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Mass Spectrometry in Structural and Stereochemical Problems. LIX.[1] Mechanism of the Formal Loss of Acetone from 2-Oxo-5α-steroids[2]

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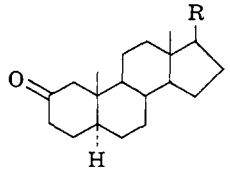
RECEIVED AUGUST 5, 1964

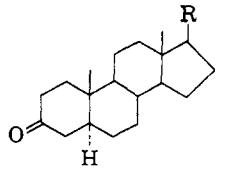
The possibility that ketones of the 2-oxo- and 3-oxo-5α-steroid type may be differentiated by mass spectrometry has been explored. A detailed examination of the fragmentation pattern of 5α-androstan-2-one and deuterated analogs has led to a delineation of the mechanism involved in the expulsion of the elements of acetone under electron impact conditions. Only hydrogen atoms attached to positions 6 and 9 are involved in the hydrogen transfers accompanying this fragmentation process. The applicability of these observations to bicyclic analogs of the β-decalone series is also discussed.

Introduction

As a result of a preliminary survey study of keto-steroids,[4] it was felt that mass spectrometry would not be useful in the differentiation of 2- and 3-oxosteroids. The extreme similarity between the mass spectra of cholestan-2-one (**1a**) and cholestan-3-one (**2a**)[4] led to the conclusion that optical rotatory dispersion measurement[5] in methanol solution (with and without hydrochloric acid[6]) was still the analytical method of choice for solving this type of problem.

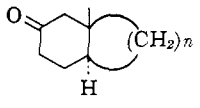


1a, R = C_8H_{17}
b, R = H

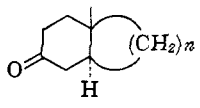


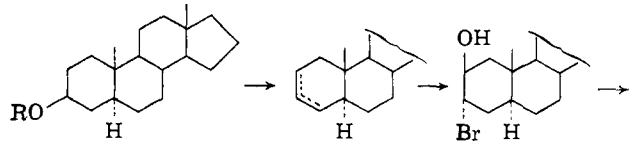
2a, R = C_8H_{17}
b, R = H

However, in connection with other work,[7] we had occasion to investigate the mass spectra of the bicyclic analogs of these steroids.[3,7] Indeed, the mass spectra of *trans*-9-methyl-2-decalone (**3a**)[8] and *trans*-10-methyl-2-decalone (**4a**)[8,9] were rather similar and this also applied to the isomeric pairs of the higher homologs (**3b, 4b** and **3c, 4c**). On closer examination of all these spectra, one difference does, however, stand out. A comparison of the relative abundances of M − 57 and M − 58 species demonstrates that the latter predominates in the 2-oxo series.[8]



3 **a**, $n = 4$ **4**
b, $n = 5$
c, $n = 6$





5, R = H
6, R = Ts
7
8

Since side-chain and ring D fragmentation dominates the mass spectra of steroids of the cholestane series,[4] 5α-androstan-2-one (**1b**) was synthesized and its mass spectrum measured in an effort to examine the feasibility of employing the above observations as a useful analytical criterion.

Of even greater interest was the elucidation of the mechanism for the process which, by the transfer of two hydrogen atoms, leads to the very prominent M − 58 ion. As will be noted from Fig. 1, this ion (*m/e* 216) is the most abundant one in the mass spectrum of 5α-androstan-2-one (**1b**). Deuterium labeling[10] proved to be the method of choice as has been the case earlier with other steroidal ketones.[11]

Synthetic Studies.—Two general routes were used for the conversion of 3-oxygenated steroids to 2-oxosteroids, the first of which represented the direct application of earlier work in the cholestane series[12] to the appropriate androstane derivative. The elements of *p*-toluenesulfonic acid were eliminated from the tosylate **6** of 5α-androstan-3β-ol[13] (**5**) either by refluxing the ester in collidine[14] or by allowing a benzene solution of the tosylate **6** to remain in contact with activated alumina for a prolonged period of time.[15] In either case, an oil was produced. This was not surprising as Fajkoš and Šorm[16] have shown that eliminations of this type produce both Δ^2- and Δ^3-olefins **7** which are frequently difficult to purify.

(1) For paper LVIII see A. M. Duffield, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 5536 (1964).

(2) Financial support by the National Cancer Institute (Grant No. CA-07195) of the National Institutes of Health is gratefully acknowledged.

(3) Taken from Part II of the Ph.D. Dissertation of J. E. G., Stanford University, 1964. The mass spectra of the various bicyclic compounds mentioned are reproduced in part I of this thesis.

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

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

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

(7) C. Djerassi and J. E. Gurst, *ibid.*, **86**, 1755 (1964).

(8) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, Chapter 8.

(9) E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1528 (1963).

(10) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, San Francisco, Calif., 1964, Chapter 2.

(11) (a) (11-ketones): D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963); (b) (3-ketones): R. H. Shapiro, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2837 (1964); (c) (7-ketones): R. Beugelmans, R. H. Shapiro, L. J. Durham, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 2832 (1964); (d) (16-ketones): C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 269 (1964).

(12) T. Nakano, M. Hasegawa, and C. Djerassi, *Chem. Pharm. Bull.* (Tokyo), **11**, 465 (1963).

(13) L. Ruzicka, V. Prelog, and P. Meister, *Helv. Chim. Acta*, **28**, 1651 (1954).

(14) R. Owyang in "Steroid Reactions: An Outline for Organic Chemists," C. Djerassi, Ed., Holden-Day, Inc., San Francisco, Calif., 1963, Chapter 5.

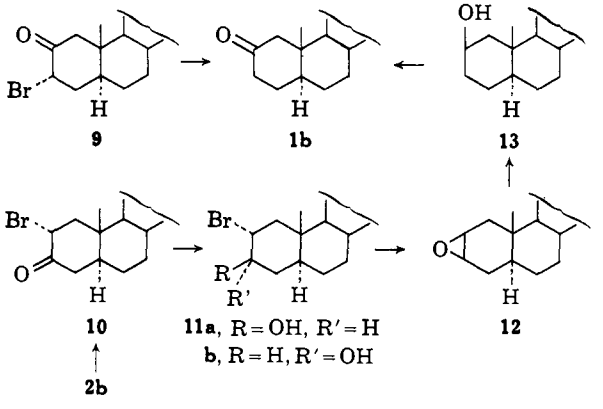
(15) G. H. Douglas, P. S. Ellington, G. D. Meakins, and R. Swindells, *J. Chem. Soc.*, 1720 (1959).

(16) J. Fajkoš and F. Šorm, *Collection Czech. Chem. Commun.*, **24**, 3115 (1959).

When the product **8** of the addition of hypobromous acid[12] to the olefin mixture was oxidized by chromium trioxide in acetic acid, a crystalline product was obtained which proved to be the desired 3α-bromo-5α-androstan-2-one (**9**). Reductive dehalogenation was carried out with zinc dust in acetic acid at room temperature to afford the pure 5α-androstan-2-one (**1b**).

Owing to the mixtures obtained in the tosylate elimination reaction[16] and the hypobromous acid addition,[12] a second synthetic sequence was investigated. 5α-Androstan-3-one (**2b**) was brominated either by bromine–acetic acid solutions or with pyridine hydrobromide perbromide.[17] The 2α-bromoketone **10**[18] was treated with lithium tri-*t*-butoxyaluminum hydride ($LiAl(t\text{-}BuO)_3H$)[19] in freshly distilled tetrahydrofuran. In reductions of other ketonic steroids, this reagent has been reported to yield quantitatively the equatorial isomer owing to its large steric requirements.[20] However, in the present instance thin layer chromatography indicated the presence of three new products with no starting material remaining (infrared spectrum). The most polar material had the same R_f value as 5α-androstan-3β-ol (**5**) and must have arisen by reductive dehalogenation along with reduction of the carbonyl function. The material of intermediate polarity was the desired 2α-bromo-5α-androstan-3β-ol (**11a**). The least polar spot was assumed to be the epimeric 2α-bromo-5α-androstan-3α-ol (**11b**). Treatment of the diequatorial bromohydrin **11a** with potassium hydroxide in methanol solution resulted in the formation of the oxide **12** by elimination of hydrogen bromide.[21] Reduction of the oxide with lithium aluminum hydride yielded the 2β-alcohol **13** by diaxial opening.[22]

In practice, it was found easiest not to separate the bromohydrins **11a** and **11b** but to treat them as a mixture with base since the bromohydrin **11b** was converted to the ketone **2b** under these conditions. If this mixture of oxide **12**[23] and ketone **2b** was then treated with lithium aluminum hydride, the axial 2β-alcohol **13** was formed along with the equatorial 3β-alcohol **5** derived from the ketone. This mixture of alcohols was separable by elution chromatography or by preparative thin layer chromatography. Jones oxidation[24] of the



(17) C. Djerassi and C. R. Scholz, *J. Am. Chem. Soc.*, **70**, 417 (1948).
(18) C. Djerassi, *J. Org. Chem.*, **12**, 823 (1947).
(19) H. C. Brown and R. F. McFarlin, *J. Am. Chem. Soc.*, **78**, 252 (1956).
(20) J. Fajkoš, *Collection Czech. Chem. Commun.*, **24**, 2284 (1959).
(21) J. Fajkoš, *J. Chem. Soc.*, 3966 (1959).
(22) A. Fürst and Pl. A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949).
(23) This oxide was sensitive to any form of chromatography—silica gel, silica gel thin-layer, and alumina (activity III)—and was probably hydrolyzed to the diol.

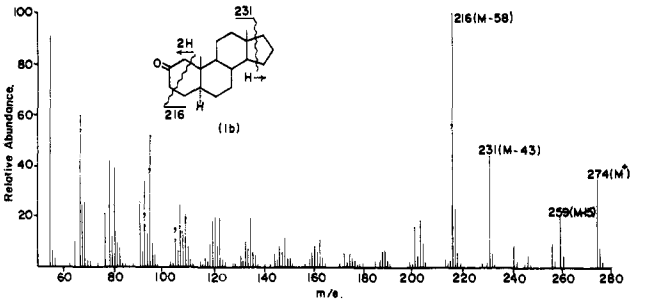
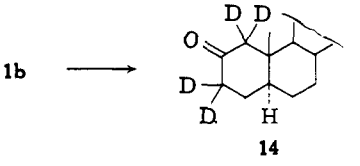


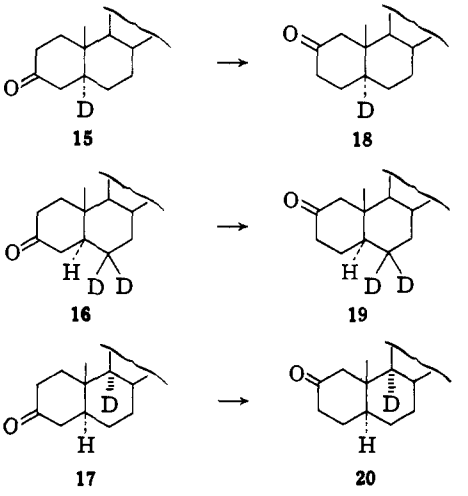
Fig. 1.—Mass spectrum of 5α-androstan-2-one (1b).

2β-alcohol **13** then led to pure 5α-androstan-2-one (**1b**) in approximately 20% yield from 5α-androstan-3-one (**2b**).

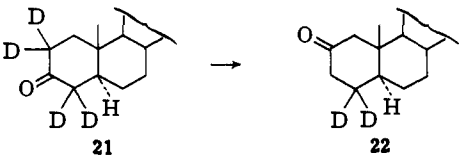
The first deuterated analog required was 1,1,3,3-d_4-5α-androstan-2-one (**14**). When the ketone **1b** was heated under reflux for 48 hr. in basic deuteriomethanol–



deuterium oxide,[10] a sample was obtained which contained predominantly two deuterium atoms. Further heating for 7 days under the same conditions was required to complete the exchange of all four hydrogen atoms. Samples of 5α-d_1-androstan-3-one[11b] (**15**), 6,6-d_2-5α-androstan-3-one[11b] (**16**), and 9α-d_1-5α-androstan-3-one[11b] (**17**) were converted to their 2-oxo isomers **18**, **19**, and **20**, respectively, *via* the β-oxide sequence described above. On the other hand, the hypobromous

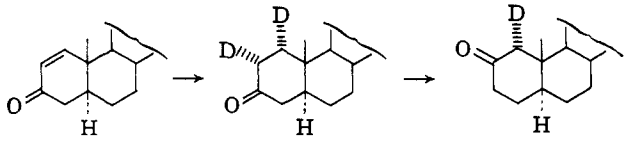


addition route was utilized for he conversion of 2,2,4,4-d_4-5α-androstan-3-one[11b] (**21**) to the 2-ketone **22**. This pathway was chosen to ensure no loss of deuterium from the ketone **21** as might occur in the initial bromination (**2b** → **10**) of the oxide route.



(24) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

The 1α-d_1-labeled ketone **23** was prepared in an unequivocal manner. Catalytic deuteration of Δ^1-5α-androsten-3-one[25] (**24**) was accomplished in cyclohexane solution with 10% palladium-on-charcoal as catalyst. The rear-side attack of the catalyst has been substantiated in three similar cases: Δ^1-5α-cholesten-3-one,[26] Δ^1-5α-androstene-3,11-dione,[27] and Δ^1-5α-androstene-3,17-dione.[28] The resulting 1α,2α-d_2-5α-androstan-3-one (**25**) can then be used to synthesize the monodeuterated ketone **23** with the β-oxide as an intermediate. This substance was most useful in the elucidation of the interesting n.m.r. spectra shown by all these 2-oxoandrostanes.[29]



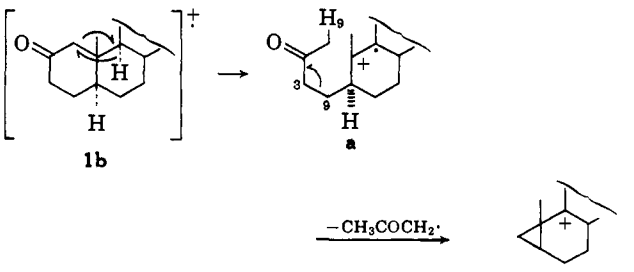
24 **25** **23**

Mass Spectrometric Results.—The mass spectrum (Fig. 1) of 5α-androstan-2-one (**1b**) clearly indicates that the removal of substituents from the D ring reduces the extent of fragmentation in that portion of the molecule (see contrasting mass spectrum[4] of cholestan-2-one (**1a**)). It is also quite evident that the loss of 58 mass units is of primary importance in compounds of this type. The M − 58 peak is the base peak of the spectrum, and an analysis of the region *m/e* 216–219 (Table I) indicates that this fragmentation accounts for 95% of the ionizing current in that area. The remaining 5% is attributed to a process yielding M − 57 as the charged species.

The first piece of evidence to be considered is the metastable peak at *m/e* ~169. This corresponds to $216^2/274$ and is evidence for a one-step process leading to the loss of 58 mass units; that is, the molecule does not lose 15 and then 43 mass units.[8] The locale of the fragmentation was pinpointed by observing a shift to M − 62 in the 1,1,3,3-d_4-5α-androstan-2-one (**14**) (Table I) and M − 59 in the 1α-d_1 compound **23**. From these data it was clear that C-1, C-2, and C-3 were eliminated along with two additional hydrogen atoms. This would formally constitute the elimination of a neutral acetone molecule.

In an attempt to determine whether this M − 58 peak arose from a complex, random, or specific transfer process, the fragmentation pattern of the 4,4-d_2 derivative **22** was examined. If hydrogens were transferred from this position, the rupture of two bonds connected to the same carbon atom would be required, which would be indicative of a complex process. As can be seen (Table I), no transfer did occur.

The details of this fragmentation became evident when it was found that for the M − 58 species, 90% of 6,6-d_2-5α-androstan-2-one (**19**) transferred a deuterium atom to the neutral departing species, while 93% of the 9α-d_1-5α-androstan-2-one (**20**) molecules lost their deuterium to the expelled group. The suggested mechanism involves ionization-rupture of the 1–10 bond with transfer of a hydrogen radical from C-9 to C-1 to yield the ion-radical, **a**. This portion of the mechanism is analogous to the transfer of the 8β-hydrogen atom during the ionization–rupture of the 9–10 bond in some 3-oxo steroids.[11b]



1b **a** **b**, M − 57

The data (Table I) would also suggest that the M − 57 moiety arises by an elimination of C-1, C-2, and C-3

TABLE I
MASS SPECTRAL DATA FOR 5α-ANDROSTAN-2-ONE[a]

	Ion current, %: M − 58 and M − 57 region, *m/e*				Molecular ion region, *m/e*				
Compound	216	217	218	219	274	275	276	277	278
d_0 (**1b**)					100	..	..	..	..
M − 58	95	..	..	..					
M − 57	..	5	..	..					
1α-d_1 (**23**)					28	65	4	3	..
M − 59	95	..	..	..					
M − 58	..	5	..	..					
1,1,3,3-d_4 (**14**)					1	2	6	29	61
M − 62	95	..	..	..					
M − 61	..	5	..	..					
4,4-d_2 (**22**)					3	11	79	7	..
M − 58	3	11	75	7					
M − 57	..	..	..	4					
Predicted	3	11	75	11					
Observed	5	11	79	5					
5α-d_1 (**18**)					16	76	8	..	..
M − 58	15	72	8	..					
M − 57	..	1	4	..					
Predicted	15	73	12	..					
Observed	24	65	9	2					
6,6-d_2 (**19**)					...	10	82	8	..
M − 59	10	78	8	..					
M − 57	..	..	..	4					
Predicted	10	78	8	4					
Observed	5	70	19	6					
9α-d_1 (**20**)					10	85	5	..	..
M − 59	10	..	..	..					
	81	5	..	..					
M − 58	..	4	..	..					
Predicted	91	9	..	..					
Observed	85	10	5	..					

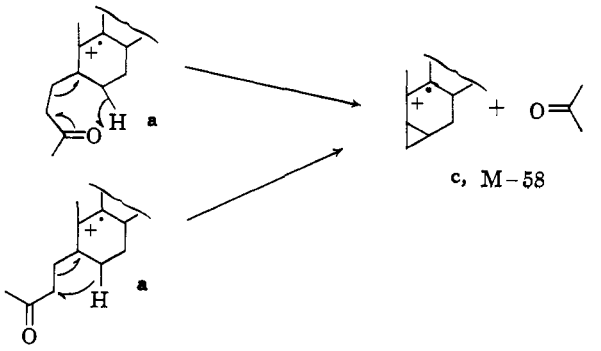
[a] These data have been corrected for isotope peaks (and rounded off to the nearest whole number) and the predictions are based on the fragmentation pattern described in the text. A sample calculation follows: The fragmentation pattern discussed in the text requires both the M − 58 (C_3H_6O) peak and the M − 57 (C_3H_5O) peak to remain unaffected in the spectrum of 5α-d_1-androstan-2-one (**18**). An analysis of the molecular ion (M^+) region gave the isotopic distribution after correction for C^{13} content.[8] This distribution is shown in Table I as 16% d_0 (*m/e* 274), 76% d_1 (*m/e* 275), 8% d_2 (*m/e* 276), 0% d_3 (*m/e* 277), and 0% d_4 (*m/e* 278).

From the first three entries in the table it is inferred that 95% of each species will lead to an M − 58 peak and 5% to an M − 57 peak. This means that 15% (95 of 16%, rounded off to the nearest whole number) of the ionizing current in the region under consideration (*m/e* 216–219) should be observed at M − 58 (*m/e* 216) while 1% (5% of 16%) should be at M − 57 (*m/e* 217). Similar treatment of the 76% of d_1 and 8% of d_2 species and addition of contributions to a particular value of *m/e* leads to the "predicted" spectrum. Comparison with the observed spectra indicates a close correlation (10%) in all cases.

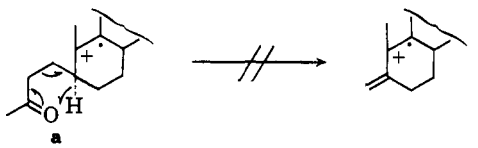
(25) R. H. Shapiro, J. M. Wilson, and C. Djerassi, *Steroids*, **1**, 1 (1963).
(26) F. J. Schmitz and W. S. Johnson, *Tetrahedron Letters*, 647 (1962).
(27) D. H. Williams, N. S. Bhacca, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2810 (1963).
(28) H. J. Ringold, M. Gut, M. Hayano, and A. Turner, *Tetrahedron Letters*, 835 (1962).
(29) N. S. Bhacca, J. E. Gurst, and D. H. Williams, *J. Am. Chem. Soc.*, in press.

with the exception that only one hydrogen atom is transferred to the departing species. Homolysis of the 3–4 linkage in ion **a** would give the M − 57 ion, which is here written as containing a cyclopropane ring because of its presumed stabilizing effect in conjugation with the tertiary carbonium ion, **b**.

The more intense M − 58 peak could arise then by transfer of hydrogen from C-6 to the carbonyl oxygen (seven-membered transition state). The charged species **c** is described as an ionized vinylcyclopropane derivative, but obviously there is no evidence as to the exact nature of this ion-radical. A similar electron migration could be written for transfer of the C-6 hydrogen to C-3 which involves a five-membered transition state and the same products.



One can visualize a cyclic six-membered transition state[30] for the transfer of the C-5 hydrogen to the C-2 carbonyl group in **a**—a reaction known[8,11d,30] to be prevalent among suitably substituted carbonyl compounds. An inspection of the mass spectrum (Table I) of 5α-d_1-androstan-2-one (**18**) contained evidence for only a token transfer (<10%) of the atom in question.



It must be recognized that these data do not preclude initial transfer from C-6 and final migration from C-9. The results only prove that C-1, C-2, and C-3 with their attached hydrogen atoms as well as the 9α- and 6-hydrogen atoms are lost in a single fragmentation procedure under electron bombardment conditions.

Two other steroidal 2-ketones have been investigated. The ketone **26** labeled with a 3α-methyl group[31] is difficult to analyze with complete certainty owing to the low intensity of this fragmentation in cholestanes, but it would seem that the loss of methyl ethyl ketone (72 mass units) does occur. 5α-Androstan-17β-ol-2-one propionate[32] (**27**) exhibits the phenomenon of acetone expulsion in two areas of the mass spectrum. An intense peak at *m/e* 288 (M − 58 from the molecular ion at *m/e* 346) appears as does a strong one at *m/e* 214 arising by successive loss of propionic acid and acetone.

Bicyclic Analogs.—Having determined the origin of the hydrogen transfers in the steroid case, we now turned our attention to the bicyclic analog **3a**. The

(30) F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959); G. Spiteller and M. Spiteller-Friedmann, *Monatsh.*, **95**, 257 (1964).

(31) R. C. Cookson and J. Hudec, *J. Chem. Soc.*, 429 (1962).

(32) R. R. Engle and C. Djerassi, Abstracts, Division of Medicinal Chemistry, 130th National Meeting of the American Chemical Society, Chicago, Ill., Sept., 1958, p. 15-O.

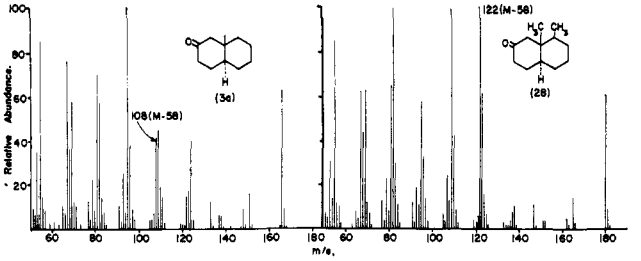
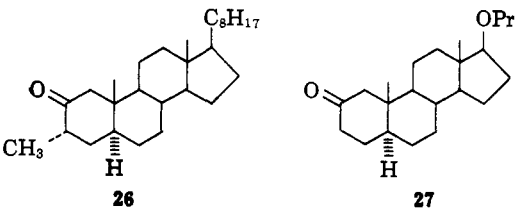


Fig. 2 (left).—Mass spectrum of *trans*-9-methyl-2-decalone (**3a**).
Fig. 3. (right).—Mass spectrum of *trans*-8,9-dimethyl-2-decalone (**28**).

1,1,3,3-d_4 compound was prepared by extended equilibration of the enolizable α-protons in **3a** with methanol-*d*, heavy water, and sodium methoxide. Other deuterated analogs were prepared from the appropriate olefin as previously described.[7] The deuterium-con-



taining olefins were synthesized according to standard procedures,[7,33] utilizing deuterium gas, lithium aluminum deuteride, or methanol-*d* in the appropriate point of the synthesis in place of the normal reagent.

High-resolution mass spectrometry[34] (Table II) indicated the complexity of the original spectrum (Fig. 2). Although the M − 58 peak in **3a** is entirely attributed to the loss of C_3H_6O (Table II), the dual nature of the M − 57 ion (Table II) precludes any quantitative estimates of the extent of deuterium transfer. However, qualitatively the results can be described easily. There was a partial transfer only in the mass spectrum of *trans*-8α-d_1-9-methyl-2-decalone (see **3a**). This is the exact bicyclic analog of the steroid **20** where 93% of the molecules transferred the corresponding atom (see above). The 8β-d_1 compound was also prepared, and the resulting spectrum was identical with that of the 8α isomer. No transfer was found in the 6,6-d_2-labeled system as one would expect, while substantial shifts to M − 59 and M − 60 were noted in *trans*-5,5,7,7-d_4-9-methyl-2-decalone.

TABLE II

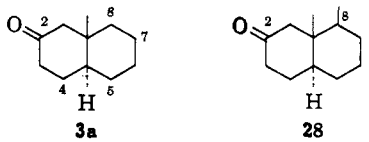
HIGH-RESOLUTION DATA FOR *trans*-9-METHYL-2-DECALONE

m/e	Oxygen-containing fragment, %	Hydrocarbon, %	Ratio
81 (M − 85)	6 (C_5H_5O)	94 (C_6H_9)	1:16
82 (M − 84)	40 (C_5H_6O)	60 (C_6H_{10})	2:3
95 (M − 71)[a]	67 (C_6H_7O)	33 (C_7H_{11})	2:1
108 (M − 58)	1 (C_7H_8O)	99 (C_8H_{12})	1:99
109 (M − 57)	26 (C_7H_9O)	74 (C_8H_{13})	1:3
124 (M − 42)	90 ($C_8H_{12}O$)	10 (C_9H_{16})	9:1

[a] It is interesting to note that the amount of oxygen-containing fragment is increased by the presence of the angular methyl group relative to β-decalone. Also, the shifts of this peak in the various deuterated compounds are consistent with the mechanism suggested in ref. 8, p. 150.

(33) J. Karliner, H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, in press.

(34) We extend our appreciation to Dr. D. A. Lightner of this laboratory for these measurements obtained with the AEI-MS-9 mass spectrometer.



3a **28**

The important feature of these results is the lack of specificity—in contrast to the steroid case—in the hydrogen transfer reaction leading to the M − 58 ion. This can be attributed to the secondary nature of the analogous atom in the bicyclic ketone **3a** as opposed to the tertiary center in the steroid **1b**. To test this hypothesis the ketone **28**[35] was examined (Fig. 3). In this case, this mode of fragmentation (loss of acetone) was greatly accentuated. The compound **28** was now resynthesized[35] utilizing deuterium gas at an early stage in the sequence. The resulting *trans*-8,9-dimethyl-8α-d_1-2-decalone underwent a very substantial loss of 59 mass units indicating the importance of the tertiary center as a driving force for the electron impact-induced reaction.

Experimental[36]

3α-Bromo-5α-androstan-2-one (**9**).—A mixture of 4 g. of 5α-androstan-3β-ol (m.p. 151–152°, $[\alpha]^{25}D$ −1.6° (*c* 1.06)),[13] 5.5 g. of *p*-toluenesulfonyl chloride, and 45 ml. of pyridine was allowed to stand in the refrigerator for 24 hr.[15] The solution was diluted with water and the precipitated **5α-androstan-3β-ol tosylate** (**6**) was collected by filtration. Recrystallization of a sample from aqueous ethanol provided white crystals, m.p. 121–125°, $[\alpha]^{26}D$ −8.6° (*c* 0.935).[37]

The tosylate **6** (1 g.) in benzene was allowed to remain in contact with 15 g. of alumina (Merck, base-washed, activity I) for 95 hr.[15] An oily product (0.4 g., 67%) was obtained by concentration of the solution after removal of the alumina. This product showed two spots on thin-layer chromatography (petroleum ether as developer). The major spot corresponded to the product obtained by refluxing the tosylate with collidine while the minor and more polar spot did not correspond to the starting material. Chromatography of this oil on 60 g. of silica gel with petroleum ether afforded a material which was homogeneous on thin-layer chromatography but still difficult to crystallize since, undoubtedly, both the product from alumina elimination and collidine treatment was a mixture of **Δ²-5α-androstene** (**7**) and the Δ³-isomer.[16]

This mixture of steroidal olefins **7** (1 g.) was dissolved in the mixed solvent consisting of 38 ml. of *t*-butyl alcohol, 20 ml. of dioxane, and 2 ml. of water. To this was added a solution of 1 g. of N-bromosuccinimide in 10 ml. of dioxane along with a second solution of 2 ml. of 60% perchloric acid in 10 ml. of water.[12] This solution was concentrated at the water pump at a temperature below 45° after standing overnight at room temperature. Ether extraction yielded 1.6 g. (theoretical, 1.38 g.) of a very viscous yellow oil. Elution chromatography (benzene on 200 g. of silica gel) yielded 492 mg. of a chromatographically homogeneous oil (**8**).

Oxidation with 8 *N* chromic acid reagent[24] at ice-bath temperature proceeded rapidly. After the addition of water, the product could be extracted with ether. Three recrystallizations from methanol–chloroform yielded 88 mg. (6.4%) of **3α-bromo-5α-androstan-2-one** (**9**), m.p. 142–143°, λ_{max}^{EtOH} 310 mμ (ε 132), λ_{max}^{KBr} 5.87 μ, $[\alpha]D$ +232° (*c* 1.015); R.D. (*c* 0.06 in methanol): $[\phi]_{589}$ +1648°, $[\phi]_{332}$ +17,419°, $[\phi]_{284}$ −16,359°, $[\phi]_{250}$ −11,700°.

Anal. Calcd. for $C_{19}H_{29}OBr$: C, 64.58; H, 8.27; Br, 22.62. Found: C, 64.48; H, 8.35; Br, 22.51.

5α-Androstan-2-one (**1b**).—The pure bromoketone **9** (45 mg.) in 15 ml. of acetic acid was treated with 1 g. of zinc dust and stirred overnight at room temperature. After filtration of the excess zinc and zinc salts, water was added and the product extracted with ether. This crude product was recrystallized from aqueous methanol to yield 19 mg. (56% yield) of material, m.p. 115–118°. A second crop of crystals brought the total yield to 78%. An analytical sample was prepared by further recrystallization from aqueous methanol: m.p. 122–123°, λ_{max}^{EtOH} 287 mμ (ε 27), λ_{max}^{KBr} 5.88 μ, $[\alpha]D$ +46° (*c* 1.03); R.D. (*c* 0.12 in methanol): $[\phi]_{589}$ −92°, $[\phi]_{306}$ +4384°, $[\phi]_{261}$ −5934°, $[\phi]_{240}$ −4700°. As expected,[6] the amplitude was unchanged upon addition of hydrochloric acid.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02; mol. wt., 274. Found: C, 83.09; H, 10.92; mol. wt. (mass spectrum), 274.

2α-Bromo-5α-androstan-3β-ol (**11a**).—The bromoketone **10**[17] (100 mg.) was dissolved in 20 ml. of freshly distilled tetrahydrofuran in an ice bath. To this was added 200 mg. of lithium tri-*t*-butoxyaluminum hydride.[19] The resulting suspension was stirred for 20 min. and then poured into 25 ml. of 5% acetic acid. Ether extraction resulted in a quantitative yield of oily product exhibiting no carbonyl absorption in the infrared spectrum. Preparative thin-layer chromatography with benzene as a developer yielded 56 mg. of the homogeneous bromohydrin **11a**. This was recrystallized from methanol to obtain a material with a double melting point: 81–83°, 93–97°; $[\alpha]^{26}D$ −12° (*c* 0.98).

Anal. Calcd. for $C_{19}H_{31}OBr$: Br, 22.49. Found: Br, 22.23.

2β,3β-Oxido-5α-androstane (**12**).—The bromohydrin **11a** (40 mg.) was dissolved in 5 ml. of methanol to which a small amount of potassium hydroxide was added.[21] The solution was heated under reflux for 12 hr., after which time the solvent was evaporated and ether–water added. Concentration of the dried ethereal extracts yielded 26 mg. of an oil. Crystallization of this oil from dilute methanol afforded the oxide **12**, m.p. 73–74°, $[\alpha]D$ −40° (*c* 1.03).

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 82.90; H, 10.87.

5α-Androstan-2β-ol (**13**).—An ether solution of the epoxide **12** (225 mg. in 50 ml.) was treated with excess lithium aluminum hydride. After 12 hr., the excess hydride was destroyed with ethyl acetate and the resulting salts dissolved in 10% sulfuric acid. The isolation of 223 mg. of crystalline alcohol, m.p. 123–130°, was achieved by ether extraction. Three crystallizations from acetonitrile afforded a sample with m.p. 134–135° and $[\alpha]D$ +12° (*c* 1.035).

Anal. Calcd. for $C_{19}H_{32}O$: C, 82.54; H, 11.67. Found: C, 82.14; H, 11.46.

5α-Androstan-2-one (**1b**).—Oxidation of the axial alcohol **13** under Jones conditions[24] at room temperature afforded in quantitative yield the ketone **1b** identical in all respects with that prepared above.

1,1,3,3-d_4-5α-Androstan-2-one (**14**).—A sample (20 mg.) of 5α-androstan-2-one (**1b**) was dissolved in 5 ml. of methanol-*d*. Sodium metal (20 mg.) was dissolved therein. Deuterium oxide was added at the boiling point of the methanol solution until it was cloudy. A minimal amount of the alcohol was used to produce again a homogeneous solution, which was then heated for 48 hr. at its boiling point. Crystallization occurred upon cooling. The product, m.p. 120–122°, had the following isotopic composition: 13% d_0, 41% d_1, 37% d_2, 8% d_3, and 1% d_4.

The sample was retreated as above for an additional 7 days. This time the sample contained 1% d_0, 2% d_1, 6% d_2, 29% d_3, and 61% d_4 species.

5α-d_1-Androstan-2-one (**18**).—5α-d_1-Androstan-3-one[11b] (**15**)[38] (100 mg.) was utilized to obtain 52 mg. of 5α-d_1-2α-bromoandrostan-3-one (m.p. 200–206°) with a bromine–acetic acid solution and recrystallization from ethanol–chloroform. This material was treated with twice its weight of $LiAl(t\text{-}BuO)_3H$ in 5 ml. of tetrahydrofuran as described above. This product (50 mg.) was dissolved in isopropyl alcohol along with potassium hydroxide and heated at the reflux temperature. After the usual work-up (see above), the oil (40 mg.) was subjected to preparative thin-

(35) L. H. Zalkow, F. X. Markley, and C. Djerassi, *J. Am. Chem. Soc.*, **82**, 6354 (1960).

(36) Melting points were determined on the micro-hot stage and are uncorrected. Microanalyses were determined by Messrs. E. Meier and J. Consul of the Stanford University Microanalytical Laboratory. Infrared spectra were obtained routinely on the Perkin–Elmer Infracord and for analytical samples on the Perkin–Elmer infrared spectrometer Model 421. Ultraviolet spectra were determined on the Cary Model 14 instrument. The optical rotatory dispersion measurements were made on a Japan Spectroscopic Co. (Jasco) automatically recording spectropolarimeter Model ORD-5 by Mrs. R. Records. All mass spectra were determined with a CEC Model No. 21-103C spectrometer using an all-glass inlet system heated to 200° while the isatron temperature was maintained at 270°. The ionizing energy was kept at 70 e.v. and the ionizing current at 50 μa. We are indebted to Dr. H. Budzikiewicz and his associates for these spectra. All specific rotations at the D line were measured in chloroform solution.

(37) Dr. Z. Pelah of these laboratories found m.p. 125.5° for an analytical sample prepared in the same manner. *Anal.* Calcd. for $C_{26}H_{38}O_3S$: C, 72.52; H, 8.90; S, 7.45. Found: C, 72.54; H, 9.03; S, 7.20.

(38) We thank Dr. Zvi Pelah for this sample which was 15% d_0, 81% d_1, and 4% d_2.

layer chromatography with benzene as the developer. Only 13 mg. of the deuterated epoxide, m.p. 66–70°, could be eluted.

Reduction of this material with lithium aluminum hydride as described above yielded 11 mg. of solid, m.p. 121–125°. Jones oxidation[24] with 1 drop of reagent led to the isolation of 9 mg. of ketone **18** which was recrystallized from aqueous methanol to yield the sample, m.p. 118–120°, employed for mass spectrometry.

6,6-d_2-5α-Androstan-2-one (**19**).—A 220-mg. specimen of 6,6-d_2-5α-androstan-3-one[11b] (**16**)[39] was dissolved in 3 ml. of acetic acid and treated with 270 mg. of pyridine hydrobromide perbromide.[18] The crystalline product was filtered after the reaction mixture had been diluted with water. A 52% yield (150 mg.) of white crystals, m.p. 206–210°, was obtained by recrystallization from ethanol–chloroform.

This sample was treated with 300 mg. of LiAl(*t*-BuO)$_3$H in 20 ml. of tetrahydrofuran. The usual work-up afforded 160 mg. of viscous oil, which was dissolved in 15 ml. of methanol to which 150 mg. of potassium hydroxide was added. After 16 hr. at reflux temperature, the solvent was evaporated and 108 mg. of yellow oil was obtained.

Lithium aluminum hydride (150 mg.) was added to an ether solution of this mixture. These conditions led to 100 mg. of an oil which was probably a mixture of 2β- and 3β-alcohols.

Preparative thin layer chromatography (benzene–ether in a ratio of 9:1) yielded 37 mg. of a homogeneous white solid. Oxidation of this substance with 8 *N* chromic acid solution[24] led to the isolation in quantitative yield of the dideuterioketone **19**, m.p. 121–123°.

4,4-d_2-5α-Androstan-2-one (**22**).—A sample (1.8 g.) of tetradeuterio-5α-androstan-3-one[11b] (**21**) was reduced to the 3β-alcohol, m.p. 151–152°, with lithium aluminum hydride (1.5 g.). This product was converted by the above-described procedure in 94% yield to the tosylate, m.p. 123–125°.

The crude tosylate was dissolved in 60 ml. of coal tar collidine and heated at the boiling point of the solution for 2 hr. About two-thirds of the collidine was removed by vacuum distillation (43° (5 mm.)). The remaining solution was diluted with 10% hydrochloric acid solution and ether. The ether layer was washed well with additional amounts of the acid solution. The crude product was filtered through a short column of alumina (activity I) with benzene. Approximately 1 g. of product was obtained by this procedure.

This was dissolved in the dioxane–water–*t*-butyl alcohol solvent described above and treated with N-bromosuccinimide (1 g.) and perchloric acid.[12] The sticky yellow oil thus obtained was dissolved in 50 mg. of acetic acid, and 1 g. of chromium trioxide was added. The solution was stirred at room temperature for 2 hr. Ether and water were added and the usual manipulations yielded 1.2 g. of another sticky oil. Crystallization was achieved with a mixed solvent of methanol–chloroform to produce 112 mg. of 4,4-d_2-3α-bromo-5α-androstan-2-one, m.p. 138–141°.

(39) We thank Dr. Zvi Pelah and Dr. Robert Shapiro for this sample, which consisted of 11% d_1, 82% d_2, and 7% d_3 species.

This steroid was dissolved in 7 ml. of acetic acid to which was added about 300 mg. of zinc dust. The suspension was stirred at room temperature for 10 hr. before the mixture was filtered, and the filtrate diluted with water. The crystallized product was obtained by filtration, and recrystallized from aqueous methanol to yield 50 mg. of long needles of 4,4-d_2-5α-androstan-2-one (**22**), m.p. 122–123°.

1α-d_1-5α-Androstan-2-one (**23**).—Catalytic deuteration of 1.1 g. of Δ^1-5α-androsten-3-one[25] (**24**) was achieved in 20 min. by using 300 mg. of 10% palladium-on-charcoal catalyst and 100 ml. of cyclohexane as solvent. Filtration of the solid materials and evaporation of the filtrate gave a quantitative yield of the saturated ketone **25**, m.p. 95–98°.

The above ketone **25** was dissolved in 25 ml. of acetic acid and treated at room temperature with small portions of pyridine hydrobromide perbromide[18] until a total of 1.4 g. (105%) had been added. The product crystallized on chilling. The suspension was filtered and the product recrystallized from ethanol–chloroform to yield 700 mg. (50%) of pure product, m.p. 210–212°.

Reduction of this bromoketone was accomplished with 1.5 g. of the complex hydride (LiAl(*t*-BuO)$_3$H) in 50 ml. of freshly distilled tetrahydrofuran. Isolation of the oily product (715 mg.) was performed as above. This material was dissolved in 50 ml. of methanol with 700 mg. of potassium hydroxide and heated at reflux temperature. The product (500 mg.) in 50 ml. of dry ether was reduced for 4 hr. with an equal weight of lithium aluminum hydride, whereupon it exhibited two spots on thin layer chromatography. Elution chromatography on 75 g. of silica gel with benzene and then 2% ether in benzene afforded 108 mg. of homogeneous material, m.p. 127–131°. Oxidation by the Jones procedure[24] converted the alcohol to the desired 1α-d_1-2-ketone **23** (90 mg., m.p. 121–122°).

9α-d_1-5α-Androstan-2-one (**20**).—A contaminated (thin-layer chromatography) sample (100 mg.) of 9α-d_1-5α-androstan-3-one[11b] was dissolved in 1 ml. of acetic acid. To this was added, at room temperature, 120 mg. (105%) of pyridine hydrobromide perbromide.[18] After the addition of water, the product was extracted with ether. Concentration of the ethereal extract after the usual washing and drying procedures yielded 100 mg. of yellow solid. Recrystallization from ethanol–chloroform afforded 36 mg. of the bromoketone (m.p. 190–200° dec.).

Lithium tri-*t*-butoxyaluminum hydride (70 mg.) was used to reduce this bromoketone in tetrahydrofuran solution at 0° with a 20-min. reaction time. The isolated product (35 mg.) was dissolved in 4 ml. of methanol to which 40 mg. of potassium hydroxide was added. Overnight heating under reflux conditions converted this to an oily mixture (20 mg.) which was treated directly with lithium aluminum hydride in dry, boiling ether for 1 hr. This product (20 mg.) could be purified by preparative thin-layer chromatography as demonstrated above and thus yielded 5 mg. of the 2β-alcohol.

Oxidation with Jones reagent[24] in 1 ml. of acetone afforded 4 mg. of homogeneous (thin layer chromatography) ketone which was recrystallized from aqueous methanol to yield the mass spectrometry sample, m.p. 115–120°.