in the chamber through a sensitive Liston-Becker infrared CO_2 analyzer and through a vibrating reed electrometer. Humidity is maintained at greenhouse level by occasional circulation of the chamber atmosphere through Drierite, and normal lighting is supplied by Gro-Lux fluorescent lights.

Isolation Procedures. The alkaloids were isolated by the usual procedure,[4] involving freezing the plants in liquid nitrogen, extraction into butanol—benzene, transfer into acid, then pH adjustment and extraction to obtain two fractions: the phenolic and nonphenolic alkaloids. In the experiments in which carrier alkaloids were added during the work-up, these were dissolved in butanol–benzene and added to the frozen plant material during the initial extraction.

Since morphine was the only phenolic alkaloid of interest, it was separated from the other phenolic alkaloids by column chromatography or tlc, system a (see Chromatography section), depending on the quantity of material. Further purification was accomplished by sublimation followed by recrystallization from methanol to constant specific activity.

In the fraction containing the nonphenolic alkaloids, codeinone was separated from the other compounds by means of its bisulfite adduct.[17] It was then converted to a more stable compound for further purification and analysis as dihydrocodeinone,[18] 6-methylcodeine,[25] or 6-phenylcodeine, as indicated in the tables. Purification of dihydrocodeinone was accomplished by sublimation and recrystallization from methanol. 6-Methylcodeine was sublimed, subjected to tlc systems a, b, and c, and recrystallized. 6-Phenylcodeine, which was the compound used to determine the specific activity of codeinone in the steady-state exposure (Table III), was analyzed by means of gas chromatography.

Codeine and thebaine were separated from each other by means of column chromatography, then sublimed and recrystallized to constant activity. In the case of the steady-state exposure (Table III), these compounds were separated and analyzed by gc.

Neopinone was immediately converted to 6-methylneopine by treating the entire nonphenolic alkaloid fraction with methyllithium. A preliminary test was conducted to establish that no contamination of 6-methylneopine occurred from other substances in the alkaloid mix. The 6-methylneopine was then separated from the other alkaloids by tlc systems a, c, and d.

Table V. R_f Values

Compound	System a	b	c	d
Thebaine	0.65		0.65	0.40
Codeine	0.40	0.25	0.15	0.10
Codeinone	0.55	0.40	0.35	
6-Methylcodeine	0.70	0.50	0.40	0.35
6-Methylneopine	0.70		0.30	0.25
Neopinone	9.53	0.40		
Morphine	0.30	0.10		
6-Phenylcodeine	0.80			
Neopine	0.40	0.25		

Chromatography Systems. Column Chromatography. Woelm silica gel for tlc (no binder), deactivated by standing 24 hr in shallow pans exposed to air, was used. Eluting solvents were $CHCl_3$ (80), CH_3OH (20), NH_4OH (0.5%).

Thin Layer Chromatography. With Camag silica gel for tlc, the solvent systems were (a) $CHCl_3$ (80), CH_3OH (20), NH_4OH (0.05%); (b) $CHCl_3$ (25), dioxane (60), ethyl acetate (10), NH_4OH (5).[31] With Merck aluminum oxide G for tlc, the solvent system were (c) benzene (70), $CHCl_3$ (15), acetone (15), shaken with 3.5% NH_4OH;[32] (d) cyclohexane (8), $CHCl_3$ (2), acetone (6). R_f values are given in Table V.

Gas chromatography was performed on 6 ft × 6 mm glass columns, packed with 4.5% OV1 on Aeropak 30 (100–120 mesh), at 210° using argon at 60 ml/min as the carrier gas and a hydrogen flame detector. The retention times were for 6-methylcodeine, 4 min 15 sec; thebaine, 6 min 45 sec; codeine, 4 min 45 sec; 6-phenylcodeine, 21 min; morphine, 5 min 30 sec.

(31) J. A. Steele, *J. Chromatogr.*, **19**, 300 (1965).
(32) L. Vignoli, J. Guillot, F. Gouezo, and J. Catalin, *Ann. Pharm. Fr.*, **24**, 461 (1966).

Mass Spectrometry in Structural and Stereochemical Problems. CCXI.[1] The Effect of Structural Variations on the Electron Impact Induced Fragmentations of Steroid Hydrocarbons[2]

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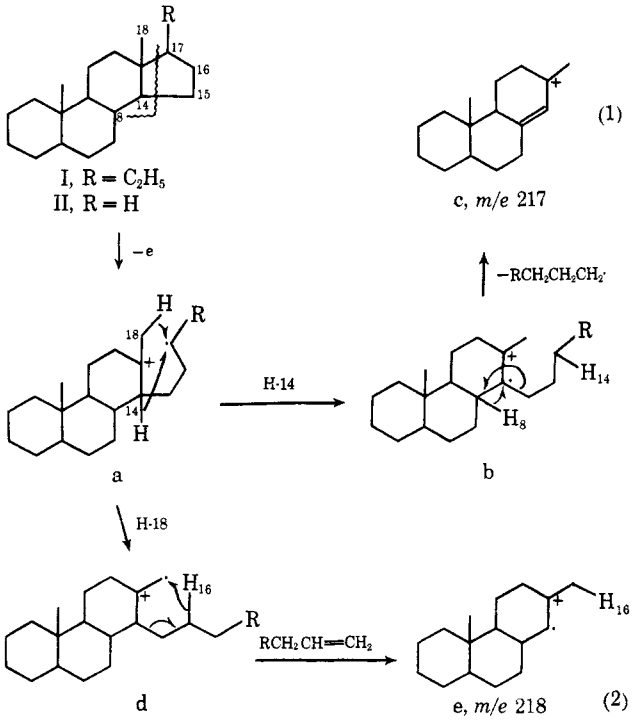
Abstract: The electron impact induced fragmentations of *D*-homoandrostane (IV) and *D*-homopregnane (III) are qualitatively similar to those of androstane (II) and pregnane (I), respectively. In particular, the characteristic ring D and ring A cleavages were subjected to close scrutiny, and were demonstrated to be mechanistically completely analogous to the processes observed in the parent compounds. In contrast, the mass spectral behavior of *D*-norandrostane (VI) and *D*-norpregnane (V) differs markedly from that of the parent compounds. For example, the very abundant *m/e* 218 ion is produced without reciprocal hydrogen transfer in these compounds. The site of charge localization, and thus of ring cleavage, is strikingly dependent on the structure of the steroid framework, particularly in the androstane series. This dependence is rationalized on the basis of relief of steric strain.

The electron impact induced fragmentation of steroids possessing an alkyl substituent at C-17 (*e.g.*, pregnane, I) characteristically involves extensive fragmentation about ring D, a process indicated schematically by the wavy line in structural formula I. The diagnostic importance of this fragmentation pattern was recognized over 15 years ago; the process reveals the molecular weight of the substituent at C-17.[5] Recently,[6] extensive deuterium labeling experiments have

(1) For paper CCX, see A. N. Yeo and C. Djerassi, *J. Amer. Chem. Soc.*, **94**, 482 (1972).
(2) Financial support by the National Institutes of Health (Grants AM 12758 and AM 04257) is gratefully acknowledged.
(3) National Institutes of Health Predoctoral Fellow, 1968–1971.
(4) Recipient of an IREX fellowship while on leave (1970–1971) from the Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia.
(5) P. de Mayo and R. I. Reed, *Chem. Ind. (London)*, 1481 (1956).
(6) L. Tökés, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

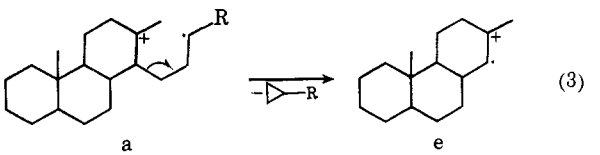
refuted a number of earlier mechanistic postulations[5,7-9] and demonstrated that these fragmentation processes (which give rise to peaks at *m/e* 217 and 218 in an unsubstituted steroid such as I or II) occur predominantly with a single hydrogen transfer (I → a → b → c) and a reciprocal hydrogen transfer (I → a → d → e), as depicted in Scheme I.[10] Additional experiments con-

Scheme I

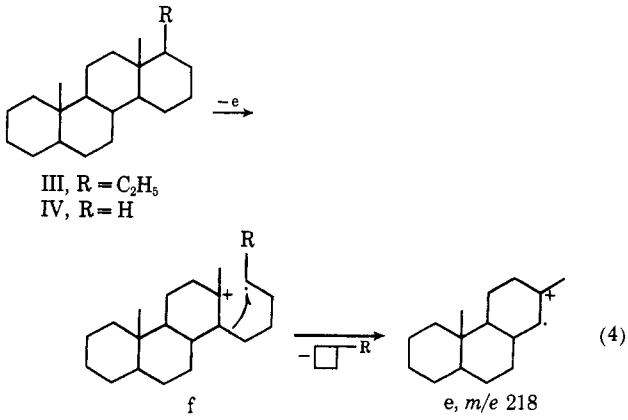


firmed the prevalence of these remarkable rearrangement processes in the electron-impact induced ring D cleavage of androstane (II).[11]

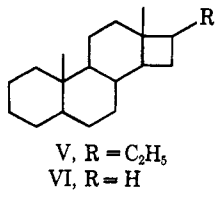
These unexpected observations, however, raise a number of interesting questions. The peak at *m/e* 218 arises through a complex reciprocal hydrogen transfer process, rather than the *a priori* plausible pathway depicted in eq 3.[12] The absence of this mechan-



istic pathway therefore must be related to the stability of the neutral species expelled. The greater stability of the olefin (eq 2) relative to the strained cyclopropane derivative (eq 3) must provide the driving force for the rearrangement; in terms of quasiequilibrium theory, the lower activation energy of the rearrangement process outweighs the higher frequency factor of the simple cleavage, even at high ionizing voltages. It is of some interest, therefore, to establish the mechanism of ring D cleavage of *D*-homopregnane (III) and *D*-homoandrostane (IV), where the alternative expelled species is a cyclobutane derivative (eq 4). The improved



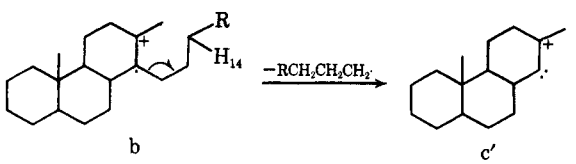
energetics of this alternative process could enable it to compete effectively with the "normal" reciprocal hydrogen transfer. Furthermore, these compounds would shed light on the importance of particular ring sizes in the transition state of the hydrogen-transfer steps (a). Another point of interest concerning the mass 218 ion relates to its origin in *D*-norpregnane (V) and *D*-norandrostane (VI). Here, the potential mechanisms include



elimination of an olefin through reciprocal hydrogen transfer, or elimination of the same olefinic species through a simple cleavage. In a similar manner, alternative mechanisms might give rise to *m/e* 217 ions in the mass spectra of the *D*-homo and *D*-nor steroids. Thus, deuterium labeling experiments were carried out on the *D*-homo- and *D*-norpregnanes and -androstanes to determine the generality of the unusual hydrogen rearrangements observed in the ring D cleavage of pregnane and androstane themselves. The results of these studies would be expected to shed considerable light on the intimate mechanistic details of these diagnostically important processes.[13] (See Figures 1–5.)

A remarkable feature of steroid mass spectroscopy revealed by the recently published deuterium labeling studies[6,11] is the preeminent importance of ions of structure a in triggering fragmentation of the steroid nucleus. This observation is partially explained by the well-established rules of radical and carbonium ion stability. Thus, cleavage of the C-13–C-17 bond in I produces a tertiary carbonium ion and a secondary radical, the

(7) R. I. Reed, *J. Chem. Soc.*, 3432 (1968).
(8) S. S. Friedland, G. H. Lane, R. T. Longman, K. E. Tenin, and M. J. O'Neal, Jr., *Anal. Chem.*, **31**, 169 (1959).
(9) H. J. M. Fitches, *Advan. Spectrom.*, **2**, 434, 454 (1962).
(10) The 1,2 shift of the C-8 hydrogen (b → c) is postulated solely to avoid the formation of the presumably high energy ionized carbene c'. Work is currently under way in these laboratories to differentiate between the pathways leading to ions c and c'.



(11) L. Tökés and C. Djerassi, *J. Amer. Chem. Soc.*, **91**, 5017 (1969).
(12) M. Spiteller-Friedmann, S. Eggers, and G. Spiteller, *Monatsh. Chem.*, **95**, 1740 (1964).
(13) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, San Francisco, Calif., 1964.

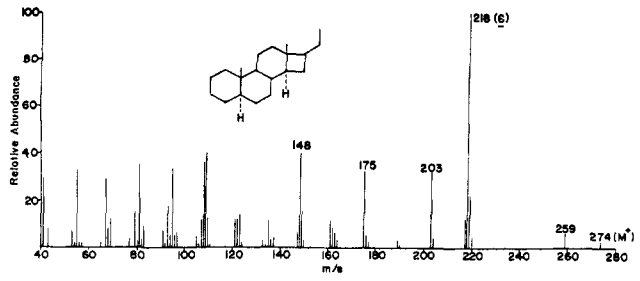


Figure 1. Mass spectrum of *D*-nor-5α,14α-pregnane (V) determined at 70 eV.

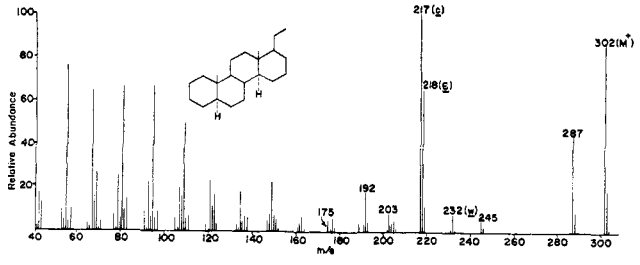


Figure 2. Mass spectrum of *D*-homo-5α,14α-pregnane (III) determined at 70 eV.

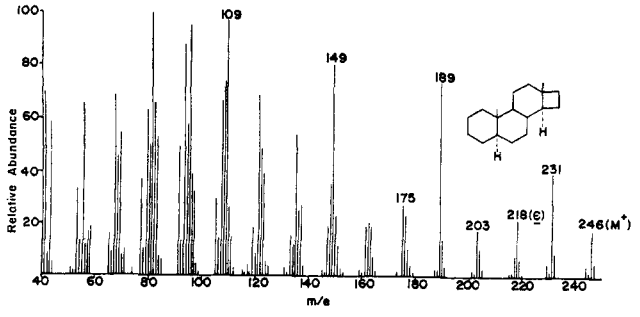
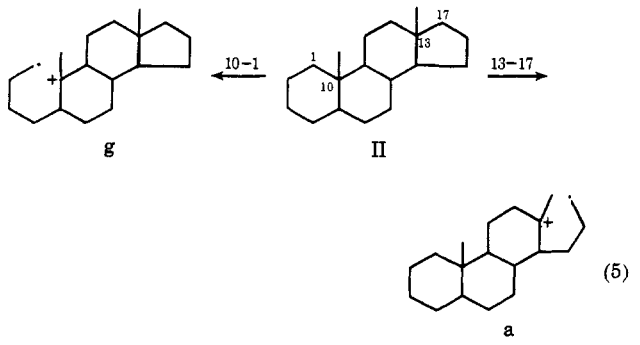


Figure 3. Mass spectrum of *D*-nor-5α,14α-androstane (VI) determined at 70 eV.

most favored situation possible in a steroid. In the androstane series, on the other hand, cleavage of the C-13–C-17 bond, or of the C-10–C-1 bond, each generates a primary radical and a tertiary carbonium ion (eq 5). However, roughly 2.0 times as much fragmentation is observed about ring A (corresponding to ion g) as about ring D (ion a) despite the added strain inherent in the trans-fused hydrindan system. In order to elucidate the factors involved in determining the site of preferred charge localization in the steroid nucleus, a number of stereochemically varied androstanes as well as *D*-nor-, *D*-homo-, and *D*-bishomoandrostane were studied.



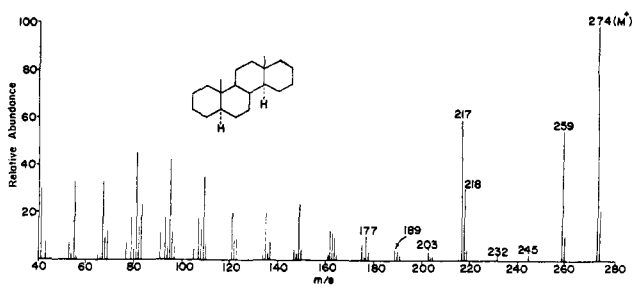


Figure 4. Mass spectrum of *D*-homo-5α,14α-androstane (IV) determined at 70 eV.

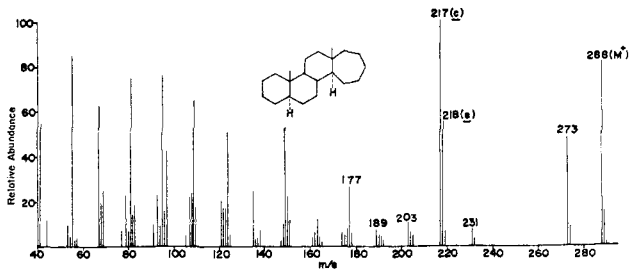


Figure 5. Mass spectrum of *D*-bishomo-5α,14α-androstane (XI) determined at 70 eV.

Results

***D*-Homosteroids.** The results of the extensive deuterium labeling experiments which bear on the genesis of the ions of mass 217 and 218 in *D*-homo-5α-pregnane (III) and *D*-homo-5α-androstane (IV) are listed in Tables I and II. The mechanisms of the processes are depicted in Scheme II; it is clear that in both cases the processes occur in a manner analogous to the five-membered ring D compounds (*cf.* Scheme I).[10]

Table I. Shifts[a] of Peaks Corresponding to Ring D Cleavage in *D*-Homo-5α-pregnane (III)

D-Homo-5α-pregnane	Isotopic purity, %	M^+	$M - C_6H_{12}$, %	$M - C_6H_{13}$, %
-d_0 (III)		302	218	217
-*21,21,21*-d_3	95% d_3	305	218	217
-*20,17,17*-d_3	50% d_3	305	218	217
-*17,17*-d_2	90% d_2	304	218	217
-*16,16*-d_2	98% d_2	304	219 (80%) 218 (20%)	217
-*14*-d_1	90% d_1	303	219	218 (34%)[b] 217 (66%)
-*18,18,18*-d_3	90% d_3	305	220 (80%) 221 (20%)	220
-*3,3*-d_2	70% d_2	304	218	217

[a] Reported shifts are corrected for isotopic impurity as well as ^{13}C contributions and are greater than 95% unless otherwise indicated. [b] These figures are not corrected for the previously reported[6,11] elimination of methyl from the *m/e* 232 ion. Consequently, the figure of 66% is a minimum value for the per cent transfer of the C-14 hydrogen in the cleavage pattern depicted in eq 6.

The specificity of these processes equals and generally exceeds that observed in pregnane (I) and androstane (II) themselves. Thus, in the formation of the mass 217 ion, the C-14 hydrogen is expelled 66% of the time in *D*-homopregnane (*cf.* 50% in pregnane), and 50% of the time in *D*-homoandrostane (33% in andro-

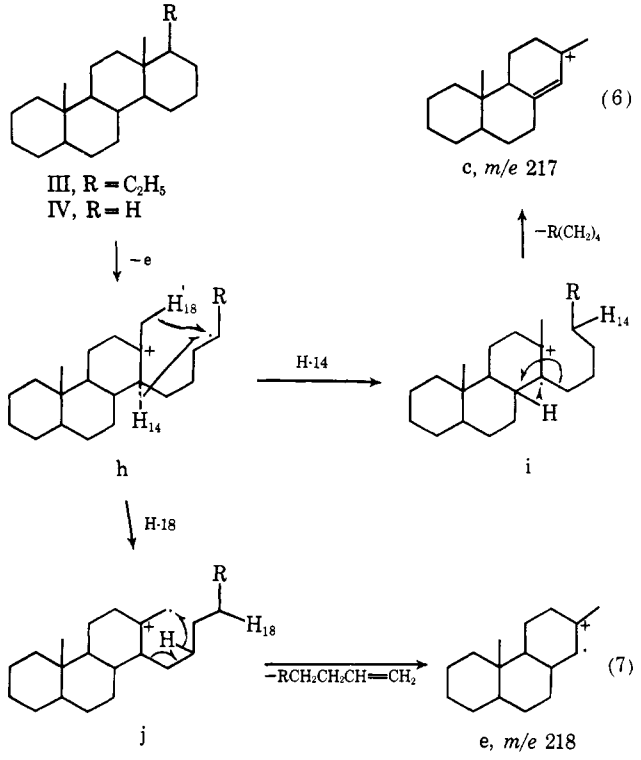
Table II. Shifts[a] of Peaks Corresponding to Ring D Cleavage in *D*-Homo-5α-androstane (IV)

D-Homo-5α-androstane	Isotopic purity, %	M^+	$M - C_4H_8$, %	$M - C_4H_9$, %
-d_0 (IV)		274	218	217
-*17a,17a*-d_2[b]	92% d_2	276	218	217
-*17,17*-d_2	93% d_2	276	218	217
-*16,16*-d_2	98% d_2	276	219	217
-*14*-d_1	90% d_1	275	218	218 (50%)[c] 217 (50%)[c]
-*18,18,18*-d_3	98% d_3	277	220 (95%) 221 (5%)	220
-*3,3*-d_2[b]	92% d_2	276	220	219

[a] Reported shifts are corrected for isotopic impurities as well as ^{13}C contributions, and are greater than 95% unless otherwise indicated. [b] In unlabeled D-homo-5α-androstane, cleavage across ring A or ring D generates ions of identical mass. The -17α,17α-d_2 and -3,3-d_2 derivatives permit calculation of the relative amount of cleavage in either ring [c] These figures are not corrected for the previously reported[6,11] elimination of methyl from the *m/e* 232 ion. Consequently, the figure of 50% is a minimum value for the per cent transfer of the C-14 hydrogen n the cleavage pattern depicted in eq 6.

stane).[14] These increases in specificity provide further support for the concept of fragmentation from molecular ions of structure h, formed by cleavage of the C-13–C-17a bond in *D*-homosteroids, and ions of structure a,

Scheme II



formed by cleavage of the C-13–C-17 bond in ordinary steroids. Abstraction of the C-14 hydrogen in ions of structure a proceeds through a five-membered ring; however, this process must compete with abstraction of other hydrogen atoms throughout the steroid nucleus.[6,11] In each case, an ion of mass 217 is formed. In *D*-homosteroids, on the other hand, abstraction of the C-14 hydrogen proceeds through a six-membered ring, while abstraction of any other hydrogen must involve less favorable ring sizes. The increase in specificity observed in the formation of the mass 217 ion in the *D*-homo series thus seems to reflect the well-known preference for six-membered rings in mass spectroscopy, and is completely consistent with the intervention of molecular ions of structures a and j in this fragmentation process.

The mass 218 ion is also formed with high specificity in the *D*-homo series. Thus, *D*-homoandrostane (IV) expels a C-18 hydrogen 90% of the time (*cf.* to 65% in androstane) and retains a C-16 hydrogen 95% of the time (70% in androstane). Similar specificities are observed in *D*-homopregnane (III) and pregnane (I) for the formation of the *m/e* 218 ion. In each case, a C-18 hydrogen is eliminated 80% of the time, and a C-16 hydrogen is retained 80% of the time.

These results exclude the significant participation of a mechanism involving the expulsion of a cyclobutane derivative in the formation of the mass 218 ion (eq 4). The driving force for the expulsion of olefin must be sufficiently great that the decrease of ring strain in the potential expelled neutral species does not tip the energetic balance in favor of the simple cleavage mechanism. It appears likely then that these hydrogen transfers also occur in steroids containing even larger ring systems (*e.g.*, in the *D*-bishomosteroids).

It should be pointed out that the crucial step for producing the aforementioned reciprocal hydrogen transfer is the migration of the C-18 hydrogen atom to the radical site at C-17a (h → j). The similarity of the ion j to that produced by the analogous step in the five-membered ring D series (a → d) is striking; it is not surprising that the structurally nearly identical ions j and d fragment identically.

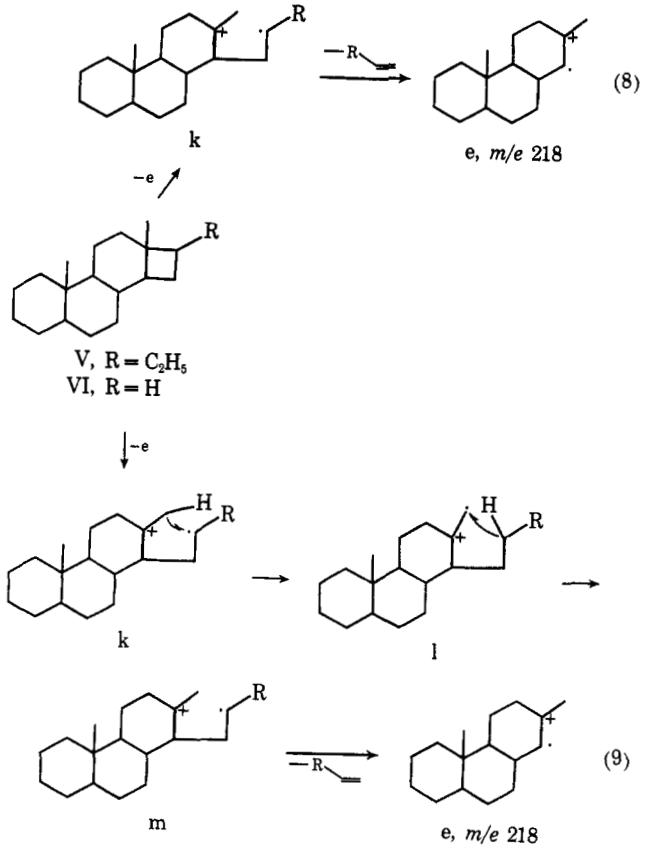
***D*-Norsteroids.** The base peak in the mass spectrum (Figure 1) of *D*-nor-5α-pregnane (V) appears at *m/e* 218, corresponding to the elimination of 1-butene from the molecular ion; similarly, the mass spectrum (Figure 3) of *D*-nor-5α-androstane exhibits a prominent peak corresponding to the elimination of ethylene at *m/e* 218. This fragmentation, which involves expulsion of ring D and any substituents on C-16, occurs without hydrogen transfer from C-16. The mechanism of the fragmentation is depicted in Scheme III. The alternative mechanism, analogous to that observed in ordinary or *D*-homosteroids, is depicted in eq 9 and was not observed.

It is clear that the charged (e) and expelled neutral species (alkene) are identical in both mechanisms. In contrast to the situation with normal steroids or *D*-homosteroids, no driving force exists to produce a reciprocal hydrogen transfer; the ion m produced after reciprocal hydrogen transfer would be identical with the ion k formed directly on electron impact.

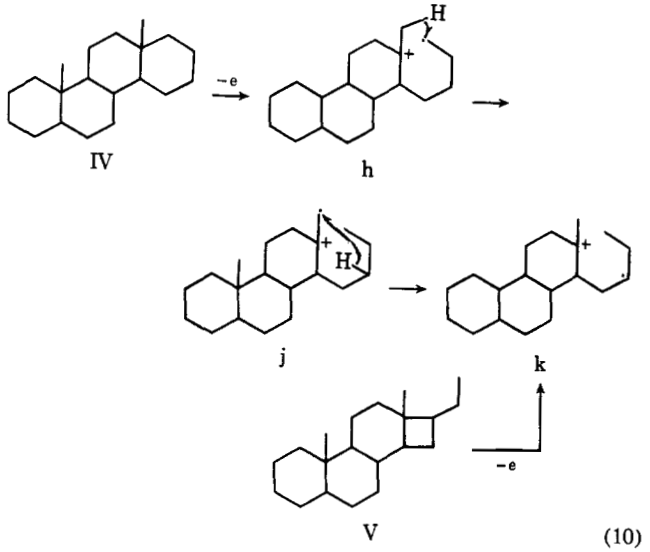
It is interesting to note that the ion k produced by simple cleavage of the C-13–C16 bond in *D*-norpregnane is structurally identical with that produced by reciprocal hydrogen transfer in the formation of the *m/e* 218 ion of *D*-homoandrostane (eq 10). Thus, the facile elimination of 1-butene from ion k provides confirmatory evidence for the earlier mechanistic postulation.

(14) The *m/e* 217 peak in the spectra of pregnane[6] and androstane[11] arises to a significant extent (*ca.* 20%) by the elimination of the C-19 methyl group from the mass 232 ion. Since the C-19 methyl group was not labeled in the *D*-homo series, it is not possible to determine the extent to which this cleavage pattern pertains. Thus, the figures quoted for per cent expulsion of the C-14 hydrogen of pregnane and androstane and of the corresponding *D*-homo compounds are not corrected for this alternative process.

Scheme III

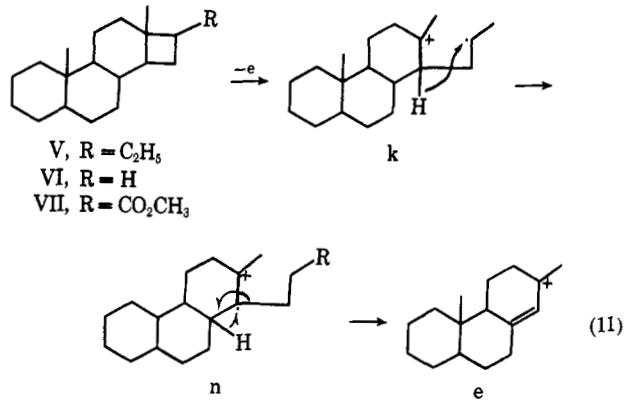


The genesis of the mass 217 ion in the mass spectrum of *D*-norpregnane and *D*-norandrostane was investigated. Deuterium labeling experiments on the hydrocarbons (V, VI) demonstrate that the fragmentation involves cleavage of ring D. Furthermore, in the ester VII the C-14 hydrogen is expelled 25% of the time. It appears then, that at least qualitatively a mechanism similar to that observed in the normal and *D*-homo steroids is operative here (Scheme IV).[10]

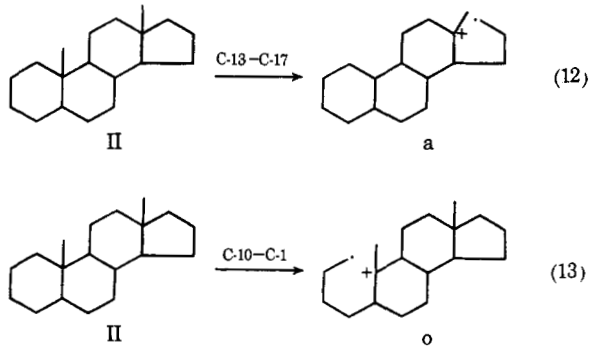


The low specificity observed for abstraction of the C-14 hydrogen atom can be readily explained. Migration of the C-14 hydrogen to the radical site at C-16 proceeds through an unfavorable four-membered transition state. Other hydrogen atoms are more favorably disposed for migration (*e.g.*, transfer of the C-12 hydrogen would involve a very favorable six-membered ring).

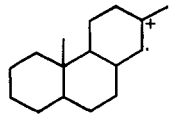
Scheme IV



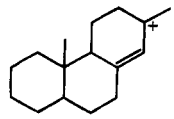
Effect of Structure on Charge Localization. The mass spectrum of 5α-pregnane is dominated by cleavage about ring D to produce ions of mass 217 and 218. Earlier deuterium labeling experiments[6] demonstrated that these fragmentations occur from a species a, with charge localized in the C-13–C-17 bond. This observation can be readily rationalized; cleavage of the C-13–C-17 bond generates a tertiary carbonium ion and a secondary radical site, relieves the interactions among the C-17 substituent, the angular methyl, and the 12-carbon, and finally releases the strain inherent in the trans-fused hydrindan system. The last should persist in the fragmentation of 5α-androstane. Inspection of its mass spectrum, however, indicates that roughly twice as much cleavage occurs in the *trans*-decalin A/B ring system as compared to the more strained *trans*-hydrindan C/D ring system (*i.e.*, (203 + 204)/(217 + 218) = 2.0). Since earlier mechanistic studies have demonstrated that the mechanisms of ring A[11] and ring D[6] cleavage are similar, and that they proceed from ions of similar structure (a and o, respectively), it seems likely that this observation is indicative of preferential charge localization in ring A, rather than a preferential reactivity of ion o over ion a. In order to better understand this apparently contradictory observation, a number of structurally modified androstanes were prepared; their mass spectra are discussed below.



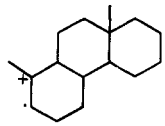
5α-*D*-Homoandrostane (IV) is a relatively symmetrical molecule. Cleavage about ring A or ring D gives rise (in the absence of deuterium labeling) to products of identical mass (*m/e* 217 and 218) and of similar structure (e and c *vs.* p and q). Because both the A/B and C/D ring systems exist in the *trans*-decalin configuration, *a priori* it might be expected that fragmentation would occur about each ring to a similar extent.



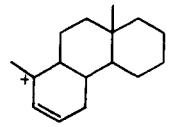
e, *m/e* 218



c, *m/e* 217



p, *m/e* 218



q, *m/e* 217

In fact, deuterium labeling experiments demonstrate (Table III) that *ring A cleavage occurred 2.7 times as*

Table III. Effect of Structure on the Ratio of Ring A Cleavage to Ring D Cleavage[a]

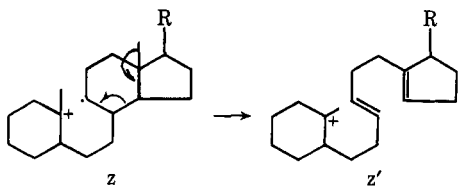
Compound	Ring A/ring D
D-Nor-5α,14α-androstane (VI)[b]	<1/15
5α,14α-Androstane (II)	2.0/1
5β,14α-Androstane (VIII)	3.3/1
5α,14β-Androstane (IX)	8/1
D-Homo-5α,14α-androstane (IV)	2.7/1
18-Nor-*D*-homo-5α,14α-androstane (X)	>20/1
D-Bishomo-5α,14α-androstane (XI)	<1/15
D-Nor-5α,14α-pregnane (V)[b]	<1/20
5α,14α-Pregnane (I)	<1/20
D-Homo-5α,14α-pregnane (III)	<1/20

[a] Only the characteristic ring D and ring A cleavages are considered in determining these ratios. [b] The characteristic ring D fragmentation of these compounds is mechanistically distinct from the others listed in the table; direct comparison is thus impossible.

readily as ring D cleavage. Apparently, fission of the 1–10 bond to form ion r releases the strain inherent in the gauche butane system encompassed by carbon atoms 1, 10, 9, and 11; in contrast, cleavage of the 13–17a bond releases only the strain inherent in an anti butane system, C-11–C-12–C-13–C-17a. (Although ring D contains a structurally analogous gauche butane interaction (C-7–C-8–C-14–C-15) breaking of the 13–17a bond would not release the strain inherent in this system. Thus, it does not contribute to preferential charge localization in ring D.) A difference in energy of about 0.9 kcal/mol[15] is sufficient to provide a strong discrimination factor between these competitive fragmentations.[16]

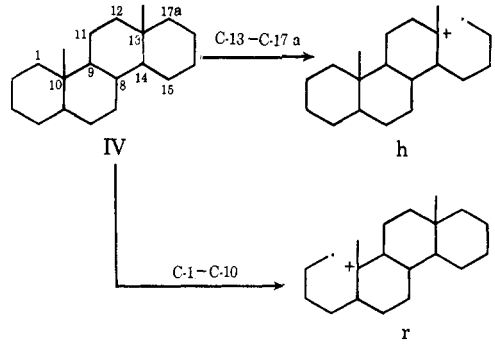
(15) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 125.

(16) The C-9–C-10 bond appears to be a favorable site for charge localization, since the resulting species z contains a tertiary carbonium ion and a secondary radical site. In addition, breaking the C-9–C-10 bond relieves two gauche interactions (C-1–C-10–C-9–C-11, C-19–C-10–C-9–C-11). Nevertheless, no major peaks can be unambiguously



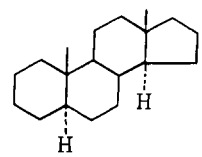
z z′

attributed to ions of this structure. For example, the low-voltage spectra of pregnane[6] and androstane[11] exhibit no major peaks near *m/e* 95, as would be produced by the expected fission of the C-5–C-6 bond of ion z. The explanation for this curious observation may be that these ions are largely consumed by the very favorable M − 15 process (z → z′) before more complicated processes, such as ring cleavages, can intervene.

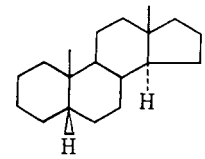


IV h r (14)

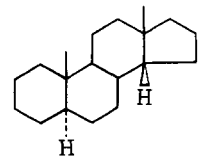
This surprising result suggests that significant differences in ring cleavage ratios might be observed in other steroidal systems, and that comparison among these systems might provide further insight into the factors affecting charge localization in steroids. Thus, since the ratio of ring A to ring D cleavage declines from 2.7/1 in *D*-homo-5α-androstane (IV) to 2.0/1 in 5α,14α-androstane (II), it is tempting to attribute this change to the increased strain inherent in a *trans*-hydrindan, as compared to a *trans*-decalin system. Alternatively, however, this observation might merely be reflective of a greater reactivity of ions with charge localized in the five-membered D ring. Comparison among "normal" steroids is less ambiguous, however, since, in these cases, the competition is always between a six- and a five-membered ring for fragmentation; differences in reactivity due to ring size would be expected to "cancel out." Thus, the 2.0/1 ratio observed for 5α,14α-androstane (II) contrasts with the 3.3/1 ratio in 5β,14α-androstane (VIII). The charge is localized even more strongly in ring A by the *cis*-decalin structure, since an additional gauche butane interaction (C-2–C-1–C-10–C-9) is relieved by cleavage of the C-1–C-10 bond. Finally in 5α,14β-androstane (IX) both the A/B and C/D ring systems are fused in their most stable forms (trans and cis, respectively). Strikingly, ring A cleavage is favored by 8/1 over ring D cleavage in compound IX; relieving the strain inherent in the trans-fused hydrindan system II decreases fragmentation about ring D by a factor of four (IX *vs.* II).



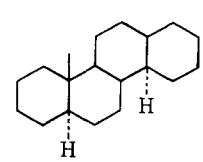
II



VIII



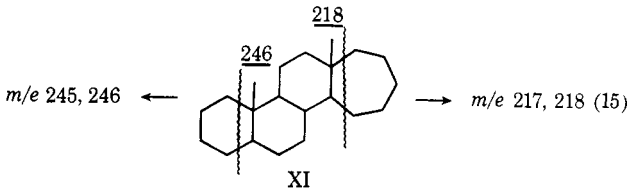
IX



X

The potent effect of the stereochemistry at C-5 and C-14 on fragmentation patterns in the androstane series provides strong evidence for the integrity of the C-5–C-10 bond and the C-13–C-14 bond after electron impact. *Since these bonds are as highly substituted as any in the androstane nucleus, this observation suggests that the entire steroid nucleus retains its stereochemistry before fragmentation.*

The mass spectrum (Figure 5) of *D*-bishomo-5α,14α-androstane (XI) provides another interesting observa-



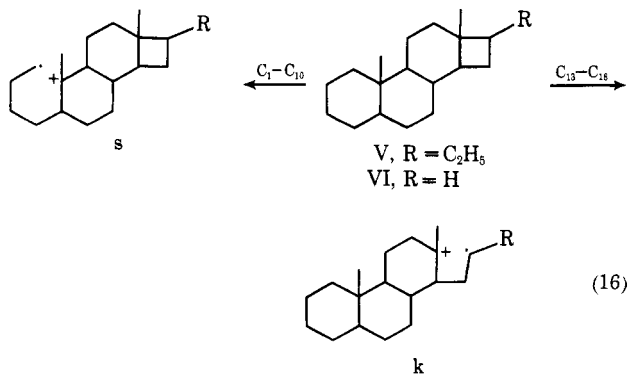
XI

tion. Ring cleavage about the seven-membered D ring predominates by more than 15/1 over ring A cleavage. This initially surprising result can be rationalized by a consideration of steric effects; severe flagpole interactions between ring hydrogens or a ring hydrogen and the angular methyl group exist in every conformation of ring D.

The spectrum of 18-nor-*D*-homoandrostane (X) indicates that cleavage occurs essentially only about ring A. This result confirms the necessity for an energetically favorable site for charge localization, if fragmentation is to occur; cleavage of the C-13–C-17a bond, because of the absence of the angular methyl group at C-13, now generates a secondary carbonium ion and a primary radical, an unfavorable result.

These results are consistent with the spectra of 5α-14α-pregnane (I) and *D*-homo-5α,14α-pregnane (III). In each case, fragmentation occurs almost exclusively about ring D. Introduction of an ethyl substituent adjacent to the angular methyl group adds two additional gauche butane interactions (C-18–C-13–C-17 (or C-17a)–C-20 and C-12–C-13–C-17 (or C-17a)–C-20) which are relieved by cleavage of the C-13–C-17 (or C-17a) bond. Thus, cleavage about this bond relieves one more gauche interaction than cleavage of the C-1–C-10 bond. In addition, of course, charge localization in the C-13–C-17 (or C-17a) bond generates a tertiary carbonium ion and a secondary radical site; charge localization in ring A generates only a primary radical and a tertiary carbonium ion. It is not surprising, therefore, that virtually no fragmentation is observed about ring A.

It would be predicted that extensive charge localization would be observed in the C-13–C-16 bond of the *D*-nor steroids, since cleavage of this bond relieves the strain inherent in the trans-fused cyclobutane system. Indeed, no evidence for ring A fragmentation is observed in the mass spectra (Figures 1 and 3) of *D*-norpregnane (V) or *D*-norandrostane (VI).[17] It must be

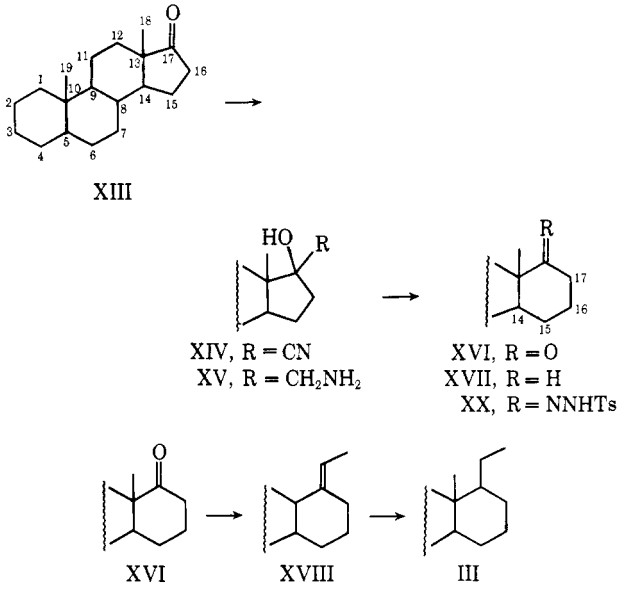


remembered, however, that the *m/e* 218 peaks observed in these spectra arise in a mechanistically distinct manner from the usual reciprocal hydrogen transfer; the ions of structure k are undoubtedly more reactive than those of structure s. Thus, the failure to observe cleavage about ring A does not unambiguously exclude the existence of significant amounts of ions of structure s.

In summary, these observations demonstrate that the competition between rings A and D for charge localization, and thus for fragmentation, is a sensitive probe for steric interaction. This concept is likely to prove valuable in interpreting the mass spectra of unknown steroids.

Synthesis of Labeled and Unlabeled *D*-Bishomo-, *D*-Homo-, and *D*-Norpregnanes and -androstanes. The *D*-homosteroids utilized in this study were generally prepared by the method depicted in Scheme V.[18]

Scheme V



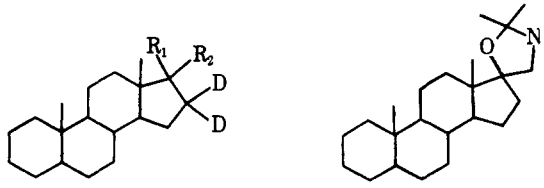
D-Homoandrostane-*17a,17a-d*$_2$ was prepared by the reduction of the corresponding tosylhydrazone derivative (XX) with sodium borodeuteride in deuteriomethanol. *D*-Homoandrostane-*17,17-d*$_2$ was prepared from the correspondingly labeled ketone; conversion to the tosylhydrazone followed by reduction with sodium borohydride in methanol gave the desired labeled hydrocarbon.

The preparation of *D*-homoandrostane-*16,16-d*$_2$ was less straightforward. Androstan-17-one-*16,16-d*$_2$ which was prepared by base-catalyzed exchange of the unlabeled ketone XIII was converted to the corresponding cyanohydrin (XXI). In order to avoid possible hydrogen–deuterium randomization, the cyanohydrin was not reduced catalytically; conversion to the *O*-acetate (XXII), reduction with lithium aluminum hydride, and conversion of the resulting hydroxyamine (XXIII) to the corresponding oxazolidine (XXIV) were accomplished by the procedure of Heusser, *et al.*[19] Tiffeneau rearrangement of oxazolidine XXIV gave

(17) The large peak at *m/e* 189 in the mass spectrum of *D*-norandrostane (Figure 3) corresponds formally to cleavage about ring A. Deuterium labeling experiments demonstate, however, that this process corresponds to cleavage about ring D. The origin of this *m/e* 189 peak is discussed in greater detail in part B of this paper.

(18) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 583.

(19) H. Heusser, P. Th. Herzig, A. Fürst, and Pl. A. Plattner, *Helv. Chim. Acta*, 33, 1093 (1950).

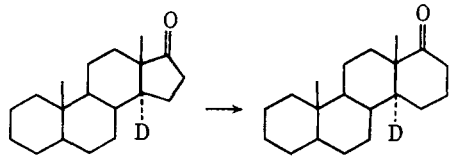


XXI, $R_1 = OH$; $R_2 = CN$
XXII, $R_1 = OC(=O)CH_3$; $R_2 = CN$
XXIII, $R_1 = OH$; $R_2 = CH_2NH_2$

XXIV

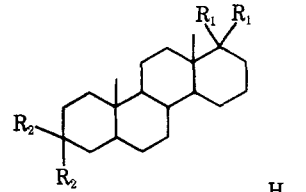
D-homoandrostan-17a-one-*16,16-d*$_2$, which was converted to *D*-homoandrostane-*16,16-d*$_2$ by Wolff–Kishner reduction.

The preparation of *D*-homoandrostane-*14α-d*$_1$ started with androstan-17-one-*14α-d*$_1$;[20] homologation to *D*-homoandrostan-*14α-d*$_1$ (XXVI) was accomplished according to the procedure already described.



XXV XXVI

D-Homoandrostane-*3,3-d*$_2$ was prepared from *D*-homoandrostan-3-ol-17a-one (XXVII).[21] Wolff–Kishner reduction, followed by oxidation, gave *D*-homoandrostan-3-one (XXVIII). Preparation of the tosylhydrazone and reduction with sodium borodeuteride in deuteriomethanol gave *D*-homoandrostane-*3,3-d*$_2$ (XXIX).



XXVII, $R_1 = O$; $R_2 = \langle^{H}_{OH}$
XXVIII, $R_1 = H$; $R_2 = O$
XXIX, $R_1 = H$; $R_2 = D$

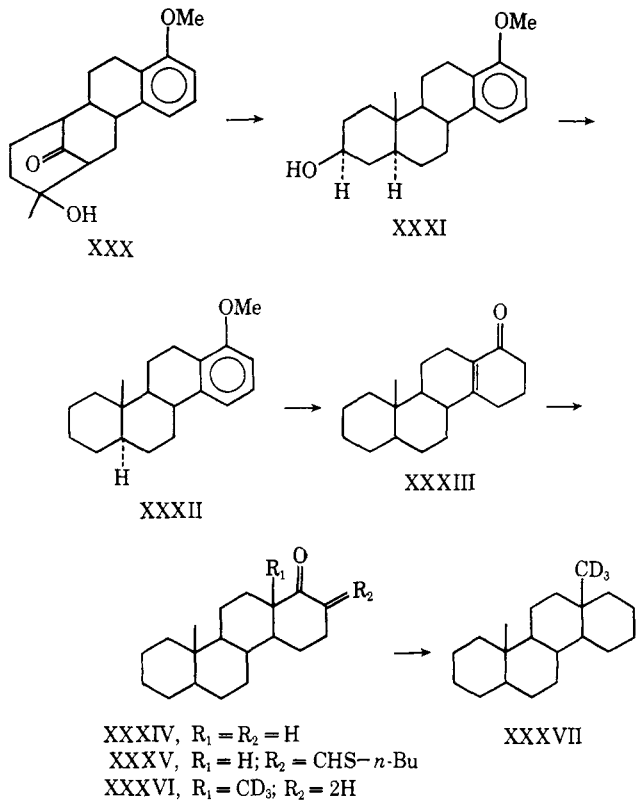
D-Homoandrostane-*18,18,18-d*$_3$ was prepared by total synthesis, as depicted in Scheme VI.

The labeled *D*-homopregananes were generally prepared from the labeled *D*-homoandrostan-17a-ones which were obtained as described above. Wittig reaction, followed by catalytic hydrogenation, gave *D*-homopregnane-*17,17,d*$_2$, *-16,16-d*$_2$, *-14α-d*$_1$, *-3,3-d*$_2$, and *-18,18,18-d*$_3$.

The *-21,21,21-d*$_3$ derivative was prepared by utilizing a Wittig reagent prepared from bromoethane-*2,2,2-d*$_3$ and triphenylphosphine. Catalytic hydrogenation of the resulting alkene gave *D*-homopregnane-*21,21,21-d*$_3$.

D-Bishomoandrostane (XI) was prepared through two different routes (Scheme VII). Conversion of *D*-homoandrostan-17a-one (XVI) to the cyanohydrin XXXVIII, catalytic hydrogenation to the hydroxyamine XXXIX, followed by Tiffeneau rearrangement, gave *D*-bishomoandrostan-17a-one (XL).[22] Alternatively, the aluminum chloride catalyzed homologation of androstan-17-one (XIII) with diazomethane gave *D*-bishomoandrostan-17b-one (XLII). Wolff–Kishner reduction of either ketone gave *D*-bishomoandrostane (XI).

Scheme VI

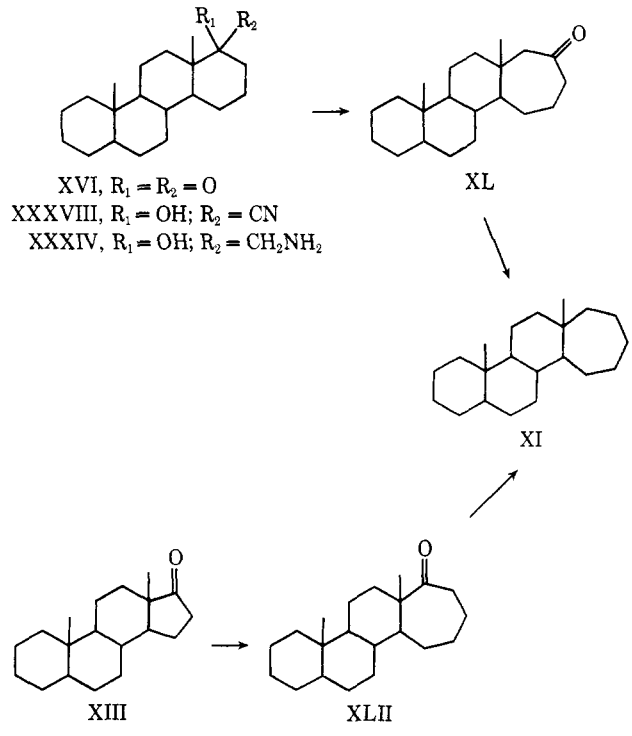


XXX XXXI XXXII XXXIII

XXXIV, $R_1 = R_2 = H$
XXXV, $R_1 = H$; $R_2 = CHS-n\text{-}Bu$
XXXVI, $R_1 = CD_3$; $R_2 = 2H$

XXXVII

Scheme VII



XVI, $R_1 = R_2 = O$
XXXVIII, $R_1 = OH$; $R_2 = CN$
XXXIV, $R_1 = OH$; $R_2 = CH_2NH_2$

XL XI XIII XLII

(20) G. Jones and C. Djerassi, *Steroids*, **10**, 653 (1967).
(21) N. L. Allinger, J. Allinger, and M. A. DaRooge, *J. Amer. Chem. Soc.*, **86**, 4061 (1964).
(22) According to an earlier report (M. W. Goldberg and S. Studer, *Helv. Chim. Acta*, **25**, 1556 (1942)), subjecting *D*-homoestr-17a-one 3-acetate (i) to a similar sequence of reactions gave the 17b ketone ii.

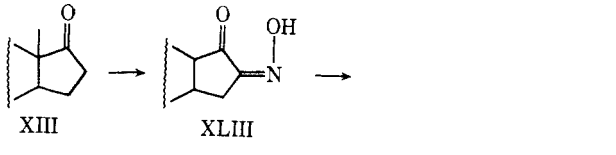
5α,14β-Androstane (IX) was prepared by the Wolff–Kishner reduction of the corresponding ketone. The ketone itself was prepared by the photolysis of 5α,14β-androstan-17-one in the presence of mercuric bromide as described by Gorodetsky and Mazur.[23]

5α,14β-Androstane VIII was prepared from 5α,14β-androstan-17-one[24] by a modified Huang-Minlon reduction.[25]

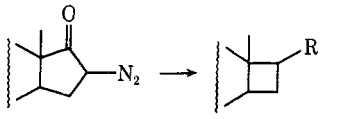
The *D*-norsteroids utilized in this study was prepared essentially according to the procedure of Meinwald, *et al.*[26] Androstan-17-one (XIII) was converted to 16-oximinoandrostan-17-one (XLIII) by treatment with isoamyl nitrite in *tert*-butyl alcohol containing potassium *tert*-butoxide. The oxime XLIII was converted to the corresponding diazo ketone (XLIV) by reaction with chloramine. Irradiation of the diazo ketone yielded *D*-norandrostane-16β-carboxylic acid (XLV).

The acid was treated with methyllithium to give the ketone XLVI. Electrolytic reduction[27] of the ketone yielded *D*-norpregnane (V).

Baeyer–Villager oxidation of the ketone XLVI gave, after hydrolysis of the intermediate acetate, *D*-norandrostan-16β-ol (XLVII). Conversion to the corresponding tosylate (XLVIII) followed by lithium aluminum hydride reduction gave *D*-norandrostane (VI).



XIII XLIII

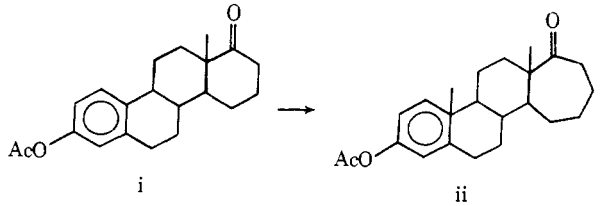


XLIV

XLV, R = CO_2H
XLVI, R = $COCH_3$
V, R = C_2H_5
XLVII, R = OH
XLVIII, R = OTs
VI, R = H

The preparation of the labeled derivative of *D*-norandrostane and -pregnane was straightforward. Base-catalyzed exchange of 16-acetyl-*D*-norandrostane (XLVI) gave the corresponding deuterated ketone. Electrolytic reduction yielded *D*-norpregnane-*16,20,20,-20-*d_4.

This structural assignment was made without the benefit of modern day spectroscopic techniques.



i ii

D-Norandrostane-*16β-*d_1 was prepared by the reduction of the tosylate XLVIII with lithium aluminum deuteride.

Experimental Section[28]

***D*-Homo-5α-androstane (IV).** A solution of 50 mg of *D*-homo-5α-androstan-17a-one[29] in 4 ml of diethylene glycol and 2 ml of hydrazine (97%) was heated at reflux for 1 hr. The solution was subsequently cooled to about 100°, treated with 100 mg of powdered potassium hydroxide, and heated under a slow stream of nitrogen (without a condenser) to 205°. After the evolution of low boiling materials ceased, the condenser was replaced and the heating was continued at 205° for 4 hr. The mixture was then cooled, poured into water, and extracted with hexane. Tlc (hexane eluent) and recrystallization (methanol) gave 35 mg of *D*-homo-5α-androstane (IV), mp 95–96° (lit.[30] mp 84.5–87°, $[\alpha]D$ −2.9° (dioxane).

Anal. Calcd for $C_{20}H_{34}$ (mol wt, 274; M^+ (obsd), 274): C, 87.51; H, 12.49. Found: C, 87.64; H, 12.43.

***D*-Homo-5α-pregn-17a(20)-ene (XVIII).** *D*-Homo-5α-androstan-17a-one (100 mg) in 2 ml of tetrahydrofuran was added to a solution of ethyltriphenylphosphonium iodide (3 g) in dry DMSO (8 ml). The mixture was stirred at room temperature in an atmosphere of dry nitrogen and a solution of potassium *tert*-butoxide (0.81 mg) in dry DMSO (5 ml) was added. After 18 hr, the solution was poured into ice water. Extraction with hexane, washing, drying ($MgSO_4$), and preparative tlc (hexane eluent) gave 70 mg of *D*-homo-5α-pregn-17a(20)-ene, mp 82–84°.

Anal. Calcd for $C_{22}H_{36}$ (mol wt, 300; M^+ (obsd), 300): C, 87.92; H, 12.08. Found: C, 88.07; H, 12.17.

***D*-Homo-5α-pregnane (III).** *D*-Homo-5α-pregn-17a(20)-ene (50 mg) was hydrogenated at atmospheric pressure in 20 ml of acetic acid over platinum black (generated *in situ* from 50 mg of platinum dioxide). After the uptake of hydrogen had ceased, the catalyst was removed by filtration and the solution was diluted with water and extracted with hexane. After washing, drying ($MgSO_4$), and removal of the solvent under vacuum, the resulting solid was purified by recrystallization from methanol.

The resulting solid exhibited mp 119–120°.

Anal. Calcd for $C_{22}H_{38}$ (mol wt, 302; M^+ (obsd), 302): C, 87.34; H, 12.66. Found: C, 87.51; H, 12.69.

***D*-Homo-5α-androstane-*17a,17a-*d_2.** To a solution of 50 mg of *D*-homo-5α-androstan-17a-one and 50 mg of *p*-toluenesulfonylhydrazide in 5 ml of anhydrous methanol was added a single drop of concentrated sulfuric acid. After 2-hr reflux, the solution was poured into water. After filtration of the crystalline precipitate, and preparative tlc (eluent, ether–benzene, 1:1), recrystallization from methanol gave 50 mg of tosylhydrazone as white crystals: mp 197–198°; ν_{max} 1600 (C=C), 1170 (S=O) cm^{-1}; no carbonyl absorption.

Anal. Calcd for $C_{27}H_{40}N_2$: mol wt, 392; M^+ (obsd), 392.

A solution of the tosylhydrazone (40 mg) in deuteriomethanol (2 ml) was treated with an excess of sodium borodeuteride (50 mg). After 15 hr at reflux, the slurry was cooled, and extracted with ether. The ether solution was washed and dried, and the residue after the evaporation of the solvent was purified by tlc (benzene eluent) to yield 30 mg of *D*-homo-5α-androstane-*17a,17a-*d_2, mp 94–95° (92% d_2).

***D*-Homo-5α-androstane-*17,17-*d_2.** *D*-Homo-5α-androstan-17a-one, (100 mg) was dissolved in 25 ml of deuteriomethanol containing 1 ml of 20% sodium deuteroxide in deuterium oxide; the solution was heated overnight at reflux. The solvent was evaporated at reduced pressure and the residue was dissolved in 25 ml of deteriomethanol. After the exchange process had been repeated four times

(23) M. Gorodetsky and Y. Mazur, *J. Amer. Chem. Soc.*, **90**, 6540 (1968).

(24) This sample was generously supplied by Syntex S.A., Mexico City, Mexico.

(25) This reaction was carried out in these laboratories by Dr. Laszlo Tökés.

(26) J. Meinwald, L. Labana, and T. Wheeler, *J. Amer. Chem. Soc.*, **92**, 1006 (1970); see also M. P. Cava and E. Moroz, *ibid.*, **84**, 115 (1962); J. L. Mateos and O. Chao, *Bol. Inst. Quim. Univ. Nac. Auton. Mex.*, **13**, 3 (1961); G. Muller, C. Huynh, and J. Mathieu, *Bull. Soc. Chim. Fr.*, 296 (1962).

(27) L. Throop and E. Tökés, *J. Amer. Chem. Soc.*, **89**, 4789 (1967).

(28) Melting points are uncorrected, and were determined in unsealed capillaries. Infrared spectra were measured in chloroform solution on a Perkin-Elmer Model 700 infrared spectrophotometer. Nmr spectra were determined in deuteriochloroform solution with tetramethylsilane as an internal reference on a Varian T-60 spectrometer, unless otherwise indicated. Mass spectra were determined on an Atlas-CH-4 spectrometer with a TO-4 ion source using the direct inlet procedure. The authors are grateful to Mr. Richard Conover for performing these measurements. All mass spectral samples were purified by preparative vpc on a Hewlett-Packard 402 gas chromatography immediately prior to submission. Thin layer chromatography was performed on silica gel H_{254}. The elemental analyses are due to E. Meier and J. Consul.

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(30) M. W. Goldberg and E. Wydler, *Helv. Chim. Acta*, **26**, 1142 (1943).

the residue was purified by preparative tlc (CH_2Cl_2 eluent) to give *D*-homo-5α-androstan-17a-one-*17,17-d*$_2$ (82 mg), 93% d_2.

The tosylhydrazone derivative was prepared by refluxing a solution of the ketone (80 mg) and *p*-toluenesulfonylhydrazide (80 mg) in 5 ml of deuteriomethanol containing 2 drops of phosphorus pentachloride. The crude tosylhydrazone was converted to *D*-homo-5α-androstane-*17,17-d*$_2$ (93% d_2) by reaction with sodium borohydride in methanol according to the standard procedure described above.

***D*-Homo-5α-androstane-*16,16-d*$_2$.** Androstan-17-one (XIII) was exchanged with sodium deuterioxide in deuteriomethanol–deuterioxide according to the procedure described above. The resulting 5α-androstan-17-one-*16,16-d*$_2$ (98% d_2) was condensed with potassium cyanide according to the usual procedure. Conversion to the *O*-acetate XXII and reduction with lithium aluminum hydride, and conversion of the resulting hydroxyamine XXIII to the oxazolidine was accomplished by the procedure of Heusser, *et al.*[19]

The oxazolidine XXIV was converted directly to *D*-homo-5α-androstan-17a-one-*16,16-d*$_2$ (98% d_2) by Tiffeneau rearrangement under the usual conditions. Wolff–Kishner reduction as described above gave *D*-homo-5α-androstane-*16,16-d*$_2$ (98% d_2).

***D*-Homo-5α-androstane-*14α-d*$_1$.** Homologation of androstan-17-one-*14α-d*$_1$ (XXV)[20] to *D*-homo-5α-androstan-17a-one-*14α-d*$_1$ (XXVI) and Wolff–Kishner reduction in the usual manner gave *D*-homoandrostane-*14α-d*$_1$ (90% d_1).

***d,l-D*-Homo-5α-androstane-*18,18,18-d*$_3$ (XXXVII).** Using procedures which have already been described in the literature,[31] *trans-anti-trans*-1-methoxy-8β-hydroxy-10a-methyl-4,5,6,6a,7,8,9,-10,10a,10b,11,12-dodecahydrochrysene (XXXI) was prepared from the tetracyclic condensation product XXX.[32]

Jones oxidation of the alcohol, followed by Wolff–Kishner reduction in the usual manner, gave *trans-anti-trans*-1-methoxy-10a-methyl-4b,5,6,6a,7,8,9,10,10a,10b,11,12-dodecahydrochrysene (XXXII), mp 83–85°.

Anal. Calcd for $C_{20}H_{28}O$: mol wt, 284; M^+ (obsd), 284.

Birch reduction, followed by hydrolysis of the intermediate enol ether according to the procedure of Johnson, *et al.*,[31] gave a complex mixture of products from which *d,l*-$\Delta^{13,14}$-dehydro-18-nor-*D*-homo-5α-androstan-17a-one (XXXIII) was isolated by preparative tlc (CH_2Cl_2 eluent). The α,β-unsaturated ketone exhibited: mp 127–129°; ir ν_{max} 1660, 1620 cm^{-1} (C=CCO); uv λ_{max} 247 mμ (log ε 4.19); nmr absence of signals below 3 ppm (no olefinic portons), angular methyl at 0.77 ppm.

Anal. Calcd for $C_{19}H_{28}O$ (mol wt, 272; M^+ (obsd), 272): C, 83.77; H, 10.36. Found: C, 83.47; H, 10.26.

The α,β-unsaturated ketone was dissolved in 95% ethanol containing a catalytic amount of potassium hydroxide. Reduction over 10% palladium on carbon at 45 psi of initial pressure of hydrogen (by analogy to the procedure of Johnson, *et al.*[31]) gave *d,l*-18-nor-*D*-homo-5α-androstan-17a-one (XXXIV): mp 113–115°; ir ν_{max} 1700 cm^{-1} (C=O).

Anal. Calcd for $C_{19}H_{30}O$ (mol wt 274; M^+ (obsd), 274): C, 83.15; H, 11.02. Found: C, 83.00; H, 11.19.

To 25 ml of a benzene solution containing 245 mg of *d,l*-18-nor-*D*-homo-5-androstan-17a-one and 245 mg of ethyl orthoformate, 100 mg of 15% ethanolic hydrogen chloride was added. The mixture was heated to reflux under a nitrogen atmosphere; after 3 and 8 hr, additional 50-mg portions of ethanolic hydrochloric acid were added. After 20 hr, the mixture was cooled, neutralized with ethanolic potassium hydroxide, washed with water, aqueous sodium bicarbonate, and water, and dried over $MgSO_4$. The crude product, 250 mg of *d,l*-18-nor-*D*-homo-5α,17-formylandrostan-17a-one exhibited an infrared absorption typical of an α-formyl ketone (ν_{max} 1640–1580 cm^{-1}).

The crude α-formyl ketone was converted, without further purification, to the *n*-butylthiomethylene adduct. Refluxing of 200 mg of the α-formyl ketone and 200 mg of *n*-butyl mercaptan plus a catalytic amount of *p*-toluenesulfonic acid dissolved in benzene with azeotropic removal of water (Soxhlet extractor containing 4A molecular sieves) was continued for 5 hr. The solution was cooled, washed with water, saturated sodium bicarbonate, and water, and dried ($MgSO_4$). The yellow oil obtained after removal of the solvent under vacuum was purified by preparative tlc (CH_2Cl_2 eluent). Recrystallization from di-*n*-propyl ether gave 146 mg of the blocked ketone XXXVII: mp 129–130°, ir 1680, 1540 cm^{-1} (C(=O)-C=CSR).

Anal. Calcd for $C_{24}H_{38}SO$ (mol wt, 374; M^+ (obsd), 374): C, 76.96; H, 10.23; S, 8.54. Found: C, 77.08; H, 10.08; S, 8.32.

Methylation and deblocking were accomplished according to the procedures of Ireland and Marshall.[33] Methylation with methyl iodide gave a mixture of C/D-*cis* *d,l*-*D*-homo-5α-androstan-17a-one and C/D-*trans* *d,l*-*D*-homo-5α-androstan-17a-one in a 4/1 ratio. Purification by preparative tlc (CH_2Cl_2 eluent) gave 48 mg of C/D-*cis* ketone (mp 146–147°) and 12 mg of C/D-*trans* ketone (mp 132–134°). The latter exhibited an nmr, infrared, and mass spectrum indistinguishable from authentic *D*-homo-5α-androstan-17a-one.

Repetition of the alkylation procedure using methyl-d_3 iodide gave *d,l*-*D*-homo-5α-androstan-17a-one-*18,18,18-d*$_3$ in an analogous manner (98% d_3). The ketone was converted to *d,l*-*D*-homo-5α-androstane-*18,18,18-d*$_3$ (98% d_3) by standard Wolff–Kishner reduction.

***D*-Homo-5α-androstane-*3,3-d*$_2$ (XXIX).** *D*-Homo-5α-androstan-3-ol-17a-one (XXVII) was prepared according to the method of Allinger, *et al.*[21] Wolff–Kishner reduction followed by oxidation according to the procedure of Ruzicka, Prelog, and Meister[34] gave *D*-homo-5α-androstan-3-one (XXVII), mp 165–168° (lit.[33] mp 164–167°).

Preparation of the tosylhydrazone and reduction with sodium borodeuteride according to the procedure described above gave *D*-homo-5α-androstane-*3,3-d*$_2$ (98% d_2).

***d,l*-18-Nor-*D*-homo-5α-androstane (X).** Wolff–Kishner reduction of *d,l*-18-nor-*D*-homo-5α-androstan-17a-one (XXXIV) (prepared as described above) in the usual manner gave a mixture of hydrocarbon steroids (vpc on a 3% OV-25 column operated at 190°) apparently epimeric at C-13. Collection of the major peak gave *d,l*-18-nor-*D*-homo-5α-androstane (X), isolated as a clear oil.

Anal. Calcd for $C_{19}H_{32}$: mol wt, 260; M^+ (obsd), 260.

***d,l*-18-Nor-*D*-homo-5α-androstane-*17a,17a-d*$_2$.** Conversion of the parent ketone XXXIV to the *p*-toluenesulfonylhydrazone, followed by reduction, gave *d,l*-18-nor-*D*-homo-5α-androstane-*17a,17a-d*$_2$, which exhibited a molecular ion at *m/e* 262 ($C_{19}H_{30}D_2$ requires a mol wt of 262).

***D*-Homo-5α-pregnane-*21,21,21-d*$_3$.** A solution of triphenylphosphine (2.60 g) and ethyl-*2,2,2-d*$_3$ bromide in 10 ml of benzene were placed in a stainless steel bomb and heated overnight at 100°. The resulting precipitate was collected by filtration and washed thoroughly with benzene and ether. Recrystallization from $CHCl_3$ gave ethyl-*2,2,2-d*$_3$-triphenylphosphonium bromide, mp 207–209° (lit.[35] 209–210.5°).

Reaction of the Wittig salt with *D*-homo-5α-androstan-17a-one in the manner described above gave *D*-homo-17a,20-pregnene-*21,21,21-d*$_3$ (98% d_3). Catalytic reduction as already described gave *D*-homo-5α-pregnane-*21,21,21-d*$_3$ (95% d_3) in quantitative yield.

***D*-Homo-5α-pregnane-*18,18,18-d*$_3$, *-16,16-d*$_2$, and *-14α-d*$_1$.** Preparation of the correspondingly labeled *D*-homo-5α-androstan-17a-ones has been described above. Wittig reactions and catalytic reductions were accomplished in the usual manner to give *D*-homo-5α-pregnane-*18,18,18*-d_3 (90% d_3), *-16,16-d*$_2$, (98% d_2), *-14α-d*$_1$ (90% d_1).

***D*-Homo-5α-pregnane-*17,17-d*$_2$.** A rapidly stirred suspension of ethyltriphenylphosphonium iodide (4.25 g, 0.01 *M*) in 50 ml of anhydrous ethyl ether was treated slowly with 10 ml of 1 *M* *n*-butyllithium. After 30 min, an ethereal solution of 100 mg of *D*-homo-5α-androstane-*17,17-d*$_2$ (whose preparation is described above) was added. The mixture was stirred for 1 hr at reflux. The excess Wittig reagent was then decomposed by the addition of water. The mixture was extracted with hexane, and the organic layer was washed with water, and dried over $MgSO_4$. After preparative tlc (hexane eluent), *D*-homo-5α-pregn-17a,20-ene-*17,17-d*$_2$ (98%) was isolated.

Catalytic reduction in the usual manner gave *D*-homopregnane-*17,17-d*$_2$ (90% d_2).

***D*-Homo-5α-pregnane-*3,3-d*$_2$.** The preparation of 5α-androstan-17-one-*3,3-d*$_2$ has already been described.[36] Conversion to *D*-

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(32) We gratefully acknowledge a gift of this material from Professor W. S. Johnson of this department.

(33) R. E. Ireland and J. A. Marshall, *J. Org. Chem.*, **27**, 1615, 1620 (1967).

(34) L. Ruzicka, V. Prelog, and D. Meister, *Helv. Chim. Acta*, **28**, 1651 (1945).

(35) H. O. House and G. H. Rasmusson, *J. Org. Chem.*, **26**, 4278 (1961).

(36) L. Tökés, R. T. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1012 (1967).

homo-5α-pregnane-*3,3-d*$_2$ (70% d_2) was accomplished in the usual manner.

***D*-Bishomoandrostane (XI).** Homologation of *D*-homo-5α-androstan-17a-one according to the usual procedure gave *D*-bishomoandrostan-17a-one (XL): mp 108.5–109.5°; $[\alpha]_D$ −87° (dioxane); infrared ν_{max} 1700 cm^{-1} (C=O); nmr, angular methyls at 0.75 and 0.80 ppm, a pair of doublets centered at 2.75 and 2.71 ppm, and a multiplet at 2.73 ppm.

Anal. Calcd for $C_{21}H_{34}O$ (mol wt, 302; M^+ (obsd), 302): C, 83.38; H, 11.33. Found: C, 83.08; H, 11.19.

Wolff–Kishner reduction of the 17α-ketone according to the usual procedure gave *D*-bishomo-5α-androstane (XI), isolated as a clear oil.

Anal. Calcd for $C_{21}H_{36}$: mol wt, 288; M^+ (obsd), 288.

Alternatively, aluminum trichloride catalyzed homologation of 5α-androstan-17-one (XIIII) with ethereal diazomethane gave 25–35% yields of *D*-bishomo-5α-androstan-17b-one (XLII): mp 75–78°; $[\alpha]_D$ +29° (dioxane); infrared ν_{max} 1700 cm^{-1} (C=O); nmr, angular methyls at 0.77 and 1.06 ppm. Exhaustive deuteration (sodium deuterioxide and D_2O in deuteriomethanol) introduced two deuterium atoms, confirming the existence of the 17b-carbonyl group.

Anal. Calcd for $C_{21}H_{34}O$ (mol wt, 302; M^+ (obsd), 302): C, 83.38; H, 11.33. Found: C, 83.42; H, 11.23.

Wolff–Kishner reduction of the 17b-ketone according to the usual procedure gave *D*-bishomo-5α-androstane (XI), whose mass spectrum and infrared spectrum were identical with those of material obtained from the 17a-ketone.

Wolff–Kishner reduction of 5α,14β-androstan-17-one[23] according to the usual procedure gave 5α,14β-androstane: mp 38–41°; $[\alpha]_D$ −47° (dioxane); nmr, angular methyls at 0.835 and 0.87 ppm.

Anal. Calcd for $C_{19}H_{32}$: mol wt, 260; M^+ (obsd), 260.

16-Diazoandrostan-17-one (XLIV). To a solution of 1.1 g of 16-oximinoandrostan-17-one (XLII),[37] 100 ml of methanol, and 10 ml of 5 *N* sodium hydroxide was added 16 ml of concentrated ammonium hydroxide; the solution was cooled in an ice bath, under a nitrogen atmosphere, and with the exclusion of light. Three 10-ml portions of potassium hypochlorite (10%) were added dropwise at 1-hr intervals. After an additional hour, the mixture was allowed to warm to ambient temperature. After an additional 2 hr, the reaction mixture was diluted with water, extracted with ether, and washed several times with saturated salt solution. The solvent was dried ($MgSO_4$) and removed under reduced pressure. The yellow residue was dissolved in pentane, and eluted through a neutral alumina column. Elution with pentane produced a yellow fraction, which, after evaporation, gave 520 mg (50%) of yellow prisms, mp 129–130°.

Anal. Calcd for $C_{19}H_{28}N_2O$: C, 75.95; H, 9.39; N, 9.33. Found: C, 76.14; H, 9.35; N, 9.09.

***D*-Norandrostane-16β-carboxylic acid (XLV).** A solution of 400 mg of diazo ketone in 90 ml of tetrahydrofuran and 50 ml of water, containing 850 mg of $NaHCO_3$, was irradiated under nitrogen with a 450-W Hanovia lamp through a Pyrex filter for 1 hr. The reaction mixture was diluted with saturated aqueous sodium chloride solution, acidified with hydrochloric acid, and extracted with ether. The combined extracts were washed several times with a saturated salt solution; the solvent was dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was dissolved in ether and extracted several times with 0.1 *N* aqueous sodium hydroxide. The combined alkali extracts were washed with ether and acidified with hydrochloric acid. The acidic solution was extracted with ether, and the combined ether extracts were washed with a saturated salt solution, dried over magnesium sulfate, and evaporated under reduced pressure, to give 335 mg of white powder. After recrystallization from methanol, 175 mg (44%) of *D*-norandrostane-16β-carboxylic acid, mp 207–209°, was obtained.

(37) C. Djerassi and D. Herbst, *J. Org. Chem.*, **26**, 4675 (1961).

Anal. Calcd for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.73; H, 10.36.

***D*-Norpregnan-20-one (XLVI).** To a solution of 150 mg of the carboxylic acid in 25 ml of dry benzene, was added dropwise, with stirring under nitrogen, 2 ml (3 mmol) of 1.75 *M* methyllithium in ether. The reaction mixture was stirred for 1 hr, quenched by pouring into ice water, and dried ($MgSO_4$), and solvent was removed under reduced pressure. The residue was chromatographed through 20 g of neutral alumina, Brockman activity grade I, using 2:1 pentane–ether as eluent. The ketone was obtained as an oil (90 mg, 60%).

Anal. Calcd for $C_{20}H_{32}O$: mol wt, 288. Found: M^+, 288.

***D*-Norpregnane (V).** A solution of *D*-norpregnan-20-one (25 mg) in 10 ml of dioxane and 10% sulfuric acid (5 ml) was placed in the cathode cell of an electrolysis apparatus. The electrolysis was carried out with a constant current of 1.2 mA until no more starting material could be detected by tlc (3 hr). The reaction mixture was diluted with water and extracted with ether. The residue from the ether phase after washing, drying, and evaporation of the solvent was purified by tlc (5:1 hexane–ether). The resulting oily compound (9 mg) was finally purified by vpc (3% OV-25 column) at 160°.

Anal. Calcd for $C_{20}H_{34}$: mol wt, 274. Found: M^+, 274.

***D*-Norandrostan 16β-ol (XLVII).** To a solution of 250 mg of *D*-norpregnan-20-one in 25 ml of methylene chloride were added 300 mg of *m*-chloroperbenzoic acid and 4 mg of *p*-toluenesulfonic acid. The reaction mixture was stirred in the dark for 5 days, then taken up in ether. The ether solution was washed with 10% sodium sulfite, 10% sodium bicarbonate, and water. The ethereal solution was dried ($MgSO_4$) and the ether removed to leave a white solid. Part of this material (20 mg) was purified by tlc (2:1 hexane–ether) to give 14 mg of the acetate as a white amorphous product. Another portion of crude acetate was refluxed under nitrogen overnight in 40 ml of 3:1 methanol–water solution containing 300 mg of potassium carbonate. The reaction mixture was extracted with ether, and the ether was washed with water, dried ($MgSO_4$), and removed. The residue was purified by preparative tlc (1:1 hexane–ether) and 150 mg (69%) of pure alcohol, mp 103–105°, was obtained.

Anal. Calcd for $C_{18}H_{30}O$: mol wt, 262. Found: M^+, 262.

***D*-Norandrostane (VI).** A solution of *D*-norandrostan-16β-ol (XLVII, 7 mg) in 1 ml of dry pyridine was treated with 15 mg of *p*-toluenesulfonyl chloride at ice-bath temperature. The colorless solution was left to stand in the refrigerator for 20 hr, and after this at room temperature for 3 hr; then it was poured into cold diluted hydrochloric acid. The resulting suspension was extracted with ether, and the ether phase was washed with dilute hydrochloric acid solution and water, dried, and concentrated under vacuum to give 8 mg of colorless product. A solution of the crude tosylate in 5 ml of dry tetrahydrofuran was added dropwise to a boiling suspension of 20 mg of lithium aluminum hydride in 5 ml of tetrahydrofuran. The reaction mixture was heated under reflux for 4 hr; then the excess hydride was decomposed with water. Extraction with ether gave, after evaporation, 4 mg of oily product which was purified by vpc (3% OV-25, at 165°).

Anal. Calcd for $C_{18}H_{30}$: mol wt, 246. Found: M^+, 246.

***D*-Norpregnan-20-one-*16α,20,20,20-d*$_4$.** *D*-Norpregnan-20-one was exchanged with deuterium oxide–deuteriomethanol containing a catalytic amount of sodium deuterioxide according to the usual procedure. Evaporation of the solvent gave a mixture of 16α and 16β epimers. After separation by tlc on benzene, 27 mg of pure 16β-acetyl-*D*-norandrostan-*16α,20,20,20-d*$_4$ (mp 121–122°) was isolated in high isotopic purity.

***D*-Norpregnane-*16α,20,20,20-d*$_4$.** A sample of 16β-acetyl-*D*-pure 16β-acetyl-*D*-norandrostan-*17α,20,20,20-d*$_4$ (10 mg) was reduced electrochemically[27] to give *D*-norpregnane-*17α,20,20,20-d*$_4$ (90% d_4).

***D*-Norandrostane-*16α-d*$_1$.** A tosylate of *D*-norandrostan-16β-ol (7 mg) was prepared as described above and reduced with 20 mg of lithium aluminium deuteride to give *D*-norandrostane-*16α-d*$_1$ (90% d_1).