	43 (46)	43 (99)	43 (14)	43 (68)	43 (100)

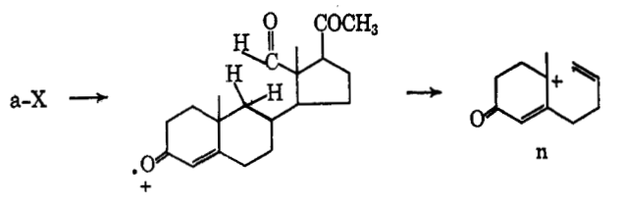
[a] Present data reported throughout. [b] In these spectra, ions f and f′ cannot be distinguished. [c] M − 60, d − 60, e − 60, f − 60, f′ − 60. [d] M − 18 − 18 (*m/e* 310) = 41%; d − 18 − 18 (*m/e* 187) = 13%; e − 18 − 18 (*m/e* 268) = 9%; f − 18 − 18 (*m/e* 225) = 7%.

TABLE II

METASTABLE ION PEAKS FOR PROGESTERONE FRAGMENTATIONS

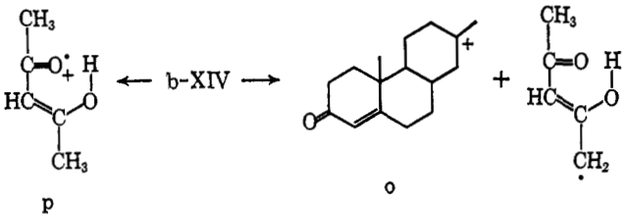
Fragmentation	m* found (calcd)																
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVII	XVIII
a → c		58.2 (58.1)	58.0	58.2	58.0	47.0 (46.9)											
a → d	116.0 (116.2)	111.2 (111.2)				127.9 (128.1)	128.0	128.0									
a → e	236.0 (235.6)	255.5 (255.6)		250.0 (249.4)	250.0			250.0									
a → f																165.5 (165.2)[a]	
e → e − 18													253.0 (253.1)				
M → M − 15				298.5 (298.7)	299.0		299.0	299.0									
M → M − 18		293.0 (293.0)		293.0	293.0	293.0	293.0	293.0	295.0 (295.0)	295.0	295.0	295.0	295.0	295.0	295.0	262.0 (261.8)[b]	
M − 18 → M − 36										277.5 (277.0)	277.0				277.0	277.0[c]	293.0 (293.0)

[a] Or b → f′. [b] M → M − 60. [c] M − 60 → M − 78.

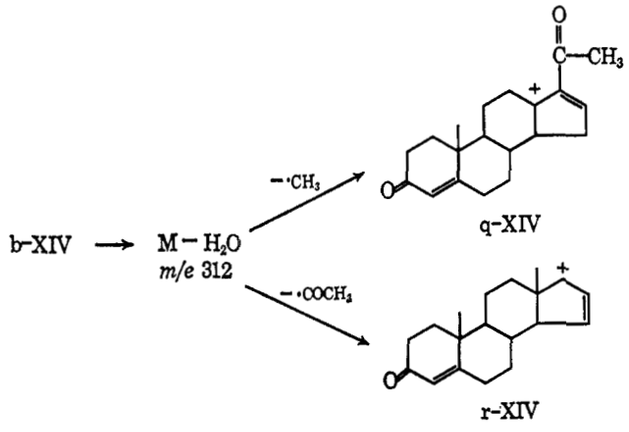


is also found, but is less intense (12%), in the spectrum of the 11α-acetoxyprogesterone (XVII).

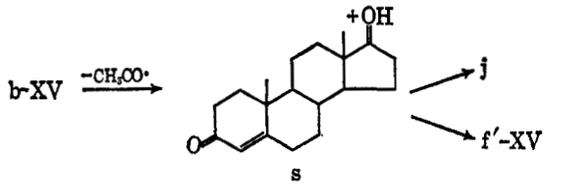
16α-Hydroxyprogesterone (XIV).—The base peak in the spectrum of XIV occurs at *m/e* 231 and can be explained by fragmentation involving the side chain and ring D. The ion at *m/e* 231 arises by loss of 99 mu from the molecular ion (m* 162.0, calcd 161.7). The structure o assigned to the ion at *m/e* 231 allows the loss of a highly stabilized radical. The intense peak at *m/e* 100 is also due to ring-D fragmentation, with charge and all hydrogen atoms remaining on the five-carbon unit (p). The origins proposed for these peaks agree with those suggested by Zaretskii, *et al.*;[11] however, their relative intensities are much stronger in the present spectra.



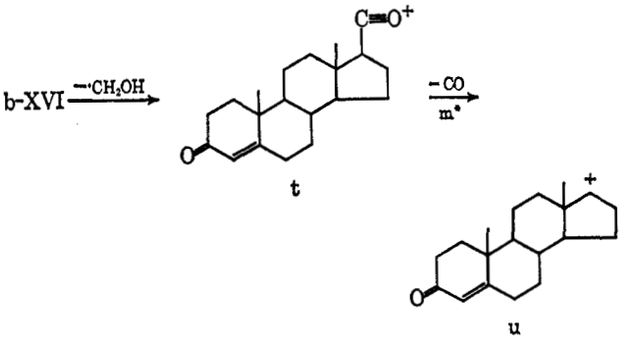
Two other peaks are relatively strong in the spectrum of XIV, although they are also found in spectra of XII and XV. These are peaks at *m/e* 297 (16%) and 269 (20%). Zaretskii, *et al.*,[11] have pointed out that the intensities of these peaks parallel those of the M − H_2O peak for 16α- and 16β-hydroxyprogesterones; thus, they are likely derived from the peak at M − 18. Possible structures are suggested as q-XIV (*m/e* 297) and r-XIV (*m/e* 269).



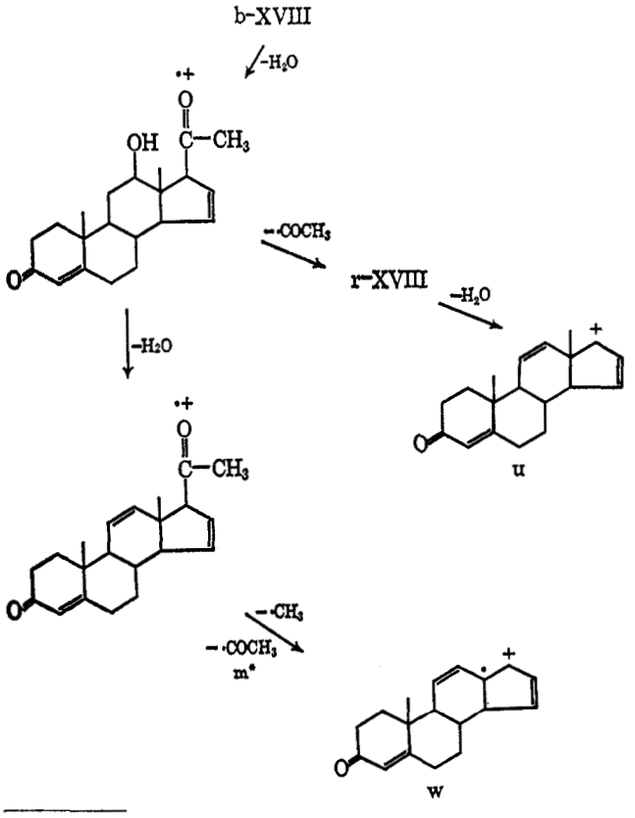
17α-Hydroxyprogesterone (XV).—The spectrum of XV contains peaks at *m/e* 297 and 269 (3 and 23%, respectively) which are presumably due to the same ions (m and n) observed in the spectrum of XIV. However, the base peak of this spectrum is at *m/e* 287, owing to the simple loss of the acetyl group to give ion s, which can lose C_2H_3O again to give ion j at *m/e* 244 and contribute to the intensity of ion f′ at *m/e* 229.[13]



21-Hydroxyprogesterone (XVI).—The principal fragmentation of the molecular ion of XVI (deoxycorticosterone) proceeds by initial loss of hydroxylmethyl to give t, followed by loss of carbon monoxide (m* 245.8, calcd 245.6) to give ion u. Fragmentation of the latter ion will be discussed in detail in the succeeding paper[1] on deoxycorticosterone derivatives.



12β,15α-Dihydroxyprogesterone (XVIII).—This compound undergoes a series of fragmentations from the anhydro and dianhydro ions (M − H_2O, M − $2H_2O$), like those of XIV and XV. Strong peaks appear at *m/e* 285 (r-XVIII) and 267 (v) owing to loss of acetyl from these ions and a peak appears at *m/e* 252 (w)



(13) The same fragmentation pathways are found in 16α-methyl- and 6α,16α-dimethyl-17α-hydroxyprogesterones,[2] where the peaks for ion g appear at *m/e* 311 and 325, those for ion r at *m/e* 283 and 297, those for ion s at *m/e* 301 and 315, and those for ion j at *m/e* 244 and 258.

owing to the simultaneous loss of acetyl and methyl (m* 205.2, calcd 204.8) from the ion of m/e 310 (M − $2H_2O$).

Experimental Section[14]

Samples.—The compounds studied are all known compounds whose properties agreed with those reported in the references indicated for individual compounds. Each sample was checked for purity by melting point determination, thin layer chromatography, and mass spectrometry.

Samples of the steroids employed in this study were obtained by published procedures: progesterone (I),[15] mp 129–131°; 2α-methylprogestrone (II),[16] mp 149.5–150°; 4-methylprogesterone (III),[17] mp 160–166°; 6α-methylprogesterone (IV),[18] mp 116–119°; 6β-methylprogesterone (V),[19] mp 172–174°; 7α-methylprogesterone (VI),[20] mp 191–199°; 16α-methylprogesterone (VII),[21] mp 134–137°; 16β-methylprogesterone (VIII),[22] mp 210–211; 9α-hydroxyprogesterone (XII),[23] mp 178–185°; 11α-hydroxyprogesterone (XIII),[24] mp 166–167°; 11β-hydroxyprogesterone (XIV),[25] mp 182–184°; 14α-hydroxyprogesterone (XV),[23] mp 199–201.5°; 15α-hydroxyprogesterone (XVI),[26] mp 226–232°; 16α-hydroxyprogesterone (XVII),[27] mp 222–228°; 17α-hydroxyprogesterone (XVIII),[28] mp 218–220°; 21-hydroxyprogesterone (XIX),[29] mp 140–141°; 11α-acetoxyprogesterone (XXII),[30] mp 171–175.5°; 12β,15α-dihydroxyprogesterone (XXIII),[31] mp 216–220°.

Registry No.—I, 57-83-0; II, 2636-91-1; III, 15981-49-4; IV, 903-71-9; V, 2300-06-3; VI, 2640-71-3; VII, 1239-79-8; VIII, 1424-09-5; IX, 15981-54-1; X, 80-75-1; XI, 600-57-7; XII, 16031-66-6; XIII, 600-73-7; XIV, 438-07-3; XV, 68-96-2; XVI, 64-85-7; XVII, 2268-98-6; XVIII, 599-14-4.

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(14) Mass spectra were determined with an Atlas CH-4 mass spectrometer equipped with a T04 ion source. All samples were introduced by the direct inlet technique employing the vacuum lock. Ionizing energy was maintained at 70 eV and ionizing current at 30 μA. Peak intensities are reported as percentages of the strongest peak in the spectrum.

(15) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 541.

(16) K. Tsuda and S. Nozoe, *Chem. Pharm. Bull.* (Tokyo), **7**, 238 (1959).

(17) F. Sondheimer and Y. Mazur, *J. Amer. Chem. Soc.*, **79**, 2906 (1957).

(18) P. F. Beal, M. A. Rebenstorf, and J. E. Pike, *ibid.*, **81**, 1231 (1959).

(19) W. J. Adams, B. Ellis, V. Petrow, and I. A. Staurt-Webb, British Patent 802,004; *Chem. Abstr.*, **53**, 9296*f* (1959).

(20) J. A. Campbell and J. C. Babcock, *J. Amer. Chem. Soc.*, **81**, 4069 (1959).

(21) E. Kaspar and R. Wiechert, *Chem. Ber.*, **91**, 2663 (1958).

(22) J. Romo, J. Lepe, and M. Romero, *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.*, **4**, 125 (1952).

(23) A. Schubert, D. Onken, R. Siebert, and K. Heller, *Chem. Ber.*, **91**, 2549 (1958).

(24) P. D. Meister, D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, and H. M. Leigh, *J. Amer. Chem. Soc.*, **75**, 55 (1953).

(25) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).

(26) A. Gubler and C. H. Tamm, *ibid.*, **41**, 301 (1958).

(27) D. Perlman, E. Titus, and J. Fried, *J. Amer. Chem. Soc.*, **74**, 2126 (1952).

(28) J. J. Pfiffner and H. B. North, *J. Biol. Chem.*, **132**, 459 (1940); see ref 15, p 610.

(29) See ref 15, p 603.

(30) D. H. Peterson, H. C. Murray, S. H. Epstein, L. M. Reineke, A. W. Weintraub, P. D. Meister, and H. M. Leigh, *J. Amer. Chem. Soc.*, **74**, 5933 (1952).

(31) Y. Kurosawa, M. Hayano, M. Gut, R. I. Dorfman, A. Schubert, and C. A. Bunton, *Agr. Biol. Chem.* (Tokyo), **25**, 424 (1961).

The Kostanecki–Robinson Acylation and Cyclization of 3-Acyl-4-hydroxy-2-pyrones

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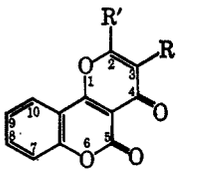
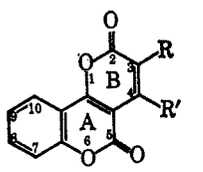
The acylation of 3-acyl-4-hydroxy-2-pyrones using the acid anhydride and salts of the corresponding acids (Kostanecki–Robinson conditions) has led to the formation of heterocyclic compounds which, depending on whether the acyl side chain has an α-CH_2 group or not, are substituted 2H,5H-pyrano[4,3-*b*]pyran-2,5-diones, or 4H,5H-pyrano[4,3-*b*]pyran-4,5-diones, respectively. The latter derivatives have been prepared by other methods, and firm spectroscopic, diagnostic methods have been established to distinguish between them.

We have investigated the acylation and cyclization of several 4-hydroxy-2-pyrones to give 4H,5H-pyrano-[4,3-*b*]benzopyran-4,5-diones I, and 2H,5H-pyrano-[4,3-*b*]benzopyran-2,5-diones II.[1]

The starting point for the well-established methods described below for preparing I or II was the observation that during the work-up of a preparation of 3-propionyl-4-hydroxycoumarin, the residues yielded a new material whose infrared spectrum was unlike the characteristic pattern of 3-acyl-4-hydroxy-2-pyrones. A new band of only moderate intensity at ~1640 cm^{-1} was observed (see Table I) in addition to the strong absorption at 1740–1750 cm^{-1} due to the lactone carbonyl which was at a slightly higher frequency than usual. The new band suggested a cyclized compound



Ia, R = R′ = CH_3
b, R = CH_3; R′ = C_2H_5
c, R = C_2H_5; R′ = CH_3
d, R = R′ = C_2H_5
e, R = $OC_6H_3Cl_2$; R′ = CH_3
f, R = H; R′ = C_6H_5
g, R = H; R′ = CH_3



IIa, R = H; R′ = CH_3
b, R = R′ = CH_3
c, R = Cl; R′ = CH_3
d, R = $CH_2C_6H_5$; R′ = CH_3
e, R = H; R′ = C_6H_5
f, R = H; R′ = OH

containing a γ-pyrone ring (I). The carbonyl stretching vibration in such a ring would be expected to give rise to an absorption near 1640 cm^{-1}. It was speculated that I could be derived from the 4-hydroxy-2-pyrone by diacylation followed by condensation. It was also realized that the condensation could proceed the other

(1) *Chemical Abstracts* describe the names used here as alternative to the preferred terminology as α-lactone derivatives of hydroxysorbic acid. We have thought it preferable to use these alternatives throughout to reveal the relationships between the different compounds.