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Mass Spectrometry in Structural and Stereochemical Problems. XIV.[1] Steroids with One or Two Aromatic Rings[2]

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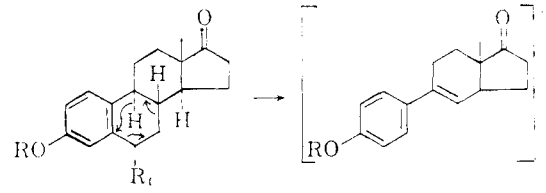
Mass spectra have been measured of a large group of steroidal estrogens and most of the principal fragmentations have been clarified by the use of suitable derivatives labeled with deuterium or other substituents. In a number of instances fragmentation patterns could also be associated with specific stereochemical features and it is likely that mass spectrometry may lend itself to analytical applications in biochemical problems involving estrogens, especially when combined with gas phase chromatography.

Systematic mass spectrometric studies in the steroid series[3] have so far been limited to bile acids[4] and to saturated steroidal mono-ketones.[5] In continuation of our investigations on the relation between mass spectrometric fragmentation patterns and structural (and/or stereochemical) features among polycyclic organic molecules,[6] we have now examined the mass spectrometric behavior of a variety of phenolic steroids (and their methyl ethers) of the estrogen series. The present article is concerned with a description of these results and a rationalization of most of the principal mass spectral fragmentation peaks.

Structural Considerations

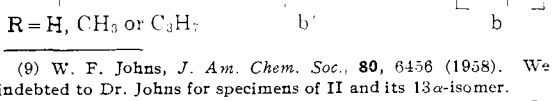
Most of the characteristic mass spectral features of ring A aromatic steroids are found in the spectrum (Fig. 1) of estrone methyl ether (I),[7] which will now be discussed in detail, starting with the high mass range. One of the typical features associated with the presence of the aromatic ring, thus making it particularly attractive in potential biochemical analytical problems, is the intensity of the molecular ion, which usually represents the strongest peak in the spectrum.[8] The M-15 peak is due in part to loss of the angular methyl group and partly to loss of the methyl ether function, since such a peak is also observed in the mass spectra of estrone and 18-norestrone methyl ether (II),[9] each of which lacks one of these substituents.

Of considerable interest is the M-28 peak at *m/e* 256, which represents the loss of carbon atoms 6 and 7 in the form of ethylene. This assignment is verified by the mass spectrum (Fig. 6) of 6β-methylestrone (III),[10] where the M-28 peak is now replaced by a substantial M-42 peak at *m/e* 242 corresponding to the loss of propylene. Further confirmation was provided by catalytic deuteration (10% palladium–charcoal in ethyl acetate) of 6-methyl-6-dehydroestrone[10] to a mixture consisting largely of 6,7-di- and *x*,6,7-tri-deuterio-6β-methylestrone,[11] the former again exhibiting an *m/e* 242 peak, while the *x*,6,7-d_3-6β-methylestrone[11] component showed the peak at *m/e* 243. Mechanistically, direct expulsion of ring B with formation of a four-membered ring B seems less likely on energetic grounds than the following process involving migration of the C-9 hydrogen atom with formation of species *a*



R = CH_3, R_1 = H, or R = H, R_1 = CH_3 *a* (R = H or CH_3)

The next two important peaks in the mass spectrum (Fig. 1) of estrone methyl ether (I) occur at *m/e* 228 and 227. The former corresponds to the loss of ring D (M-56) with generation of ion b and is most easily visualized through the following process (b′)[12] the neutral fragment presumably being cyclopropanone or acrolein



R = H, CH_3 or C_3H_7 b′ b

(1) Paper XIII, C. Djerassi, Y. Nakagawa, H. Budzikiewicz, J. M. Wilson, J. LeMen, J. Poisson and M.-M. Janot, *Tetrahedron Letters*, 653 (1962).

(2) We are indebted to the National Institutes of Health for financial support (grants No. CRTY-5061 and No. A-4257).

(3) Incidental studies of diverse steroid types have also been recorded by (a) R. I. Reed, *J. Chem. Soc.*, 3432 (1958); (b) S. S. Friedland, G. J. Lane, R. T. Longman, K. E. Train and M. J. O Neal, *Anal. Chem.*, **31**, 169 (1959); (c) H. J. M. Fitches, "Symposium on Mass Spectrometry," Oxford, 1961, Pergamon Press, London, in press; (d) L. E. Peterson, *Chemistry & Industry*, 264 (1962).

(4) S. Bergström, R. Ryhage and E. Stenhagen, *Svensk Kem. Tids.*, **73**, 566 (1961), and earlier references cited therein.

(5) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

(6) In particular steroids (ref. 5), triterpenoids (C. Djerassi, H. Budzikiewicz and J. M. Wilson, *Tetrahedron Letters*, 263 (1962)) and alkaloids (ref. 1 and preceding articles).

(7) Estrone (XV) itself exhibited essentially the same mass spectrum except for the obvious 14 mass unit shift. The reason that the methyl ether spectrum (Fig. 1) is used for purposes of illustration is to have available a direct comparison for the mass spectra (Figs. 2–5) of the various stereoisomers of estrone methyl ether (I).

(8) The 17-alkylated tertiary carbinols, such as 17α-ethynylestradiol methyl ether (XXVIII), where the molecular ion is the second most intense peak, the base peak occurring at M-57 (analogous to ion c), are exceptions. The spectrum is reproduced in C. Djerassi, H. Budzikiewicz and J. M. Wilson "Recent Applications of Mass Spectrometry in Steroid Chemistry," *Proceed. Internat. Congress Hormonal Steroids*, Milano, May, 1962 (Academic Press, Inc., New York, N. Y., in press).

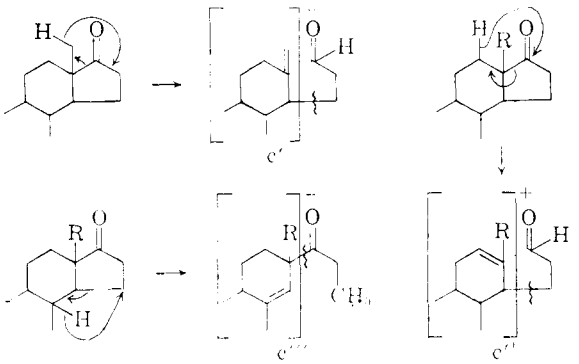
(9) W. F. Johns, *J. Am. Chem. Soc.*, **80**, 6456 (1958). We are indebted to Dr. Johns for specimens of II and its 13α-isomer.

(10) E. Velarde, J. Iriarte, H. J. Ringold and C. Djerassi, *J. Org. Chem.*, **24**, 311 (1959).

(11) The third deuterium is attached to the aromatic ring just as was observed in the preparation of 15-deuterioestrone methyl ether (XII). Insufficient material was available in this instance to remove aromatically bound deuterium by catalytic equilibration with hydrogen.

(12) For the sake of simplicity only, all bond cleavages are written as homolytic fissions.

The adjacent *m/e* 227 peak involves the loss of ring D with migration of one hydrogen atom (M-57), the three most likely ions being c′, c″ and c‴ arising from rupture of the allylically activated bond indicated by the wavy line



That these two peaks involve only the loss of carbon atoms 15, 16 and 17 is demonstrated by the occurrence of *m/e* 228 and 227 peaks in the mass spectra of as diverse a group of substances as 16,16-d_2-estrone methyl ether (V), 16,16-difluoroestrone methyl ether (VIII)[13] and 15-*d*-estrone methyl ether (XII), as well as of the *m/e* 227 peak in estradiol methyl ether (IX, Fig. 15) and its 16,16-d_2 (VI) and 16,16,17-d_3 (VII) analogs. Some information on the hydrogen-rearrangement[14a] step associated with the M-57 peak can be gained from a comparison of the mass spectra of 18-norestrone methyl ether (II),[9] estrone methyl ether (I, Fig. 1) and 18-nor-13-propylestrone (IV,[14b] Fig. 7). In general, the fragmentation patterns of estrone methyl ether (I) and its 18-nor analog II are very similar, except that the relative intensity of the M-56 and M-57 peaks is reversed in II as compared to I (Fig. 1). Nevertheless, the existence of a M-57 peak in the 18-norestrone methyl ether (II) spectrum demonstrates that migration of one of the hydrogen atoms of the angular methyl group cannot be the only path (c′) to the M-57 peak and that the hydrogen atoms at positions 12 (c″) or 8 (c‴) must also be considered. A definite decision among these possibilities can, of course, be reached only by deuterium labeling in these positions. In the 18-nor-13-propylestrone (IV)[14b] spectrum (Fig. 7), the M-56 (b) and M-57 (c) peaks occur at *m/e* 242 and 241 and are accompanied by peaks at *m/e* 213 and 212, which apparently represent the further loss of ethyl (29 mass units) from b and c, although no metastable peaks for this transition could be detected because of overlap with other peaks. This additional loss of an ethyl fragment from the angular propyl group is most consistent with the formulation c″ for the M-57 ion, where allylic activation can be invoked. It will be noted from a comparison of Figs. 1 and 7 that the relative intensities of the M-56 and M-57 peaks are much

(13) C. H. Robinson, N. F. Bruce, E. P. Oliveto, S. Tolksdorf, M. Steinberg and P. L. Perlman, *J. Am. Chem. Soc.*, **82**, 5256 (1960).

(14) (a) For general survey of hydrogen rearrangements in mass spectrometry see F. W. McLafferty in "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1962, Vol. 2, pp. 129–149. (b) L. Velluz, G. Nominé, R. Bucourt, A. Pierdet and Ph. Dufay, *Tetrahedron Letters*, 127 (1961). We are indebted to these authors for a specimen of IV.

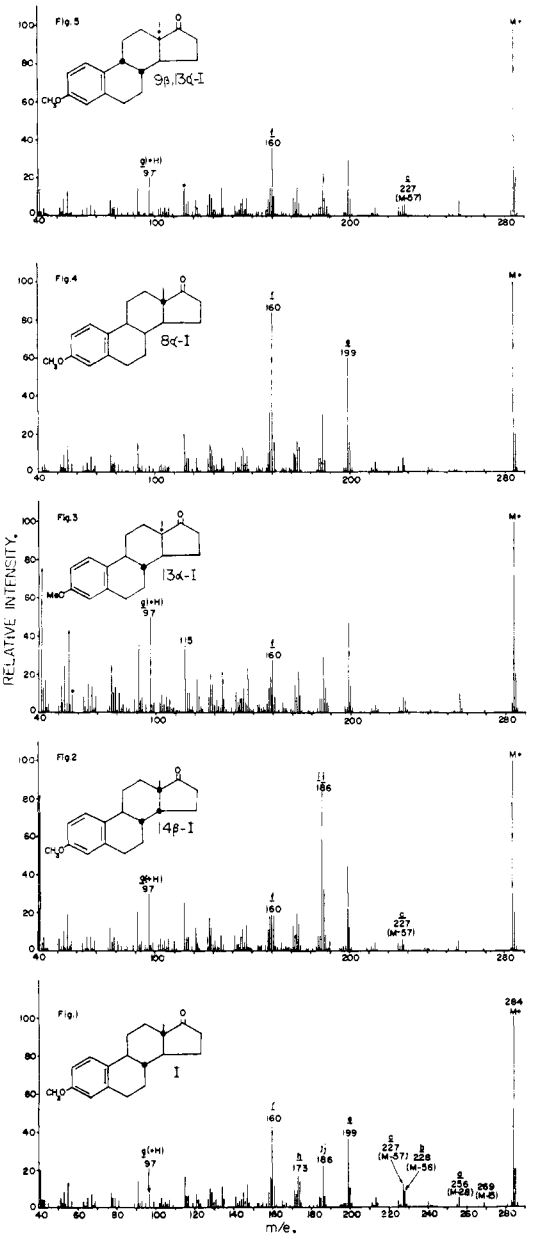


Fig. 1.—Mass spectrum of estrone methyl ether (I).

Fig. 2.—Mass spectrum of 14-iso(β)-estrone methyl ether (14β-I).

Fig. 3.—Mass spectrum of 13-iso(α)-estrone methyl ether (13α-I).

Fig. 4.—Mass spectrum of 8-iso(α)-estrone methyl ether (8α-I).

Fig. 5.—Mass spectrum of 9-iso(β)-13-iso(α)-estrone methyl ether (9β, 13α-I).

greater in IV than in I. Apparently, this type of fission is favored with bulkier angular substituents and, in accordance with this observation, it is observed that the M-56 and M-57 peaks are less intense in 18-norestrone methyl ether (II) as compared to its higher homolog I.

The substantial M-42 peak observed at *m/e* 256 in the mass spectrum (Fig. 7) of 18-nor-13-propylestrone (IV), but not with estrone methyl ether (Fig. 1) or 18-norestrone methyl ether (II), can

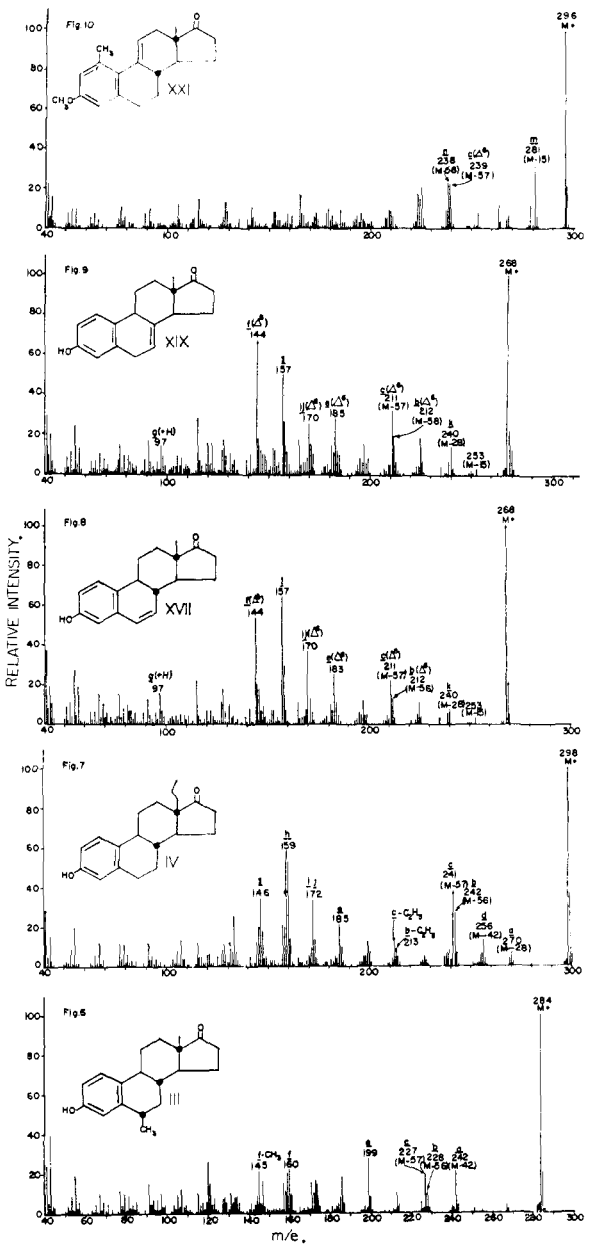
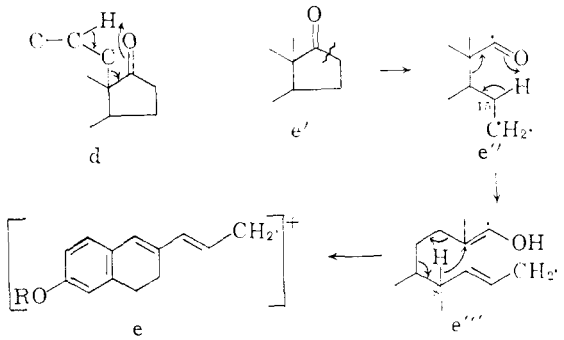


Fig. 6.—Mass spectrum of 6β-methylestrone (III).
Fig. 7.—Mass spectrum of 18-nor-13-propylestrone (IV).
Fig. 8.—Mass spectrum of 6-dehydroestrone (XVII).
Fig. 9.—Mass spectrum of equilin (XIX).
Fig. 10.—Mass spectrum of 9-dehydro-1-methyl estrone methyl ether

almost certainly be attributed to the loss of propylene *via* the six-membered cyclic transition state d.

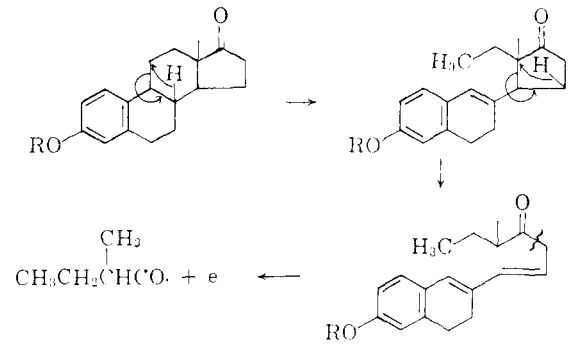
One of the most characteristic peaks in the mass spectrum (Fig. 1) of estrone methyl ether (I) is the one appearing at *m/e* 199. It is encountered in all of its stereoisomers (*e.g.*, Figs. 2–5), as well as in 18-norestrone methyl ether (II) and is shifted to *m/e* 185 in the spectrum (Fig. 7) of 18-nor-13-propylestrone (IV) because of the absence of the methyl ether function. On the other hand, it is moved to *m/e* 201 in 16,16-d_2-estrone methyl ether (V) and to *m/e* 235 in the 16,16-difluoro analog VIII. It follows, therefore, that the angular substituent is not present in this ion, while C-16 is still attached. In view of the fact that all of the principal peaks above *m/e* 160 still retain rings A and B intact, this *m/e* 199 peak almost certainly involves these two rings as well as three additional carbon atoms and, as one of them is C-16, the other two carbon atoms must be C-15 and C-14. In order to arrive at the correct mass number (199), additional loss of two hydrogen atoms must be invoked, and we propose the following path[12] involving initial cleavage[15] of the 16–17 bond (e′), followed by transfer of the C-15 hydrogen atom to oxygen (e″) and finally migration of the C-8 hydrogen atom (e‴), the net result being the formation of the stable, conjugated ion e (*m/e* 199 in Fig. 1; *m/e* 185 in Fig. 7).[16]



Conclusive proof for the correctness of the first hydrogen transfer step (e″) of this mechanism could be provided by labeling position 15 with deuterium. This was effected by catalytic deuteration of 15-dehydroestrone methyl ether (X),[17] which provided tetradeuterioestrone methyl ether, accompanied by about equal amounts of tri- and pentadeuterioestrone methyl ether. Two of the deuterium atoms were located at positions 15 and 16 (XI), while the remainder was attached to the aromatic rings (see discussion below of peaks f, h and i). Removal of the C-16 deuterium atom was effected by equilibration with sodium in methanol–water, while the aromatic deuterium atoms were almost completely

(15) Cleavage of one of the bonds adjacent to the carbonyl group is a common occurrence in mass spectra of ketones (*e.g.*, A. G. Sharkey, J. L. Schultz and R. A. Friedel, *Anal. Chem.*, **28**, 934 (1956)) and is favored because of stabilization of the resulting carbonium ion through participation of the electrons on oxygen ($RC{\equiv}O^+$).

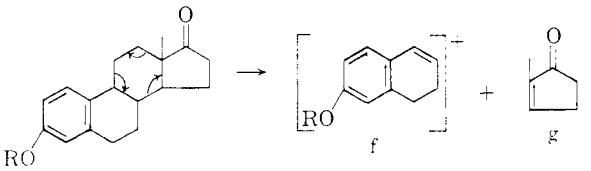
(16) The following alternate mechanism, involving rearrangement of the same hydrogen atoms, has been proposed recently by Dr. F. W. McLafferty (Dow Chemical Co.).



(17) W. S. Johnson and W. F. Johns, *J. Am. Chem. Soc.*, **79**, 2005 (1957).

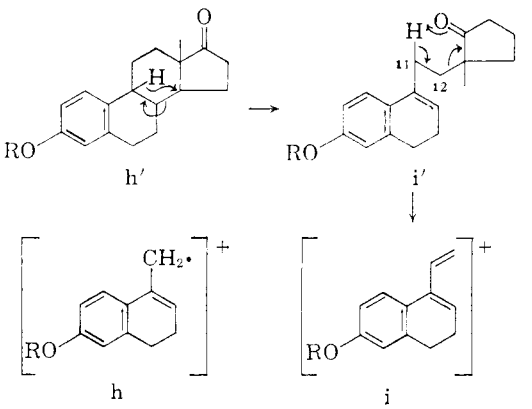
removed by two catalytic exchanges with hydrogen. The resulting 15-d-estrone methyl ether (XII) spectrum exhibited a molecular ion peak at *m/e* 285 (estrone methyl ether (I) = 284) and contained M-57 and M-58 peaks (rather than M-56 and M-57 as observed in Fig. 1 for estrone methyl ether). Most importantly, the *m/e* 199 peak (e) of estrone methyl ether (Fig. 1) was now distributed[18] between *m/e* 199 and *m/e* 200, thus proving that migration of the C-15 hydrogen is involved in the genesis of the *m/e* 199 ion. Experimental verification for the second postulated hydrogen transfer (e''') from C-8 would require the synthesis of 8-deuterioestrone methyl ether, which was not attempted in our laboratory.

The remaining three characteristic peaks in the higher mass range of the estrone methyl ether (I) spectrum (Fig. 1) occur at *m/e* 186, *m/e* 173 (accompanied by *m/e* 174) and especially at *m/e* 160. The latter is most easily rationalized through the following collapse of ring C with formation of ethylene, 1-methylcyclopent-1-en-2-one (g) and the dihydro-β-naphthol methyl ether ion f (*m/e* 160). This peak remains unchanged in ring D substituted relatives such as II, V, VIII, XII, but is shifted by 14 mass units in the mass spectrum of 1-methylestrone methyl ether (XIII),[19] the spectrum of which is otherwise virtually identical (except for the appropriate 14 mass unit shifts) with that (Fig. 1) of its lower homolog I. In the catalytic deuteration product of 15-dehydroestrone methyl ether (X), where, prior to catalytic hydrogen exchange, one, two and three of the aromatic hydrogens of XI and XII had been exchanged for deuterium, the *m/e* 160 peak was distributed between *m/e* 161, 162 and 163 in exact proportion to the corresponding molecular ion peaks.

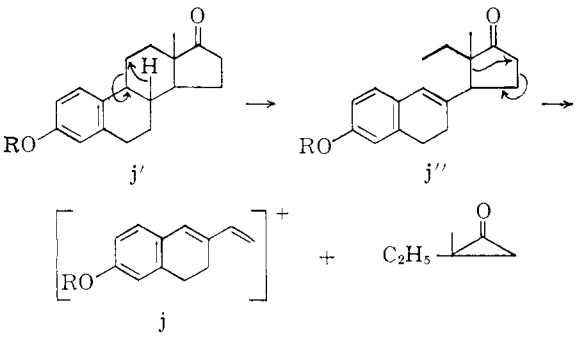


The *m/e* 173 and 174 peaks (Fig. 1) obviously represent the *m/e* 160 ion together with one additional carbon atom, which can only be C-11 or C-14. The latter is less likely since its retention would have to involve the cleavage of two bonds attached to one carbon atom and we propose a fragmentation path[12] involving rearrangement of the C-9 hydrogen (h') followed by fission of the activated 11–12 bond in *i'* with formation of ion h (*m/e* 173). An analogous mechanism (rupture of 8–14 and 11–12 bonds) without hydrogen transfer would account for the *m/e* 174 peak.

The origin of the *m/e* 186 peak (Fig. 1) is somewhat more complicated and an inspection of the 15-deuterioestrone methyl ether (XII) spectrum indicates that it is made up of two different ions since, in the spectrum of XII, the original *m/e* 186 peak of I is almost exactly divided between *m/e* 186 and 187. Since the *m/e* 186 peak certainly encompasses rings A and B (as confirmed by the spectra of III (Fig. 6) and XIII) as well as two additional carbon atoms, the 15-deuterioestrone methyl ether spectrum indicates that part of the ion consists of rings A and B together with C-11 and C-12, while the other portion is made up of rings A and B together with C-14 and C-15. The generation of the former is most readily rationalized[12] through a transfer of the C-11 hydrogen in the six-membered cyclic intermediate i' to yield the conjugated diene i (*m/e* 186).



A plausible mechanism for the production of an *m/e* 186 ion containing C-14 and C-15 is proposed below, in which initial cleavage of the 9–11 bond and migration of the C-8 hydrogen (j')[20] would yield a conjugated species j''. Further fission of the allylically activated 13–14 bond (see j'') eventually provides the conjugated diene j (*m/e* 186) together with a neutral cyclopropanone. It must be emphasized that the above mechanisms are only hypothetical and that confirmation of the postulated hydrogen transfers (h', i' and j') would have to be proved by synthesis of the rather inaccessible 8-, 9- and 11-monodeuterioestrone methyl ethers.



One additional peak in the spectrum (Fig. 1) of estrone methyl ether (I) merits attention. This is the rather small one at *m/e* 97, which becomes more important in some of the stereoisomers to be discussed below (see Fig. 3). It definitely represents ring D (including the angular substituent) together with one additional hydrogen atom and is found at *m/e* 83 in the 18-nor analog II, at *m/e* 98 in the 15-deuterio derivative XII and at *m/e* 99 in 16,16-

(18) Complete movement to *m/e* 200 would, of course, be observed only with a 15,15-d_2 derivative.

(19) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *J. Am. Chem. Soc.*, **78**, 2477 (1956).

(20) The close similarity to the mechanism in ref. 16a proposed by Dr. F. W. McLafferty should be noted.

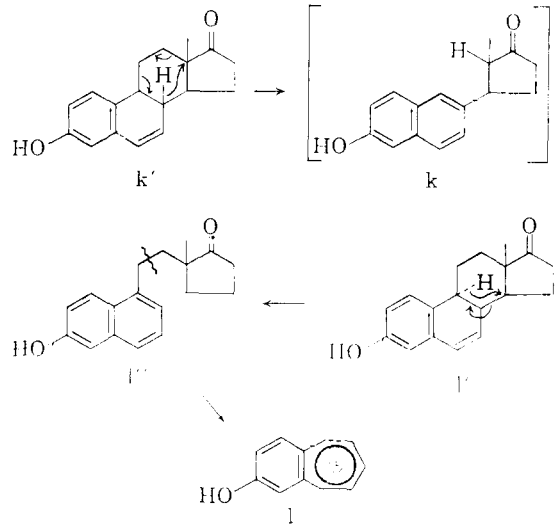
d_2-estrone methyl ether (V). For the sake of simplicity, it is identified on the mass spectra as peak g+H, but several alternate plausible formulations can be suggested for this ion (*e.g.* fission of 12–13 bond in species i′).

In connection with the above-detailed discussion of the mass spectrum of estrone methyl ether (I), reference was made to the mass spectra of a number of other relatives (II–XIII). Two additional ring A aromatic 17-ketones may be mentioned before proceeding to more highly unsaturated estrogens. In connection with the mass spectrum of 1-methylestrone methyl ether (XIII), which—as was mentioned above—closely resembled that (Fig. 1) of estrone methyl ether (I) (except for the necessary 14 mass unit shift), it was of interest to examine the isomeric hetero-4-methylestrone methyl ether (XIV).[21] In point of fact, the mass spectra of XIII and XIV closely resembled each other except for an over-all reduced intensity of all peaks in the spectrum of XIV and a reversal in intensity of the *m/e* 187 (corresponding to h) and *m/e* 200 (corresponding to i, j) peaks, the latter being more intense in XIII.

Another substituted estrone derivative that was investigated is 16β-methylestrone (XVI).[22] Its mass spectrum was very similar to that[7] of estrone (XV), the principal difference being that the *m/e* 185 peak (e) of estrone (XV) was shifted to *m/e* 199 in 16β-methylestrone, since this peak retains the C-16 substituent. The M-57 and M-56 peaks of estrone (XV) corresponded to M-71 and M-70 in XVI because of the 14 mass unit difference in their respective molecular ions.

Attention was directed next to a study of the mass spectra of some representative estrogens with additional unsaturation in rings B or C. Of particular pertinence is 6-dehydroestrone (XVII),[23] the mass spectrum of which is reproduced in Fig. 8. The spectrum is in general quite similar to that[7] of estrone (XV), except for the two mass unit shifts associated with the additional double bond. The peaks at *m/e* 212, 211, 183, 170 and 144 thus simply represent the Δ^6-analogs of b, c, e, f, i and j and require no further comment. The M-28 peak at *m/e* 240 cannot be associated with the loss of ethylene from ring B, as demonstrated above (see a) for ring B saturated estrogens, and is most readily rationalized by an analogous process (k′) involving transfer of the C-8 hydrogen with expulsion of ethylene and genesis of the naphthalenic ion k.

The second strongest peak in the spectrum (Fig. 8) of 6-dehydroestrone (XVII) is the one at *m/e* 157, which corresponds to the much less intense h peak (*m/e* 173) in the spectrum (Fig. 1) of estrone methyl ether. The explanation for the favored production of this *m/e* 157 ion is straightforward and again utilizes (see h′) initial migration of the C-9 hydrogen atom followed by cleavage at the benzylic C-11 position (1″), thus yielding the favored[24] hydroxybenztropylium ion (1).



The other ring B-unsaturated estrogen that was available to us was equilin (XIX)[25] and its mass spectrum (Fig. 9) differed from that (Fig. 8) of 6-dehydroestrone (XVII) only in the intensity relationships of the principal peaks, the most striking feature being the reversal in intensity of the *m/e* 157 (1) and 144 (f) peaks, apparently reflecting the greater difficulty in generating the hydroxybenztropylium ion (1). The formation of this latter ion and indeed of most of the other identified ion peaks in Fig. 9 presumably involves migration of the 7–8 double bond, which is not surprising in view of the great mobility[25] of this double bond in equilin (XIX).

The importance of the fragmentation process (1′ → 1″) leading to the hydroxybenztropylium ion (1) is reflected in the mass spectrum of 1,2-dimethyl-6-dehydroestrone (XVIII).[26] Aside from the 28 mass unit shift, due to the two additional methyl groups, its mass spectrum is qualitatively very similar to that (Fig. 8) of 6-dehydroestrone (XVII). The most important difference lies in the complete reversal in intensity of the *m/e* 172 and *m/e* 185 peaks in XVIII, which correspond to the *m/e* 144 and *m/e* 157 peaks in the spectrum (Fig. 8) of XVII. In this case steric factors due to the presence of the 1-methyl group may favor initial cleavage of the 9–11 bond. Similarly, the ratio of the intensities of peaks f and h is much greater in the spectrum of 1-methylestrone methyl ether (XIII) than in that of estrone methyl ether I.

Another type of unsaturated estrone derivative is exemplified by 9-dehydroestrone methyl ether

(21) C. Djerassi and C. R. Scholz, *J. Org. Chem.*, **13**, 697 (1948), where this substance is referred to as "1-methylestrone methyl ether." For structure revision see C. Djerassi, G. Rosenkranz, J. Romo, J. Pataki and St. Kaufmann, *J. Am. Chem. Soc.*, **72**, 4540 (1950); A. S. Dreiding and W. J. Pummer, *ibid.*, **75**, 3162 (1953); R. B. Woodward, H. H. Inhoffen, H. O. Larson and K. H. Menzel, *Ber.*, **86**, 594 (1953).

(22) F. A. Kincl and M. Garcia, *ibid.*, **92**, 595 (1959).

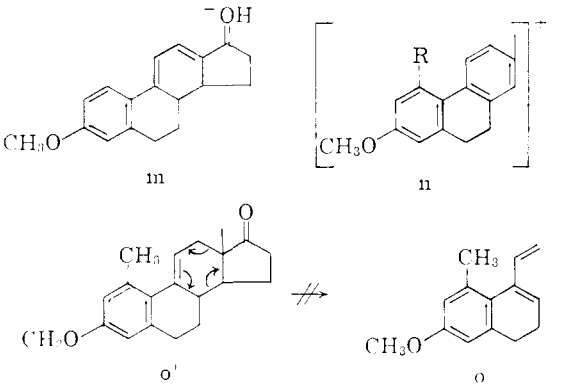
(23) W. H. Pearlman and O. Wintersteiner, *J. Biol. Chem.*, **132**, 605 (1940); S. Kaufmann, J. Pataki, G. Rosenkranz, J. Romo and C. Djerassi, *J. Am. Chem. Soc.*, **72**, 4531 (1950).

(24) P. N. Rylander, S. Meyerson and H. M. Grubb (*J. Am. Chem. Soc.*, **79**, 842 (1957)) have shown that electron impact upon toluene results in loss of one hydrogen and formation of the tropylium rather than benzyl ion. See also J. M. S. Tait, T. W. Shannon and A. G. Harrison, *ibid.*, **84**, 4 (1962).

(25) See J. A. Zderic, A. Bowers, H. Carpio and C. Djerassi, *ibid.*, **80**, 2596 (1958).

(26) J. Iriarte and H. J. Ringold, *Tetrahedron*, **3**, 28 (1958).

(XX)[27] and 1-methyl-9-dehydroestrone methyl ether (XXI).[28] Three aspects of their mass spectra (see Fig. 10 of XXI) deserve special comment. First, the most intense peak—aside from the molecular ion—is the one corresponding to M-15. This can be used as a diagnostic tool for Δ^9-17-keto estrogens[29] and its appearance is evidently associated with the loss of the angular methyl group to produce a fully conjugated ion such as *m*. Second, the other unique feature (see Fig. 10) is the presence of a strong M-57 peak (Δ^9-analog of c) accompanied by an equally intense M-58 peak,[29] which is absent in all of the other estrone mass spectra (Figs. 1–9). This additional loss of hydrogen (from a Δ^9-analog of c) is understandable in this group since it leads to aromatization of ring C with production of an ion (n) possessing an aromatic ring C. Third, while the absence of a peak corresponding to the *m/e* 160 ion (f) of the estrone methyl ether (I) spectrum (Fig. 1) is understandable, the absence in Fig. 10 of a peak at *m/e* 200 (ion o) expected from a reverse Diels–Alder fragmentation (o′)—so pronounced among triterpenoid olefins[6]—is remarkable.



The mass spectrum of a steroid with two aromatic rings, equilenin methyl ether (XXIII), is reproduced in Fig. 11. Noteworthy is the more intense nature of the M-56 and M-57 peaks when contrasted with the spectrum (Fig. 1) of estrone methyl ether (I), which is reasonable since the resulting double bond (see b and c) would be in conjugation with the naphthalene ring. The *m/e* 165 peak does not contain the angular substituent, since this peak is also observed in the mass spectra (Figs. 13 and 14) of the angular ethyl-substituted homologs XXV and XXVII. It may involve the loss[30] of methyl and carbon monoxide from a 2-methoxyphenanthrene ion. At the present time we can offer no plausible assignments for the *m/e* 211 and *m/e* 237 (M-43) peaks. The latter represents the loss of either

(27) B. J. Magerlein and J. A. Hogg, *J. Am. Chem. Soc.*, **80**, 2220 (1958).

(28) E. J. Bailey, J. Elks, J. F. Oughton and L. Stephenson, *J. Chem. Soc.*, 4535 (1961). We are indebted to Dr. Elks for a sample of XXI.

(29) The corresponding 17-alcohol XXII (prepared by Dr. P. Crabbé by methylation of 9-dehydroestradiol: see J. S. Mills, J. Barrera, E. Olivares and H. Garcia, *J. Am. Chem. Soc.*, **82**, 5882 (1960)) did not show this peak.

(30) Such a fragmentation has been observed in aromatic methyl ethers such as rotenoids and isoflavones by J. M. Wilson, Ph. D. Thesis, University of Glasgow, 1961.

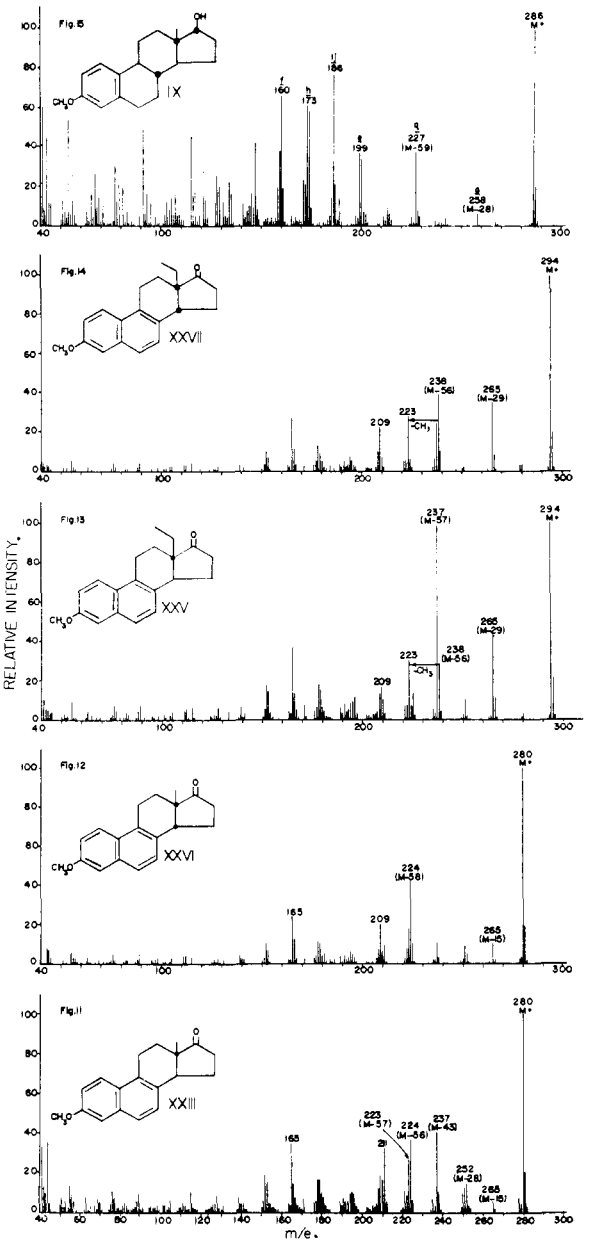


Fig. 11.—Mass spectrum of equilenin methyl ether (XXIII).

Fig. 12.—Mass spectrum of 14-iso(β)-equilenin methyl ether (XXVI).

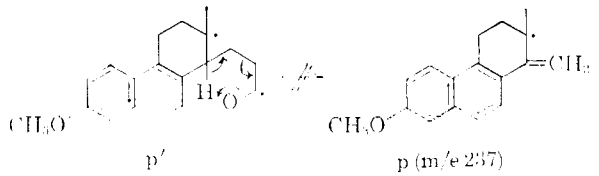
Fig. 13.—Mass spectrum of 18-nor-13-ethylequilenin methyl ether (XXV).

Fig. 14.—Mass spectrum of 18-nor-13-ethyl-14-iso(β)-equilenin methyl ether (XXVII).

Fig. 15.—Mass spectrum of estradiol methyl ether (IX).

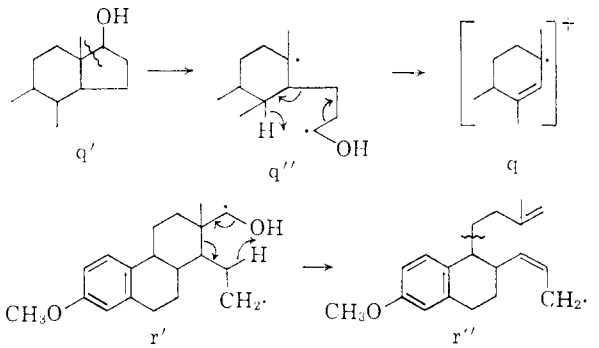
C_2H_3O or of C_3H_7, but since the *m/e* 237 ion must still encompass C-16 (peak moved to *m/e* 239 in 16,16-d_2-equilenin methyl ether (XXIV)), the most obvious mechanism[12] involving initial rupture (see b′) of the 13–17 linkage followed by transfer of the C-14 hydrogen to oxygen (p′ → p) is excluded.

Until now, the discussion has been limited largely to estrogenic steroids with a 17-keto group. A number of 17β-hydroxy estrogens have also been examined, including estradiol methyl ether (IX), its 16,16-d_2 (VI) and 16,16,17-d_3 (VII) analogs, 9-



p′ p (m/e 237)

dehydroestradiol methyl ether (XXII),[29] 17α-ethynylestradiol 3-methyl ether (XXVIII) and 17α-methylestradiol (XXIX). As shown in Fig. 15, the mass spectrum of estradiol methyl ether (IX) shows in general the same principal fragmentation peaks as observed in Fig. 1 with estrone methyl ether (I), the main difference being that the intensity of most peaks in the estradiol spectrum is more pronounced. The rather substantial M-59 peak at *m/e* 227 is analogous to the M-57 peak (c) in the estrone methyl ether (I) spectrum and may proceed[12] through the usual (see b′) 13–17 cleavage (q′) *via* the cyclic transition state q″ to the ion q (see also c′, c″ and c‴). Similarly, the characteristic *m/e* 199 peak (moved to *m/e* 201 in VI and VII) can again be ascribed to ion e, its formation being explained readily through intermediates[12] such as r′ and r″. In contrast to the situation obtaining in the 17-ketone I, in the estradiol series (IX), the last step (r″ *vs.* e‴) can evidently proceed also without hydrogen transfer thus producing an equally intense *m/e* 200 ion (moved to *m/e* 202 in VI and VII).



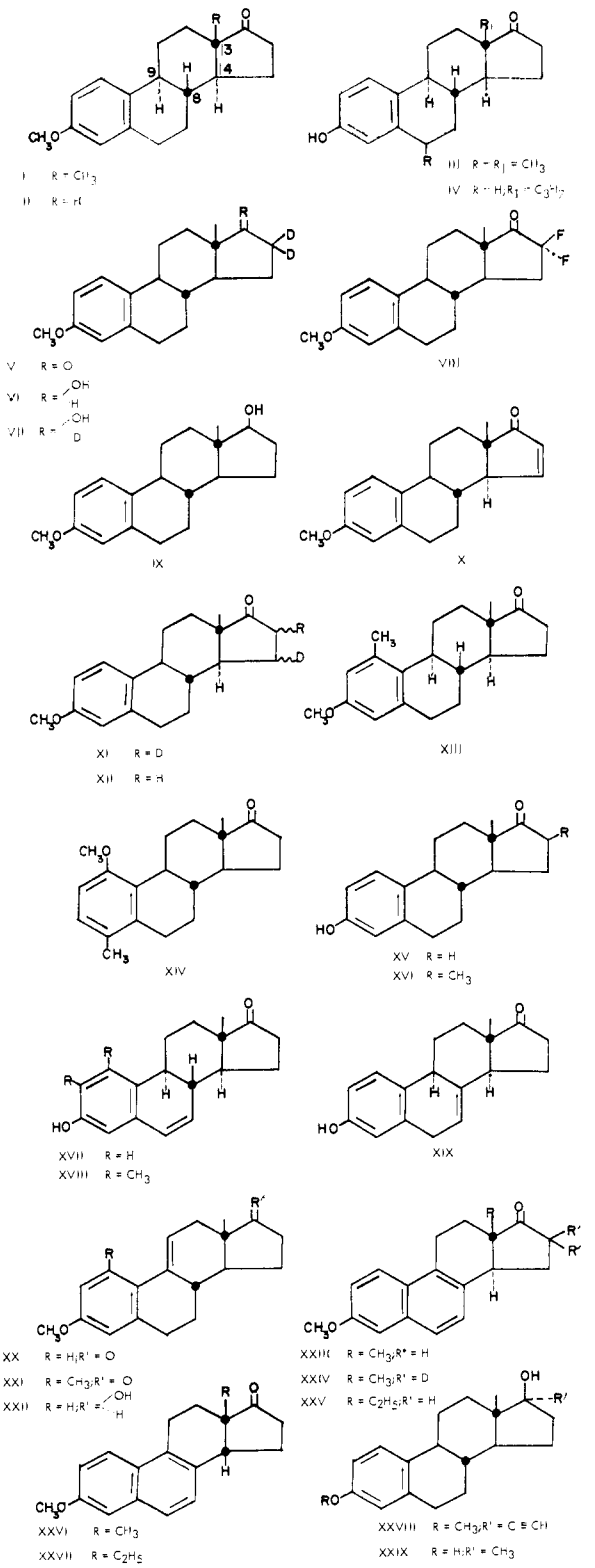
q′ q″ q

r′ r″

As has already been noted elsewhere,[8] even tertiary carbinols such as 17α-ethynylestradiol methyl ether (XXVIII) or 17α-methylestradiol (XXIX) exhibit very strong molecular ion peaks, dehydration (M-18 peak) being of relatively minor importance.

Stereochemical Considerations

It has already been noted in the past that certain stereochemical features may be related to characteristic mass spectrometric fragmentation processes, although it is usually desirable to measure mass spectra of isomers consecutively under identical conditions. Thus, Biemann and Seibl[31] were able to utilize intensity differences in the molecular ions of pairs of epimeric cyclic alcohols or acetates for stereochemical purposes (axial *vs.* equatorial), while recent investigations with steroid ketones,[5] bicyclic systems[32,33] and ring-heterocyclic yohimbine-type alkaloids[34] indicated that the nature of the ring fusion (*cis vs. trans*) can often be elucidated from an inspection of the mass spectral fragmentation patterns. In view of the availability of



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

(32) R. I. Reed, "Ion Production by Electron Impact," Academic Press, Inc., London, 1962, Chapter IX.

(33) E. Lund, H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Am. Chem. Soc.*, submitted for publication.

(34) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *ibid.*, **84**, 2161 (1962).

several isomers, the present group of estrogens appeared to represent an admirable test case to examine the applicability of mass spectrometry to stereochemical problems and the remaining discussion is concerned with this subject.

A comparison between the mass spectra of equilenin methyl ether (XXIII) (Fig. 11) and its 14β-isomer XXVI[35] (Fig. 12) shows notable intensity differences, especially in the 200–240 mass range. Even more striking is the intensity difference (see especially *m/e* 237) in the spectra of Bachmann's[36] isomeric 18-nor-13-ethylequilenin methyl ethers (XXV, XXVII). In Fig. 14 is reproduced the spectrum of the lower melting (m.p. 125.5°) "α"-isomer which exhibits an M-56 peak (*m/e* 238) of intensity comparable to that of the M-56 (*m/e* 224) peaks in the spectra (Figs. 11, 12) of the isomeric equilenin methyl ethers (XXIII, XXVI). In the spectrum (Fig. 13) of the higher melting (m.p. 173°) 18-nor-13-ethylequilenin methyl ether, the intensity relation of the M-56 and M-57 peaks is completely reversed and the one at *m/e* 237 (M-57)[37] is now as intense as the molecular ion. The substantial M-29 peaks (*m/e* 265) in Figs. 13 and 14 evidently involve the loss of the angular ethyl function, while the *m/e* 223 peaks most likely reflect the loss of methyl from the angular substituent in the *m/e* 238 species, although no metastable peak for this transition could be detected (because of strong *m/e* 208 and 209 peaks in the expected region).

On the basis of biological activity, Bachmann and Holmes[36] assigned the "normal" 14α-configuration (XXV) to the higher melting "β"-isomer (Fig. 13) and hence the "iso"-14β-orientation (XXVII) to the lower melting "α"-isomer (Fig. 14). It will be noted that the mass spectra are in concordance[37] with this conclusion since one of the principal differences in the spectra of equilenin methyl (Fig. 11) and 14β-equilenin methyl ether (Fig. 12) is the relatively strong *m/e* 209 peak in the latter, as compared to the *m/e* 211 peak in Fig. 11. A similar feature is noted in the spectrum (Fig. 14) of Bachmann's[36] lower melting 18-nor-13-ethyl-14β-equilenin methyl ether (XXVII). Furthermore, when the intensity of the molecular ion is expressed as percentage of the total ionization of the molecule (covering the range *m/e* 41 → M$^+$), then it is found that for the C/D *trans*-ketones equilenin methyl ether (XXIII) and its higher homolog XXV values of 9.9% and 11.9% are observed, while the *cis* isomers XXVI and XXVII yield values of 18.1% and 17.5%. These results are in agreement with the lower stability of the *trans* fused hydrindanone system.

The availability[38] in pure form of seven of the eight possible stereoisomers of estrone methyl ether made possible an examination of the effect of stereochemical alterations at positions 8, 9, 13 and 14 upon the mass spectral fragmentation pattern (Fig. 1) of estrone methyl ether (I), all spectra being run consecutively under identical conditions. The mass spectra of four estrone methyl ether isomers are reproduced in Figs. 2–5 and only those peaks are identified on the spectra which are notable for purposes of differentiation.

Inversion of the C-14 orientation yields 14-isoestrone methyl ether (14β-I), the mass spectrum of which is strikingly different from that (Fig. 1) of estrone methyl ether (I) in the greatly increased intensity of the *m/e* 186 (i,j) and *m/e* 97 (g + H) peaks at the expense of the *m/e* 160 (f) peak, which is substantially reduced. The only spectrum closely resembling that (Fig. 2) of 14β-I is that of 8-iso-13-isoestrone methyl ether (8α,13α-I), which is not reproduced since its principal difference is a doubly intense *m/e* 160 ion.

The lumi-estrone methyl ether (13α-I) spectrum (Fig. 3) differs principally from that (Fig. 1) of estrone methyl ether (I) in the reduced intensity of the *m/e* 160 (f) peak and the greatly increased *m/e* 97 peak, the 13α,14α-*cis* C/D juncture apparently favoring particularly fragmentation with detachment of the D ring with the angular methyl group. Precisely the same situation was observed in a comparison of the mass spectra of 18-norestrone methyl ether (II) and its 13α-isomer[9] where the intensity of the corresponding peak at *m/e* 83 (g without methyl group) is quintupled in the spectrum of the C/D *cis* isomer (13α-II).

8-Isoestrone methyl ether (8α-I) can be distinguished readily from estrone methyl ether (I) as well as the other isomers because its mass spectrum (Fig. 4) exhibits the most intense *m/e* 160 (f) ion observed in any of the spectra, coupled also with a strong *m/e* 199 (e) peak.

The mass spectra of 9-isoestrone methyl ether (9β-I, spectrum not reproduced) and of 9-iso-13-isoestrone methyl ether (9β,13α-I, Fig. 5) resemble each other closely and the relatively minor intensity differences (Fig. 1 *vs.* Fig. 5) between their spectra and that of estrone methyl ether (I) are rather striking, considering the substantial conformational differences among these three isomers.

In conclusion it can be stated that, while a great deal of additional work is required before firm predictions can be made from a mass spectrum about the stereochemical nature of ring junctures of the types of polycyclic structures under investigation, there is no doubt that in a number of instances mass spectrometry offers a sensitive and very convenient criterion for differentiating between closely related stereoisomers.

Experimental[39]

16,16-Dideuterio Estrogens.—A mixture of 50 mg. of estrone methyl ether (I) or equilenin methyl ether (XXIII) was heated under reflux for 6 hr. with 5 cc. of deuteriomethanol, 100 mg. of sodium and 0.32 cc. of heavy water.

(35) W. E. Bachmann, W. Cole and A. L. Wilds, *ibid.*, **62**, 824 (1940).

(36) W. E. Bachmann and D. W. Holmes, *ibid.*, **63**, 595 (1941). We are indebted to Prof. R. E. Ireland of the University of Michigan for these two specimens from the collection of the late Prof. W. E. Bachmann.

(37) If the *m/e* 237 ion in the equilenin methyl ether spectrum (Fig. 11) does not contain the angular substituent, then the *m/e* 237 peak in Fig. 13 consists of that species as well as the M-57 ion. This would be consistent with the stereochemical assignments of Bachmann and Holmes (ref. 36) since the *m/e* 237 ion is much more intense in Fig. 11 than in Fig. 12.

(38) W. S. Johnson, I. A. David, H. C. Dehm, R. J. Highet, E. W. Warnhoff, W. D. Wood and E. T. Jones, *J. Am. Chem. Soc.*, **80**, 661 (1958).

(39) All of the mass spectra were measured in precisely the same fashion as recorded in an earlier paper (ref. 5), always utilizing an inlet temperature of 200°.

In the case of 16,16-d_2-estrone methyl ether (V), the material crystallized directly upon cooling and was recrystallized once to afford the desired material, m.p. 167–169°, which contained 11% of monodeuterio ketone as contaminant as determined by mass spectrometric examination of the molecular ion peaks. In the equilenin methyl ether series, the 16,16-d_2 analog XXIV was isolated by methylene chloride extraction and filtration in benzene solution through a short column of neutral alumina (activity II). Recrystallization from acetone provided the dideuterio derivative XXIV, m.p. 184° dec., containing 13% of the monodeuterio contaminant.

16,16-d_2-Estradiol methyl ether (VI) was prepared by lithium aluminum hydride reduction of V in ether solution (1 hr. steam-bath), while the 16,16,17-d_3 derivative VII was synthesized in an analogous fashion employing lithium aluminum deuteride.

15-Deuterio-estrone Methyl Ether (XII).—A solution of 35 mg. of 15-dehydroestrone methyl ether (X)[17] in 4 cc. of purified ethyl acetate was stirred with 50 mg. of 10% palladized charcoal catalyst in an atmosphere of deuterium[40] for 45 min. Filtration followed by evaporation of the solvent provided 27 mg. of crystalline material (m.p. 160–167°) which consisted of the following mixture as determined from the mass spectral molecular ion peaks (corrected for the natural abundance isotope peaks): 3% d-, 9% d_2-, 27% d_3-, 40% d_4-, 19% d_5- and 2% d_6-estrone methyl ether. The excess deuterium beyond d_2 (XI) is located in the aromatic ring as is demonstrated below.

The above material was equilibrated at C-16 by standing overnight with 10 cc. of methanol, 50 mg. of sodium and 0.8 cc. of water followed by heating under reflux for 45 min. The product was isolated by dilution with water and filtration and consisted of 6% d-, 26% d_2-, 46% d_3-, 21% d_4- and 1% d_5-estrone methyl ether, all excess deuterium beyond one (XII) being located in the aromatic ring. This was removed by stirring the material for 1 hr. in ethyl acetate solution with hydrogen in the presence of 50 mg. of 10% palladized charcoal catalyst, whereupon mass spectral analysis indicated the presence of 10% estrone methyl ether (I), 58% of 15-d-estrone methyl ether (XII), 27% of d_2- and 5% of d_3-derivative. The reaction was repeated once more whereupon the product (m.p. 160–165°) consisted of 76% of the desired 15-d-estrone methyl ether (XII), contaminated by 13% of non-deuterated material (I) and 11% of d_2-estrone methyl ether.

Catalytic Deuteration of 6-Methyl-6-dehydroestrone.[10]—A sample (2.3 mg.) of 6-methyl-6-dehydroestrone was shaken in a deuterium atmosphere in a micro-hydrogenation apparatus in 5 cc. of ethyl acetate in the presence of 10 mg. of 10% palladized charcoal catalyst. The uptake of deuterium (0.24 cc.) ceased within 2 min. and the mixture was filtered immediately and the solvent evaporated to dryness. The residue was shown by mass spectrometry to consist of 42% 6,7-d_2- and 30% x, 6,7-d_3-6-methylestrone,[11] the remaining contaminants being 11% monodeuterio, 7% d_4-, 6% d_5- and 3% d_6-6-methylestrone.

Acknowledgment.—In addition to the sources indicated in refs. 9, 14b, 28 and 36, we are particularly indebted to Dr. Pierre Crabbé (Syntex, S.A., Mexico City) and Prof. W. S. Johnson (Stanford University) for fulfilling numerous requests for samples.

(40) The course of the catalytic deuteration of steroidal olefins, dienes and enones will be discussed in detail in another paper.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF.]

Optical Rotatory Dispersion Studies. LXXVIII.[1] Comparative Studies of Circular Dichroism and Rotatory Dispersion Curves. Some Observations on Sulfur-containing Chromophores[2]

BY CARL DJERASSI, H. WOLF AND E. BUNNENBERG

RECEIVED JULY 23, 1962

Comparative ultraviolet absorption spectra, optical rotatory dispersion and circular dichroism curves are reported for a number of representative sulfur-containing chromophores such as xanthates, dithiocarbamates, thionocarbethoxy-α-amino acids, thiohydantoins, acylthioureas and cyclic disulfides (a cyclic diselenide also being included for comparison). These results are used to indicate that, for most stereochemical applications in organic chemistry, optical rotatory dispersion and circular dichroism measurements may be used interchangeably. The operation of background rotation effects in optical rotatory dispersion is discussed and it is pointed out, through examples among steroid ketones, that such background effects may often represent a desirable feature for structural work by optical rotatory dispersion, which is not as conveniently possible by means of circular dichroism. In other situations, especially where recognition of Cotton effects is masked by such background rotations or when overlapping, optically active absorption bands enter into operation, circular dichroism measurements are to be preferred, the recognition of very weak or hidden absorption bands being a particularly useful application.

Introduction

The phenomena of circular dichroism (C.D.) and of optical rotatory dispersion (O.R.D.) are intimately related.[3] The former refers to unequal absorption of right and left circularly polarized light by the optically active medium, while the latter corresponds to the change in optical rotation (unequal refractive indices of medium for right and left circularly polarized light) with wave length. Of particular interest are those wave length regions corresponding to absorption bands of the chromophore under discussion and, for most (colorless) organic substances, this means the ultraviolet range.

Both optical rotatory dispersion and circular dichroism have been determined in the ultraviolet in the past by physical chemists,[4] using laborious measurements on a few isolated organic substrates. The availability, in the early nineteen fifties, of a relatively simple spectropolarimeter led to the rapid determination of several thousand optical rotatory dispersion curves and to the now well-known[3] applications of this tool in organic chemistry, notably in stereochemistry and conformational analysis. If, instead of a spectropolarimeter, there had been available a convenient instrument

(1) Paper LXXVII, C. Djerassi and W. Klyne, *J. Chem. Soc.*, in press (1962).

(2) Supported by the National Science Foundation (grant No. G-19905) and the National Cancer Institute (grant No. CRTY-5061) of the National Institutes of Health, U. S. Public Health Service.

(3) C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960; see especially chapters 1 and 12.

(4) See especially T. M. Lowry, "Optical Rotatory Power," Longmans, Green and Co., London, 1935; and W. Kuhn, *Ann. Rev. Phys. Chem.*, **9**, 417 (1958).