(5.25 mmole) of sodium azide in 90 ml. of 0.1 *N* hydrochloric acid in methanol was stirred at room temperature for 2 hours. The solution was poured into 2.0 l. of cold ether and the solid that separated was filtered and dried; yield 2.5 g. This material was recrystallized from methanol–ether; yield 1.25 g., $[\alpha]^{25}D$ −185°, R_f 0.77; $\lambda_{max}^{0.1\ N\ HCl}$ 255, 340, log ε 4.33, 3.92.

Anal. Calcd. for $C_{21}H_{22}N_6O_7 \cdot H_2SO_4 \cdot H_2O$: C, 44.1; H, 4.6; N, 12.2; S, 5.6. Found: C, 43.9; H, 4.7; N, 11.9; S, 5.6.

9-Azido-6-demethyl-6-deoxytetracycline Hydrochloride[14] (XVII).—Sodium azide (0.75 g., 11.5 mmoles) was added to 5.0 g. (10.4 mmoles) of 6-demethyl-6-deoxytetracycline-9-diazonium oxide hydrochloride in 125 ml. of 0.1 *N* methanolic hydrochloric acid (8.3 ml. of concentrated hydrochloric acid in 1 liter of methanol). The solution was stirred at room temperature for 45 minutes, and the solid which precipitated was filtered and dried to give 2.5 g. The crude product was recrystallized from methanol–ethyl ether; 1.5 g. of a yellow, crystalline solid was obtained, $[\alpha]^{25}D$ − 230°, R_f 0.74; $\lambda_{max}^{0.1\ N\ HCl}$ 260, 345 mμ, log ε 4.30, 4.10.

Anal. Calcd. for $C_{21}H_{21}N_5O_7 \cdot HCl \cdot H_2O$: C, 49.6; H, 4.7; N, 13.7; Cl, 7.0. Found: C, 50.07; H, 4.21; N, 13.35; Cl, 7.33.

9-Ethoxythiocarbonylthio-6-deoxytetracycline[14] (XVIII).—A solution of 6-deoxytetracycline-9-diazonium disulfate (1.0 g., 1.5 mmoles) in water (20 ml.) was added dropwise with stirring to a solution of potassium ethyl xanthate (1.7 g., 11 mmoles) in water (20 ml.). The mixture was stirred for 1 hour and the solid which precipitated out of solution was collected and dried in the vacuum desiccator; yield 70%.

The product was purified by partition column chromatography (solvent system: ethyl acetate, 60; heptane, 40; 0.2 *M* phosphate buffer, *p*H 2.0, 20), $[\alpha]^{25}D$ − 174°, R_f 0.81; $\lambda_{max}^{0.1\ N\ HCl}$ 255, 275, 350, log ε 3.23, 3.23, 3.10.

Anal. Calcd. for $C_{25}H_{28}N_2O_8S_2$: N, 5.1; S, 11.7. Found: N, 4.7; S, 12.1.

7-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline[14] (XIX).—A solution of 2.0 g. (3.4 mmoles) of 6-demethyl-6-deoxytetracycline-7-diazonium hydrochloride sulfate (XIII) and 0.54 g. (3.4 mmoles) of potassium ethyl xanthate in 30 ml. of water was stored at room temperature until the evolution of nitrogen ceased (10 min.) and the solution was freeze-dried; yield 2.0 g. This material was purified by partition chromatography using the solvent system chloroform–butanol–*p*H 2 buffer (200:5:100) and diatomaceous earth as the support for the stationary phase; $[\alpha]^{25}D$ − 96.5°, R_f 0.77; $\lambda_{max}^{0.1\ N\ HCl}$ 245, 270, 355, log ε 4.39, 4.25, 4.10.

Anal. Calcd. for $C_{24}H_{26}N_2O_8S_2 \cdot H_2O$: C, 52.2; H, 5.4; N, 4.7; S, 11.6. Found: C, 52.2; H, 5.1; N, 5.0; S, 11.0.

9-Ethoxythiocarbonylthio-6-demethyl-6-deoxytetracycline[14] (XX).—A solution of 1.5 g. (9.4 mmoles) of potassium ethyl xanthate in 25 ml. of water was slowly poured into 1.9 g. (3.6 mmoles) of 6-methyl-6-deoxytetracycline-9-diazonium oxide hydrochloride in 40 ml. (4.0 mmoles) of 0.1 *N* hydrochloric acid. After 45 minutes of stirring at room temperature, the *p*H of the solution was adjusted from 3.2 to 4.5 with 1 *N* sodium hydroxide to precipitate the free base. Filtering off the precipitate and drying gave a 1.2 g. yield, $[\alpha]^{25}D$ − 51°, R_f 0.86; $\lambda_{max}^{0.1\ N\ HCl}$ 265, 345 mμ, log ε 4.34, 4.10.

Anal. Calcd. for $C_{24}H_{26}N_2O_8S_2$: N, 5.3; S, 12.0. Found: N, 5.23; S, 12.47.

Reduction of 6-Demethyl-6-deoxytetracycline-9-diazonium Disulfate (XIII) to 6-Dimethyl-6-deoxytetracycline (III).—A solution of 100 mg. of XIII in 10 ml. of methanol was heated to reflux for 10 minutes and evaporated to dryness *in vacuo*. The residue was identical to 6-demethyl-6-deoxytetracycline as compared by ultraviolet and infrared spectroscopy and paper chromatography.

Reduction of 6-Deoxytetracycline-9-diazonium Disulfate (XII) to 6-Deoxytetracycline Sulfate (II).—A solution of 6-deoxytetracycline-9-diazonium disulfate (0.10 g., 0.55 mmole) in methanol (10 ml.) was refluxed under anhydrous conditions for 30 minutes. The reaction mixture was added dropwise to 400 ml. of ethyl ether and the solid which separated was filtered and dried. This material was identical to 6-deoxytetracycline sulfate as shown by paper chromatography and infrared and ultraviolet spectroscopy.

Acknowledgment.—We wish to thank Dr. A. S. Kende for helpful discussions, Mr. L. Brancone and staff for the analytical data and Mr. W. Fulmor for the spectroscopic determinations.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIFORNIA]

Mass Spectrometry in Structural and Stereochemical Problems. I. Steroid Ketones[1]

BY HERBERT BUDZIKIEWICZ AND CARL DJERASSI

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Mass spectra have been measured for steroids with keto groups in all possible eleven nuclear positions. Characteristic features have been noted (largely due to primary cleavage of the carbon–carbon bond in the ring adjacent to the carbonyl group and retention of the charge with the oxygen-containing fragment) which in most instances permit a decision as to the location of the carbonyl group in a steroid skeleton. It should now be possible to locate in an unambiguous manner a carbonyl group in a saturated, monoketonic steroid by the combined use of mass spectrometry and optical rotatory dispersion.

In spite of the extensive applications of mass spectrometry[2] to a wide variety of organic compounds, only very few systematic studies have been performed with steroids. Reed[3] has concentrated on the use of low-voltage spectra for the determination of the molecular weight and the size of the side chain in sterol and triterpene types, but he has also listed the most important fragments in the high voltage spectra of a few members of these classes. Friedland and collaborators[4] discussed the principal fragmentation processes in a rather heterogeneous group of steroid alcohols, while Bergström and his colleagues[5] emphasized the use of mass spectrometry for the determination of the molecular weights of bile acids. A more detailed discussion of the fragmentation patterns observed with such bile acids has been published recently by Ryhage and Stenhagen,[6] who have also carried out a very extensive mass spectro-

(1) This study has been supported by grant No. A-4257 from the National Institutes of Health, U. S. Public Health Service.

(2) See J. H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier Publ. Co., Amsterdam, 1960.

(3) P. de Mayo and R. I. Reed, *Chemistry & Industry*, 1481 (1956); R. I. Reed, *J. Chem. Soc.*, 3432 (1958).

(4) S. S. Friedland, G. H. Lane, R. T. Longman, K. E. Train and M. J. O'Neal, *Anal. Chem.*, **31**, 169 (1959).

(5) S. Bergström, R. Ryhage and E. Stenhagen, *Acta Chem. Scand.*, **12**, 1349 (1958).

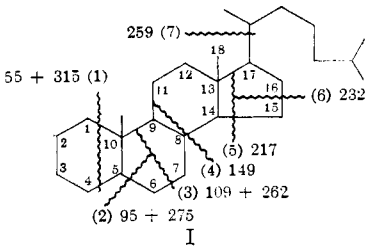
(6) R. Ryhage and E. Stenhagen, *J. Lipid Research*, **1**, 361 (1960).

metric investigation with ordinary fatty acid methyl esters.[7] Finally, Biemann, who has pioneered in the use of mass spectrometry in the alkaloid[8] and amino acid[9] fields, mentioned[10] that the orientation of the hydroxyl group in 3-hydroxyandrostanes can be determined by mass spectrometry.

The almost unique advantage of mass spectrometry among the various physical tools employed in organic chemistry is the minute amount of material required. With the advent of special heated inlet systems, it has been possible to handle relatively non-volatile substances and the above cited references[3-10] illustrate this point. Our own interest in mass spectrometry has been motivated by the belief that a semi-empirical study of the mass spectrometric fragmentation patterns of a group of closely related substances (together with certain deuterated analogs) would lead to generalizations, which might prove very fruitful in structural and stereochemical investigations of natural products, currently under way in our laboratory.[11] Just as with optical rotatory dispersion,[12] we selected steroids as the first test case and, for reasons outlined in the sequel, we started with steroid ketones.

The fragmentation process of the steroid skeleton itself has been discussed in detail[3,4] in terms of the hydrocarbon cholestane (I), the generation of the principal fragments (often accompanied by the further loss of one hydrogen atom) being indicated by the wavy lines,[13] which, however were not necessarily meant to be of mechanistic significance or to represent one-step processes. In addition, there is observed the loss of one methyl group (M-15) as well as the fragmentation of the side chain itself (M-29, M-43, etc.), but these latter peaks are rather weak.

Since many of the substances investigated by us are ketonic analogs of androstane (II), we have measured its mass spectrum (Fig. 1). As was to be expected, only those cholestane peaks were shifted which still retained the side chain and the shift in the androstane spectrum (Fig. 1) amounted to 112 mass units (C_8H_{16}). Thus, in rupture 1, the *m/e* 55 peak is observed in both cholestane (I) and androstane (II), while the former's 315 peak

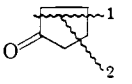


I

is now found at *m/e* 203 in androstane (II). The assignment of various structural fragments (see wavy numbered lines in Fig. 1) to the principal peaks does not imply any mechanistic preferences (nor single-step *vs.* multi-step decompositions) but is largely based on a comparison with the recorded spectra of cholestane, the C_8-side chain serving in part as a label. For a detailed mechanistic study, especially as it pertains to the loss or gain of one hydrogen atom, it would be necessary to examine androstane labeled with deuterium in many portions of the molecule. In the present paper, we have purposely refrained from possible mechanistic proposals (other than to mention one-step in lieu of multi-step decompositions where these are indicated by observable metastable peaks), since a detailed study is under way in our laboratory on the mass spectral fragmentation behavior of a series of bicyclic analogs labeled with deuterium. In Fig. 1, a one-step cleavage typified by wavy line 5 is indicated by the presence of a metastable peak at *m/e* 182.5 (corresponding to the change *m/e* 260 → 217). Probable metastable peaks at *m/e* 207 and 70 could not be recognized unambiguously, but, if correct, they would suggest one-step processes for cleavages 6 (260 → 232) and 8 (260 → 135).

With cholestane (I) and androstane (II) as reference standards, we now turned to substrates which contained a keto group in various positions of these two steroid skeletons. The reason for concentrating initially on ketonic steroids was the following.

Aliphatic ketones of type R_1COR_2 give strong peaks[14] corresponding to R_1CO^+ and R_2CO^+ (α-cleavage) as well as to $R_1COCH_3^+$ and $R_2COCH_3^+$ (β-cleavage with rearrangment of one hydrogen atom). The most important feature is that the charge remains predominantly with the oxygen-containing fragment, which has proved to be of substantial advantage in the steroid series (*vide infra*). In the case of cyclic ketones, very exact mass measurements[15] as well as studies[16] with "labeled" (deuterated) analogs revealed that the main fragments were due to cleavage of type 1 (CH_2CH_2CO; CH_2CHCO; CH_2CH_2) and 2 (CH_2-CO; $CH_2CH_2CH_2$; CH_2CH_2CH) as indicated by the wavy lines in the structural formula



(7) See R. Ryhage and E. Stenhagen, *Arkiv. Kemi.*, **15**, No. 50, 545 (1960), and earlier papers.

(8) K. Biemann, *Tetrahedron Letters*, No. **15**, 9 (1960); K. Biemann and M. Friedman-Spiteller, *ibid.*, 68 (1961).

(9) K. Biemann and C. G. J. Deffner, *Biochem. Biophys. Res. Comm.*, **4**, 283 (1961); see also K. Biemann, *Chimia*, **14**, 393 (1960).

(10) K. Biemann and J. Seibl, *J. Am. Chem. Soc.*, **81**, 3149 (1959).

(11) The use of mass spectrometry in the structure elucidation of the antibiotic filipin is a case in point (C. Djerassi, M. Ishikawa, H. Budzikiewicz, J. N. Shoolery and L. F. Johnson, *Tetrahedron Letters*, 383 (1961).

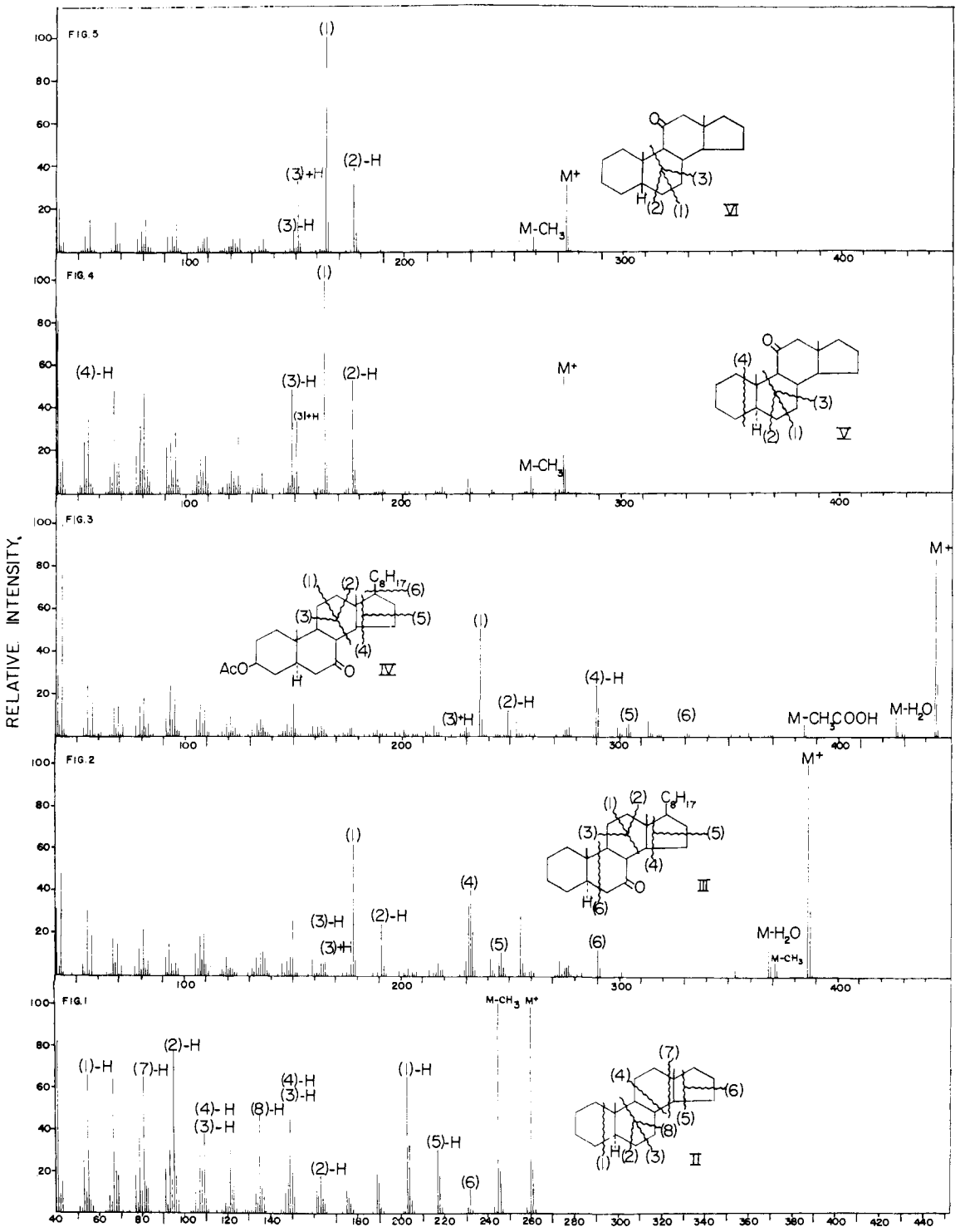
(12) C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(13) The rupture indicated by wavy line 5, involving the loss of the side chain plus three carbon atoms, has been interpreted in the manner shown by Ryhage and Stenhagen (ref. 6). Reed (ref. 3) assumed that the three carbon atoms should be represented by C-13, C-17 and C-18, while Friedland, *et al.*, (ref. 4) proposed C-16, C-17 and C-18. We favor the suggestion of the Swedish workers (ref. 6), as this involves the rupture of only two bonds. It is pertinent to mention that the experience of the Swedish workers has shown that the presence of an actual or potential (by dehydration of a hydroxyl group) double bond between C-11 and C-12 favors the loss of the C-17 side chain without carbon atoms 15, 16 and 17.

(14) A. G. Sharkey, J. L. Schultz and R. A. Friedel, *Anal. Chem.*, **28**, 934 (1956).

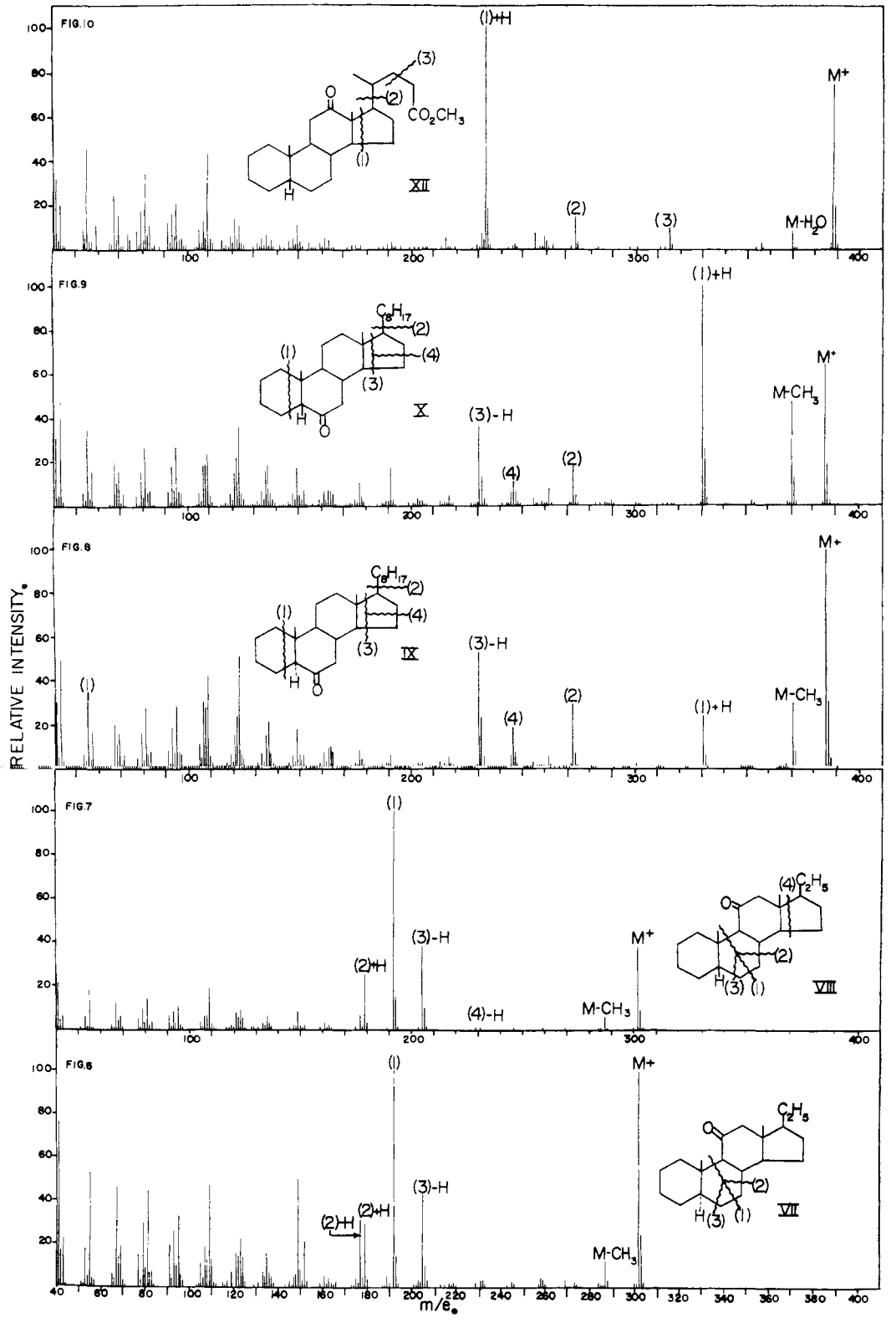
(15) J. H. Beynon, R. A. Saunders and A. E. Williams, *Appl. Spectros.*, **14**, 95 (1960); see also pp. 354–361 in ref. 2.

(16) P. Natalis, *Bull. soc. chim. Belg.*, **67**, 599 (1958).



Figs. 1–5.—Mass spectra of androstane (II), cholestan-7-one (III), 3β-acetoxycholestan-7-one (IV), 5α-androstan-11-one (V) and 5β-androstan-11-one (VI).

In cyclohexanone, the formation of the ion $C_3H_3O^+$ as well as the loss of C_2H_4 and $C_2H_4 + H$ are the principal processes.[15] Only two bicyclic ketones (*cis*- and *trans*-β-decalone) have been discussed in the literature[15]; their fragmentation is rather complex and includes rearrangement peaks (*e.g.*, pronounced loss of C_2H_4O). Furthermore, they exhibit the loss of water, a feature which can also be observed among the steroid ketones (*e.g.*, Fig. 15). An extensive study of the mass spectrometric behavior of bicyclic ketones of the decalone and hydrindanone series is under way in our laboratory and will be published in a future paper. For investigating the effect of a keto group upon



Figs. 6–10.—Mass spectra of 5α-pregnan-11-one (VII), 5β-pregnan-11-one (VIII), cholestan-6-one (IX), coprostan-6-one (X) and methyl 12-ketocholanate (XII).

the mass spectral fragmentation pattern of the steroid skeleton, we selected either unsubstituted monoketonic cholestanes and androstanes, or such ketones where additional substituents were sufficiently far away from the expected centers of fragmentation so as not to cause complications.

Steroids with Keto Groups in Rings B and C. (a) **7-Ketones.**—The presence of a ketonic func-

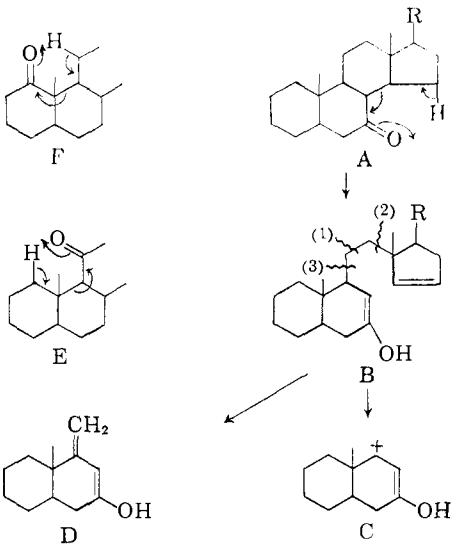
tion at position 7 as in cholestan-7-one (III) yields a spectrum (Fig. 2) in which the main fragmentation process is represented by β-cleavage of the most heavily substituted bond (8–14) followed by breakage of the 11–12 bond, the charge remaining with the oxygen-containing fragment (*vide* supra). The strong peak at *m/e* 178 thus encompasses rings A and B together with C-11 and *should not be affected by differences in the side chain at C-17.* On the other hand, additional substituents in rings A and B should shift this peak and this is borne out by the spectrum (Fig. 3) of 3β-acetoxycholestan-7-one (IV) where this peak is shifted to *m/e* 236 (addition of $C_2H_2O_2$). The correctness of our conclusion that the principal cleavage involves rupture of the 8–14 and 11–12 linkages is further substantiated by the mass spectra (not shown) of 6α-deuterio-3β-acetoxycholestan-7-one[17] and 6,6-dideuterio-3β-acetoxycholestan-7-one,[17] since the 236 peak in IV (Fig. 3) is now shifted to 237 and 238, respectively.

Turning again to the spectrum (Fig. 2) of cholestan-7-one (III), the strong peak at *m/e* 178 is accompanied by weaker ones at *m/e* 165 and *m/e* 191, which are attributed to cleavage (together with rearrangement of one hydrogen) of the 9–11 or 12–13 bonds. Metastable peaks at *m/e* 358 (associated with the loss of the methyl group: M-CH_3) and at *m/e* 337.5 (further loss of water) can be recognized unequivocally. Analogous metastable peaks could also be found in the mass spectra of most of the other keto steroids.

Mechanistically, the cleavage of the 8–14 bond most likely proceeds as in A to give initially species B, which decomposes further to yield the characteristic positively charged fragments indicated by wavy lines 1, 2 and 3 in Fig. 2. Cleavage 3 of the 9–11 bond is readily understandable, since the resulting ion C is stabilized by allylic conjugation. Fission 2 of the 12–13 bond is probably similarly facilitated because the radical (at C-13) is now stabilized by a 14,15-double bond. Finally, if the allylic hydrogen atom at C-9 is lost followed by rupture (1) of the 11–12 bond, a species (D) results which would appear to be especially favored because of complete conjugation. In the absence of studies with deuterated analogs (at positions 9, 11, 12 and 15) it would be premature to discuss the possible processes of hydrogen loss which seem to accompany some of these fragmentations.

The same explanations (see E and F) also apply to rationalize the appearance of three characteristic peaks in the mass spectra of 11-ketones (Figs. 4–7) and 1-ketones (Figs. 18–19).

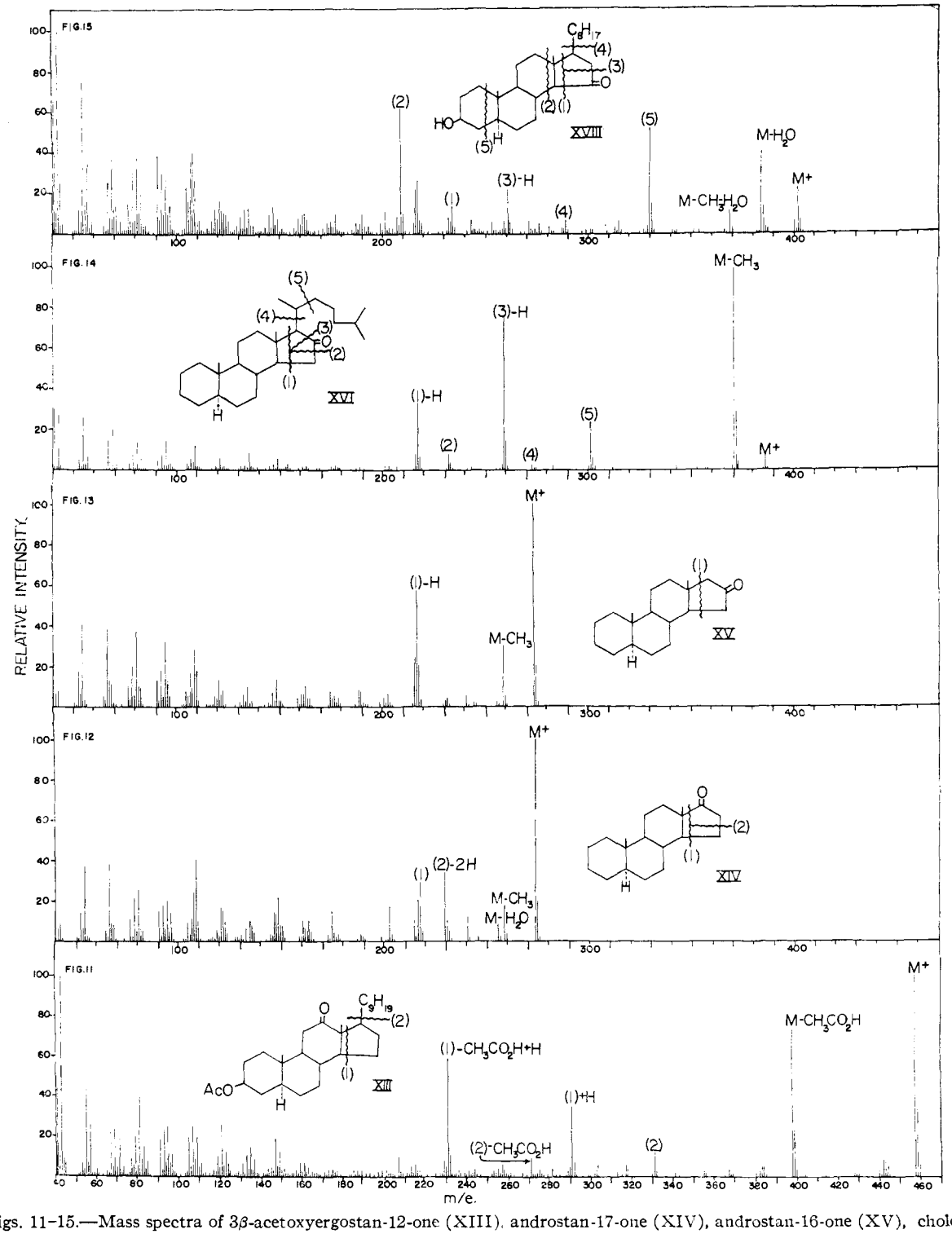
(b) **11-Ketones.**—11-Keto steroids resemble the 7-keto analogs in that the primary process is again β-cleavage of the most heavily substituted bond (9–10) and splitting of the 6–7 linkage, the charge again remaining with the ketone-containing fragment. As a result, the base peak in the mass spectrum (Fig. 4) of androstan-11-one (V) occurs at *m/e* 164 and includes rings C and D together with C-7. Consequently, in contrast to 7-keto steroids, *substitution in ring A of 11-keto steroids will not affect the base peak, while additional groups in ring D will cause a shift.* This is demonstrated in Fig. 6 with the mass spectrum of 5α-pregnan-11-one (VII) where the base peak has been shifted by 28 mass units (corresponding to the C_2H_5 substituent at C-17) to *m/e* 192.



Again, just as with 7-keto steroids, the base peak in 11-keto steroids such as the 164 peak of androstan-11-one (V) is surrounded by two satellites at *m/e* 164 ± 13 (see Fig. 4) due to alternate cleavage (and rearrangement of one hydrogen) between C-5 and C-6 (*m/e* 177) or C-7 and C-8 (*m/e* 151). An associated peak at *m/e* 149 appears to be related to the stereochemistry at C-5 and will be discussed below. The identical picture is observed (Fig. 5) in the mass spectrum of the A/B *cis* isomer 5β-androstan-11-one (VI), the only difference associated with the altered C-5 stereochemistry being of a quantitative nature at lower mass units (and the lower intensity of the molecular ion). The same relationship has also been noted in the mass spectra of 5α-pregnan-11-one (VII, Fig. 6) and its 5β-isomer (VIII, Fig. 7). A not very well defined metastable peak at *m/e* 98.5 in the spectrum (Fig. 5) of VI would suggest a one-step process for fragmentation (1). The spectra (Figs. 6 and 7) of the corresponding 11-ketopregnanes (VII and VIII) appear to possess metastable peaks at *m/e* 139.5, which could be attributed to a one-step fragmentation indicated by wavy line 3 (*m/e* 302→205).

(c) **6-Ketones.**—With the above background at our disposal, one may expect in 6-keto steroids that one of the principal fragmentation processes should involve cleavage of the 4–5 bond with loss of ring A. Indeed, the mass spectra of cholestan-6-one (IX, Fig. 8) and especially coprostan-6-one (X, Fig. 9) exhibit an important peak at *m/e* 331, which represents a loss of 55 mass units. This is due to the removal of the structural fragment encompassed by C-1 to C-4 and the picking up of one hydrogen by the oxygen-containing fragment. It should be noted that in the mass spectra of the hydrocarbons (*e.g.* androstane (II) in Fig. 1), loss of ring A results in M-57 (carbon atoms C-1 to C-4 together with one hydrogen).

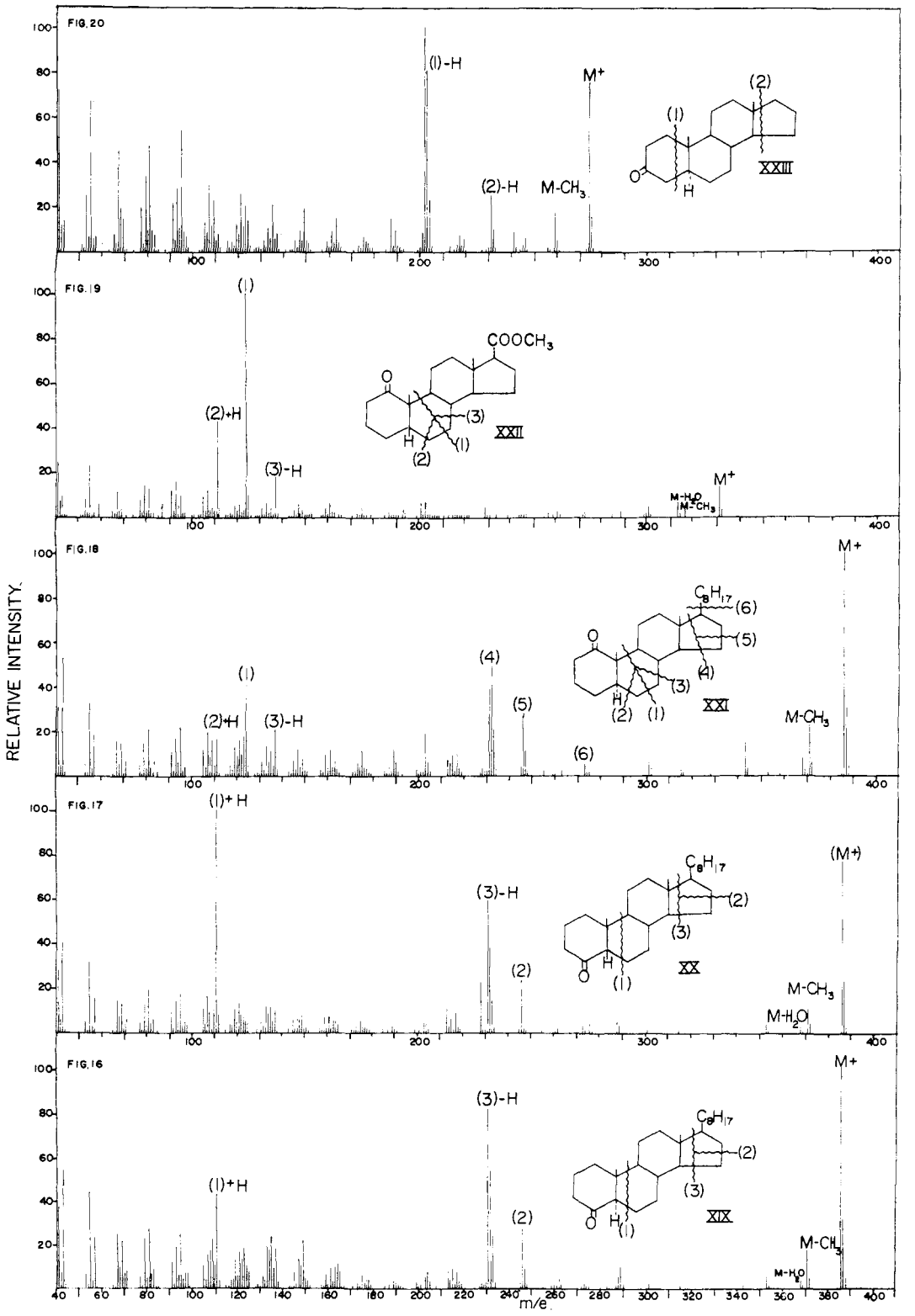
(17) E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).



Figs. 11–15.—Mass spectra of 3β-acetoxyergostan-12-one (XIII), androstan-17-one (XIV), androstan-16-one (XV), cholestan-16-one (XVI) and 3β-hydroxycholestan-15-one (XVIII).

The correctness of assigning the *m/e* 331 peak to the loss of the four-carbon fragment of ring A is demonstrated by the fact that the mass spectrum (not reproduced) of 3β-acetoxycholestan-6-one (XIa) still retains this same peak (albeit of reduced intensity because the 3β-acetoxy group favors further fragmentation in this area of the molecule), while the spectra of both 5α-deuterio-(XIb)[17] and 7α-deuterio-(XIc)[17] 3β-acetoxy-cholestan-6-one now contain a peak at *m/e* 332. It follows that the hydrogen atoms adjacent to the carbonyl group are retained in this fragmentation process.

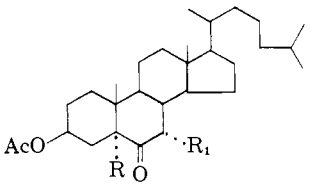
(d) **12-Ketones.**—By analogy to the 6-keto steroids, 12-ketones with their similar environment should suffer loss of ring D (C-15, C-16, C-17 and side chain) with capture of one hydrogen by the



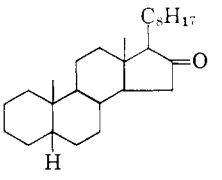
Figs. 16–20 —Mass spectra of cholestan-4-one (XIX), coprostan-4-one (XX), cholestan-1-one (XXI), methyl 1-ketoetianate (XXII) and androstan-3-one (XXIII).

oxygen-containing fragment. In other words, 12-keto steroids should show a peak at M-C_3H_4R (R = side chain), while ordinary steroids lacking a 12-keto group usually exhibit M-C_3H_6R. Differences in the nature of the C-17 side chain of 12-keto steroids, therefore, should not be of any significance[17a] as far as this important peak is concerned while substituents in ring A should shift

this peak by a mass equivalent to that substituent. Both these predictions are substantiated in Figs. 10 and 11 by the mass spectra of methyl 12-ketocholanate (XII) and 3β-acetoxyergostan-12-one (XIII). The former exhibits a strong peak at *m/e* 233, equivalent to rings A, B, C (including C-18 methyl group) and one additional hydrogen atom, while in the spectrum (Fig. 11) of 3β-acetoxyergostan-12-one (XIII), this peak appears now at 58 mass units higher, *i.e.*, *m/e* 291. A second strong peak at *m/e* 231 is due to the further loss of acetic acid, which is a common occurrence among acetates (see also M-CH_3CO_2H at *m/e* 398).



XIa, R = R′ = H
b, R = D; R′ = H
c, R = H; R′ = D



XVII

Steroids with Keto Groups in Ring D.—The mass spectra of androstan-17-one (XIV, Fig. 12) and androstan-16-one (XV, Fig. 13) are on the whole very similar. The principal difference is the strong M-57 (C_3H_5O) peak in androstan-16-one (XV) while the 17-keto analog XIV exhibits substantial ones at M-44[18] and M-56 as well as a smaller one at M-57. The M-44 peak represents a characteristic feature which we have noted among bicyclic ketones, where this rather unusual loss of two additional hydrogen atoms (*e.g.*, fragmentation 2 in Fig. 12) has also been noted. Details of this process are now being studied in our laboratory through the mass spectral behavior of a number of suitably deuterated hydrindanone model compounds.

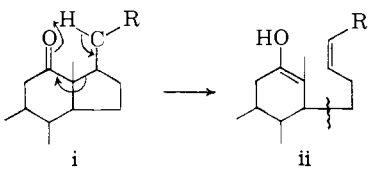
The generation of this M-44 peak involves a one-step process from the molecular ion as indicated by a metastable peak at *m/e* 194 (corresponding to 274→230) found in the spectrum (Fig. 12) of androstan-17-one (XIV). Cleavage 1 in androstan-16-one (XV, Fig. 13) is also a one-step process as evidenced by a metastable peak at *m/e* 172 (*m/e* 274→217). The presence of a hydroxyl function at C-3 appears to complicate somewhat the analysis[4] of the fragmentation pattern and a further refinement has to be made when considering ring D ketones of the cholestane series.

Thus, predominant cleavage in cholestan-16-one (XVI) occurs again in ring D, the peak (1) at *m/e* 217 (Fig. 14) arising from loss of carbon atoms 15, 16, 17 and the side chain. Cleavage adjacent to the carbonyl group is particularly favored leading to a very strong peak (3) at *m/e* 259, which represents the loss of the side chain and the equivalent of CH_2.[19] Minor peaks may also be observed at *m/e* 232 and 273, which can be attributed respectively to alternate cleavage alpha to the carbonyl group, or to loss of the side chain. The base peak in cholestan-16-one (XVI)—contrary to all the other keto steroids reported in this paper—occurs at M-15 which we associate with loss of the C-21 methyl group. Labilization of this portion of the molecule is also indicated by the *m/e* 301 peak involving cleavage of the 20–22 bond and, apparently is caused by the proximity of the C-16 keto function. A similar observation was made in the mass spectrum of coprostan-16-one (XVII)[20] which proved to be very similar throughout the entire mass range and, therefore, is not reproduced. An investigation is now underway in our laboratory on the synthesis and mass spectral fragmentation behavior of 16-keto steroids with deuterium in various positions in the side chain, since this should clarify many of the outstanding questions in this series.

The only 15-keto steroid at our disposal was 15-ketocholestan-3β-ol (XVIII). Its mass spectrum is shown in Fig. 15 and several of the higher molecular weight fragments can again be rationalized by ruptures promoted through the proximity of the keto group. Cleavage at either one of the α-positions of the keto group together with rupture of the 13–17 bond leads to fragments of mass 234 and 261. In addition, there is observed a very strong peak at *m/e* 209, the genesis of which can be explained readily through hydrogen transfer in the cyclic intermediate G and then cleavage of the allylically activated 12–13 bond (H), the charge remaining with the oxygen-containing ring D fragment.

In summary, while the course of the principal fragmentation of individual ring D keto steroids

(17a) NOTE ADDED IN PROOF.—This appears to be true only if there is present a hydrogen atom at C-20 which is transferred to the oxygen atom (see i), thus explaining the gain of one hydrogen in peaks (1) in Figs. 10 and 11. The justification for this statement is that the mass spectrum of 5β-pregnan-12-one shows a strong peak at M-69 (cleavage of the allylically activated C-14 center in ii), while no analogous strong M-41 peak is noted in the mass spectrum of 5β-androstan-3α-ol-12-one acetate (kindly provided by Prof. T. Reichstein).

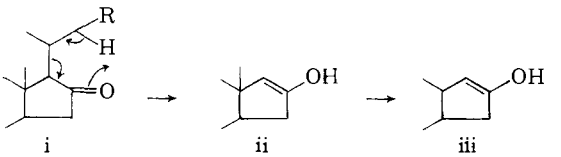


i ii

A number of suitably deuterated substrates now are being synthesized in this series to settle this point unambiguously.

(18) It should be noted that in the ring D-unsubstituted androstane (II), there exists no M-44 peak, but only one at M-43 (rupture 5 in II involving carbon atoms C-15, C-16, C-17 and one additional hydrogen).

(19) This important peak has been attributed tentatively to fragmentation process 3 in fig. 14. The following alternate explanation has been proposed by Prof. K. Bieman (private communication).

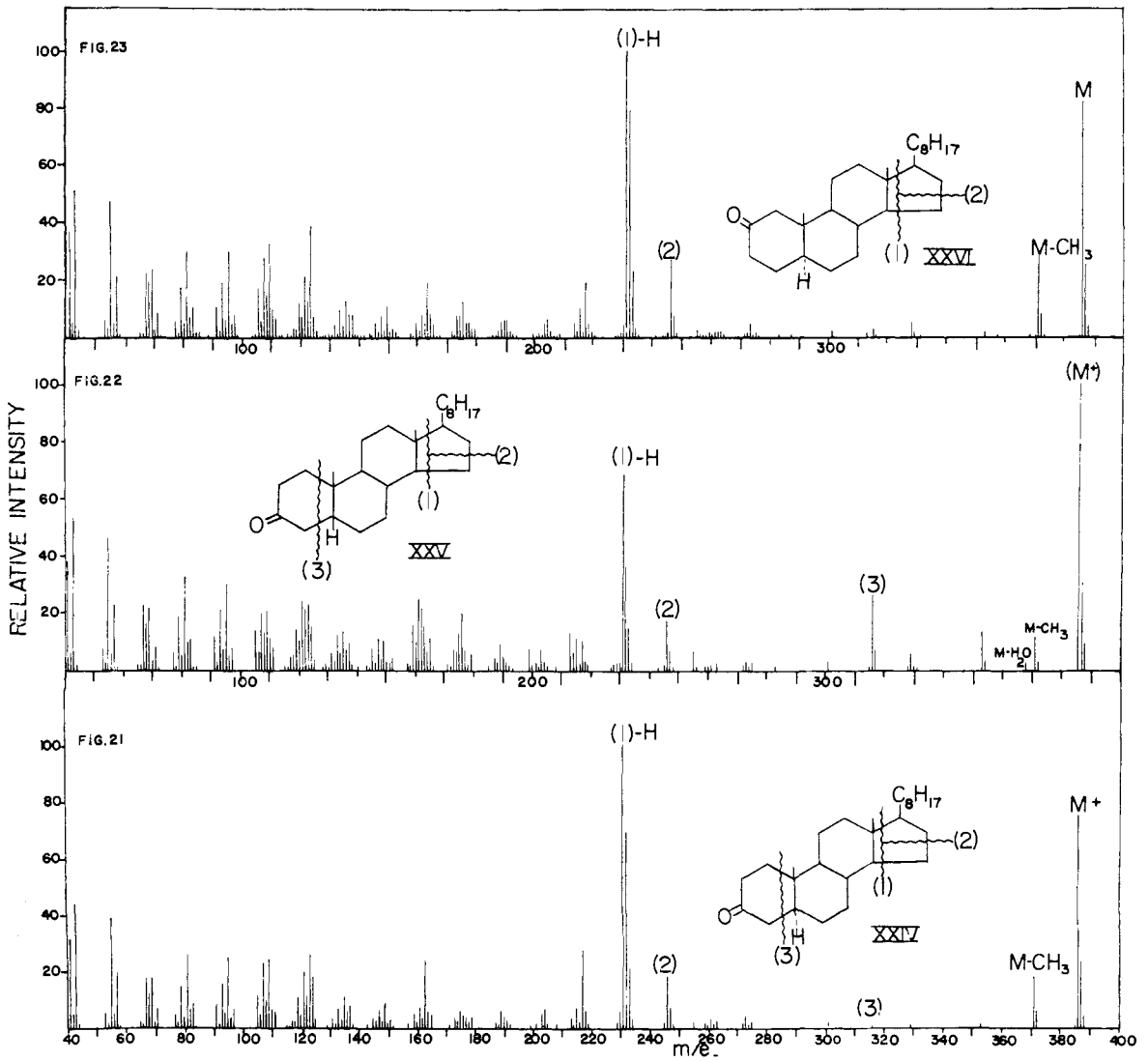


i ii iii

NOTE ADDED IN PROOF.—This explanation rather than the one indicated in (3)—Fig. 14, has now been supported by the observation that in the 15,15,17-trideuterio analog, all three deuterium atoms are retained in the cation corresponding to *m/e* 259.

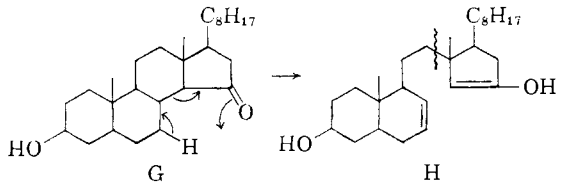
Aside from a metastable peak at *m/e* 371 due to the loss of a methyl group, the mass spectrum (Fig. 14) of cholestan-16-one (XVI) contains a second one at *m/e* 157.5 (301 → 217) which indicates that the fragment associated with 1 arises, at least partially, by further decomposition of fragment 5.

(20) I. Scheer and E. Mosettig, *J. Am. Chem. Soc.*, **77**, 1820 (1955).



Figs. 21–23.—Mass spectra of cholestan-3-one (XXIV), coprostan-3-one (XXV) and cholestan-2-one (XXVI).

can be rationalized satisfactorily, it is preferable to base firm conclusions in an unknown steroid concerning the location of a keto group in that region of the molecule on a combination of physical measurements. Thus, an infrared spectrum will immediately locate the carbonyl group in the cyclopentane ring, while the optical rotatory dispersion curve[12] will then usually differentiate between 15-, 16- and 17-keto steroids.



Steroids with Keto Groups in Ring A. (a) **4-Ketones.**—4-Keto steroids, such as cholestan-4-one (XIX, Fig. 16) and coprostan-4-one (XX, Fig. 17), behave in the anticipated manner, one of the principal cleavages occurring β to the carbonyl group and rupture of the most highly substituted bond (between C-9 and C-10) of ring B. This results in a strong peak at *m/e* 111 (ring A including angular methyl group and capture of one hydrogen atom), *which will be characteristic of 4-keto steroids irrespective of the presence of non-ketonic substituents in rings C and D* and which was not observed with any of the other steroids discussed above. The peaks at *m/e* 231 and 246 are not associated with the presence of the 4-keto group, but rather represent the usual fragmentation of ring D in cholestane derivatives (see ruptures 5 and 6 in I).

(b) **1-Ketones.**—1-Keto steroids should behave like 7- and 11-ketones in that the principal peak associated with the ketone fragmentation process should be accompanied by two smaller peaks at 13 mass units higher and lower. Thus in cholestan-1-one (XXI, Fig. 18) or methyl 1-ketoetianate (XXII, Fig. 19), this peak occurs at *m/e* 124 due to rupture 1 of the 9–10 and 6–7 bonds. The oxygen-containing fragment retains the charge and represents, therefore, ring A together with C-6 and the angular methyl group, the additional substituents (see XXI *vs.* XXII) not playing any role.

In addition to the *m/e* 124 peak, there are found again the two smaller satellites at *m/e* 111[21] (fission 2 in XXI and XXII involving the 9–10 and 7–8 bonds). A metastable peak at *m/e* 306 in the spectrum (Fig. 18) of cholestan-1-one shows that the peak at *m/e* 343 (due to loss of C_2H_3O) arises from the molecular ion rather than from the M-CH_3 species.

(c) **2- and 3-Ketones.**—In androstan-3-one (XXIII), the principal cleavage (including rearrangement of one and two hydrogen atoms) involves loss of carbon atoms 1, 2, 3 and 4, the charge remaining with the tricyclic ring system lacking the oxygen atom and resulting in a strong peak at *m/e* 203 (Fig. 20).[22] Apparently, this is diagnostic only of 3-keto steroids lacking a side chain at C-17 or of those C-17-substituted steroids, where loss of ring A is favored for steric grounds. The reason for these two reservations becomes obvious from an inspection of Figs. 21 and 22 of the mass spectra of cholestan-3-one (XXIV) and coprostan-3-one (XXV).

In cholestan-3-one (XXIV), the loss (one-step process as indicated by a metastable peak at *m/e* 260) of ring A with its 3-keto group is practically negligible (*m/e* 316 in Fig. 21), when compared with the base peak of *m/e* 231 representing the typical ring D fragmentation of cholestanes (see I). On the other hand, in its 5β-isomer, coprostan-3-one (XXV), while the ring D cleavage (*m/e* 231 in Fig. 22) still predominates, the loss of the four carbon atoms and one oxygen from ring A becomes again very noticeable (*m/e* 316).

The mass spectrum (Fig. 23) of cholestan-2-one (XXVI) is so similar to that (Fig. 21) of cholestan-3-one (XXIV) that this method cannot be used for purposes of differentiation. Infrared spectroscopy is also of no help in this instance, but optical rotatory dispersion measurements in methanol solution (with and without hydrochloric acid[23]) are decisive.

Stereochemical Differentiations.—When a pair of C-5 epimeric steroid ketones is available, then mass spectrometry can often be used as a useful adjunct in deciding upon the stereochemistry at C-5. Among the three pairs of ring A oxygenated steroids (1-, 3- and 4-keto steroids) studied by us, cleavage of ring A is invariably favored in the 5β-(rings A/B *cis*) isomer over its 5α-analog (XXII *vs.* XXI, XXV *vs.* XXIV, XX *vs.* XIX).[24]

(21) While this *m/e* 111 peak is also observed in 4-ketones, these do not possess (see Figs. 15 and 16) the much stronger one at *m/e* 124.

(22) The mass spectrum of 5β-androstan-3-one (not shown) is very similar to that of its 5α-isomer XXIII, except that, as anticipated, cleavage of ring A is greatly favored. The *m/e* 203 peak (see Fig. 20) is now by far the highest one, while the ratio of the 202 peak to that of the molecular ion is not altered to any marked extent. It is for this reason that we assign the 203 peak to the loss of ring A (C-1 to C-4) plus one hydrogen.

(23) C. Djerassi, L. A. Mitscher and B. J. Mitscher, *J. Am. Chem. Soc.*, **81**, 2383 (1959).

(24) Dr. R. I. Reed of the University of Glasgow mentioned a similar conclusion in a lecture at the British Chemical Society Meeting in Liverpool, April, 1961.

Among ring **B** substituted steroid ketones, it will again be noted (X *vs.* IX) that loss of ring **A** is much more pronounced in the 5β-series.

No particular generalization appears possible in the 12-keto group, but a possible means of differentiation in C-5 epimeric 11-ketones is offered by the relative intensities of the lower member of the two ketone satellite fragmentation peaks. As can be seen in Figs. 4 and 5, the 5α-11-ketone V exhibits in addition to the ketone peak (1)—13, a second and even stronger companion −15 peak. In the 5β-series (Fig. 5), this relationship is altered in that the second peak is noticeably smaller, a generalization which was also found to hold in the 11-ketopregnane series (Figs. 6 and 7).

Conclusion.—The above study of the mass spectrometric fragmentation patterns of steroidal monoketones shows that many structural conclusions are possible, which can locate or at least narrow down the possible points of attachment of a carbonyl group. Indeed, when mass spectrometry is combined with optical rotatory dispersion measurements,[12] then a firm decision can be made in virtually every instance with a total amount of less than 1 mg. of substance. Our present results have encouraged us to undertake a more extensive investigation of the mass spectrometric behavior of polysubstituted steroid ketones, notably those with additional methyl and carbonyl substituents. Furthermore, bicyclic analogs as well as triterpenoid ketones are also currently under investigation and the results will be reported in future publications.

Experimental

All mass spectra were measured with a Consolidated Electrodynamics Corp. mass spectrometer No. 21-103C using an all-glass heated inlet system capable of reaching a temperature of 350°. In order to avoid possible interaction with the metal in the conventionally used gallium seals, the solid samples were placed in an electrically heated glass finger, which was directly attached to the reservoir through a ground joint with a Teflon seal. In our experience, no appreciable spectral background was caused by the use of such Teflon seals. For all of the above measurements, the sample in the inlet system was heated to 200° (except for 3-hydroxy steroids where a temperature of 250° was employed), while the isatron temperature was maintained at 270°. The ionizing voltage was kept at 70 eV. and the ionizing current at 10 μa.

Acknowledgment.—All of the spec mens used in the present study had been synthesized in our laboratory or had been obtained in connection with our earlier recorded[12] optical rotatory dispersion measurements. We are indebted to Syntex, S. A. (Mexico City), as well as to the following individuals for gifts of samples: D. H. R. Barton (London), S. Bergström (Stockholm), E. J. Corey (Cambridge, Mass.), W. G. Dauben (Berkeley), E. Mosettig (Bethesda), C. W. Shoppee (Sydney) and C. Tamm (Basel).