plane with the central phosphorus atom and the unpaired electron being contained in an almost pure p orbital perpendicular to this plane.

The radicals chosen for the present work include examples from most of the known major structural types of phosphorus centered radicals. Thus, the phosphinyl radicals **1** and **2** are planar, the phosphoranyl radicals **3–10** have a distorted trigonal bipyramidal structure with the unpaired electron occupying an equatorial position,[5c,9,10,14,23] and the phosphonyl radicals **11** and **12** are close to tetrahedral.[14] It is clear that in the absence of significant steric effects all three structural types react at, or close to, the bimolecular diffusion-controlled limit (*ca.* 2×10^9 M^{-1} sec^{-1})[24] as do other simple unhindered radicals that dimerize (or disproportionate) with the release of ≥ 20 kcal/mol.[25]

Steric retardation of the reaction is only significant in the phosphoranyl radicals. It is interesting to note that **9** is more reactive than **3** or **4**. Since the bimolecular reaction almost certainly involves the formation of a P–P bonded dimer it must be concluded that steric repulsion between spirophosphoranyls is reduced relative to that for analogous nonspiro radicals by appropriate orientation of the ring systems.

The tetraalkoxyphosphoranyls **3** and **4** eliminate *tert*-butyl radicals in their unimolecular decay process as is indicated by the observation of the *tert*-butyl radical under appropriate conditions,[5,a,c,11] *e.g.*

$$t\text{-BuO}\dot{\text{P}}(\text{OMe})_3 \xrightarrow{\beta} t\text{-Bu}\cdot + \text{O}{=}\text{P}(\text{OMe})_3$$

The trialkoxychlorophosphoranyls **5** and **6** are considerably more stable toward unimolecular decay than are the tetraalkoxyphosphoranyls, but the reason for this remains to be determined. The first-order decay process for **5** (and presumably **6**) involves the elimination of either a *tert*-butyl radical or a chlorine atom, the two processes being of approximately equal importance.[15a] If the radical which is eliminated reacts rapidly with a second phosphorus radical the reported first-order decay rate constants will be twice the rate constants for the actual β-scissions.

Conclusion

In the absence of steric effects, phosphorus centered radicals having planar, tetrahedral, and trigonal bipyramidal geometries undergo bimolecular self-reactions at rates equal (or close) to the diffusion-controlled limit. There does not appear to be much likelihood that an unconjugated, long-lived, neutral phosphorus centered radical will be prepared.[26]

(23) These radicals may undergo "pseudorotation" in which ligands in the apical and equatorial positions in the bipyramid exchange positions. Except for **7** and **9**, these radicals may therefore exist in more than one form.[5b,8b,13]

(24) Dialkoxydialkylphosphoranyl radicals have remarkably long lifetimes[6,10] which indicates that *not all phosphoranyls* react at this rate.

(25) See, *e.g.*, G. B. Watts and K. U. Ingold, *J. Amer. Chem. Soc.*, **94**, 491 (1972); J. R. Roberts and K. U. Ingold, *ibid.*, **95**, 3228 (1973).

(26) See G. D. Mendenhall and K. U. Ingold, *Chem. Brit.*, in press.

Mass Spectrometry in Structural and Stereochemical Problems. CCXXXIX.[1] Elucidation of the Ring D Cleavage in Lanostane[2]

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Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. *Received August 24, 1973*

Abstract: Through extensive deuterium labeling of the steroid nucleus the electron impact induced fragmentation of ring D in lanostane (and 14α-methylcholestane) has been elucidated in order to provide a secure basis for the interpretation of the mass spectra of tetracyclic triterpenes. Major mechanistic differences were noted as a result of the additional methyl group at C-14. Unlike cholestane, the dominant ring D cleavage process of lanostane involves methyl loss from a partial ring D fragmentation ion; the minor cleavage process occurs with a single hydrogen transfer (the C-32 position being the main contributor). Reciprocal hydrogen transfer is a minor process. Contrary to the situation among steroids lacking a 14α-methyl group this partial ring D cleavage ion is found to play a dominant role in directing all the subsequent fragmentations. The results of the deuterium labeling have made it possible to explain other important fragmentations as well. The synthesis of the analogs labeled with deuterium at positions 1, 2, 3, 6, 7, 8, 9, 11, 12, 15, 16, 18, 19, 30, 31, and 32 is described, with special emphasis on the use of the tetramethylphosphorodiamidate reduction (Ireland reaction).

The electron impact induced fragmentation of steroids possessing an alkyl side chain at C-17, such as cholestane (I) or pregnane (II), characteristically involves extensive fragmentation of ring D. In addition to being of considerable mechanistic interest, the fragmentations are of particular diagnostic importance in determining the nature of the C-17 side chain.[4] Extensive deuterium labeling experiments[5] have revealed the precise course of these fragmentations and demon-

(1) For paper CCXXXVIII, see J. H. Block, D. H. Smith, and C. Djerassi, *J. Org. Chem.*, submitted for publication.

(2) Financial assistance from the National Institutes of Health (Grants No. GM-06840 and AM-04257) is gratefully acknowledged.

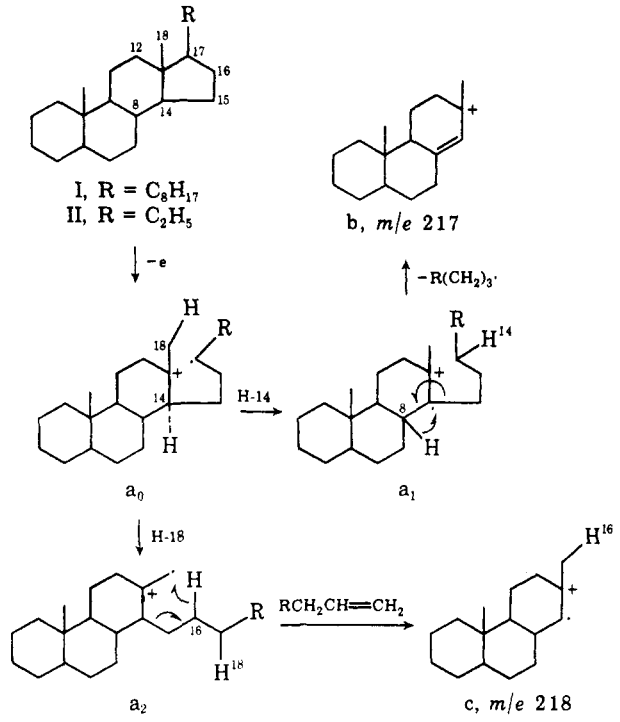
(3) National Institutes of Health Postdoctoral Fellow, 1971–1973.

(4) P. de Mayo and R. I. Reed, *Chem. Ind. (London)*, 1481 (1956).

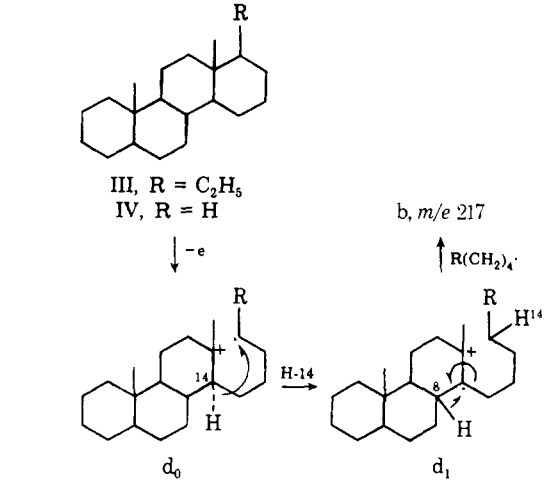
(5) L. Tökes, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

strated that these processes (which give rise to peaks at *m/e* 217 and 218 in steroids such as I or II) occur predominantly with a single hydrogen transfer (I → a_0 → a_1 → b) and a reciprocal hydrogen transfer (I → a_0 → a_2 → c), as illustrated in Scheme I.

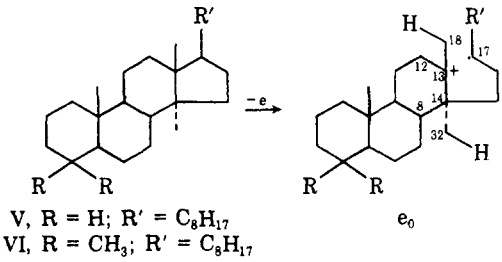
Scheme I



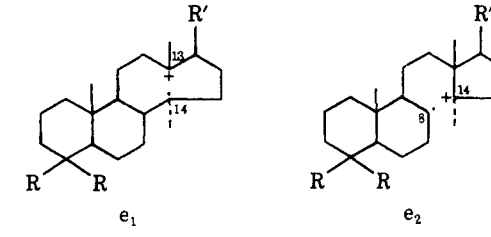
These results raise a number of interesting questions. The abstraction of hydrogen, in the genesis of the mass 217 ion, could be expected, *a priori*, to come from the 8β, 12β, and 18 positions since transfer from these sites would yield allylically stabilized carbonium ions. The predominant abstraction from the 14α position might initially not be expected since formally it requires cleavage of two bonds attached to one carbon atom.[6] Similar deuterium labeling studies confirmed these striking hydrogen transfer processes in the electron impact induced ring D cleavage of androstane.[7] Even greater site specificity was observed in the rearrangement processes in the ring D cleavage of *D*-homopregnane (III) and *D*-homoandrostane (IV).[8] The increased specificity for hydrogen transfer from the C-14 position in the formation of the mass 217 ion (d_0 → d_1 → b) in these *D*-homosteroids apparently reflects the preference for a six-membered ring abstraction.



In this connection, it was of considerable interest to investigate the mechanism of ring D cleavage in 14α-methylcholestane (V). A 14α-methyl substituent



would, by necessity, force hydrogen abstraction in molecular ion e_0 to occur from another site within the molecule. Prime candidates for hydrogen transfer would be the 8β, 12β, 18, and 32 positions. Another point of interest is the effect of the 14α-methyl function on the subsequent fragmentation of molecular ion e_0. The increased substitution at C-14 could trigger alternative cleavages to those already established for a_0. The strong influence of the 14α-methyl group has already been noted in the mass spectral fragmentations of 4,4-dimethyl-3-ketosteroids.[9] Furthermore, cleavage of the highly substituted 13–14 or 8–14 bonds rather than the 13–17 bond could give rise to molecular ions e_1 and e_2, respectively. In a similar manner, the influence of the 14α-methyl group upon the reciprocal hydrogen transfer process would shed further light on the nature of this intriguing reaction.[5,8]



In addition to being of mechanistic importance, the presence of the 14α-methyl substituent is characteristic of tetracyclic triterpenes (*e.g.*, lanostane, VI); elucidation of the mass spectral fragmentation of the fundamental tetracyclic triterpene skeleton would provide the basic information upon which the interpretation of more highly substituted steroidal triterpenes rests. Comparison of the mass spectra of 14α-methylcholestane (V) and lanostane (VI) shows only slight quantitative differences in the principal peaks (aside from expected mass shifts due to the extra methyl groups at C-4). Metastable defocusing measurements on these principal peaks revealed that they originate from the same parent ions in both compounds (and when more than one parent was involved, derived from the same parents in the same proportions). Thus the same mechanisms appeared operative in both compounds, the 4,4-

(6) To avoid formation of a (presumably high energy) ionized carbene, a 1,2 shift of the C-8 hydrogen was postulated prior to ring D cleavage to yield allylic carbonium ion b.

(7) L. Tökes and C. Djerassi, *J. Amer. Chem. Soc.*, **91**, 5017 (1969).

(8) G. Eadon, S. Popov, and C. Djerassi, *J. Amer. Chem. Soc.*, **94**, 1282 (1972).

(9) R. H. Shapiro and C. Djerassi, *Tetrahedron*, **20**, 1987 (1964).

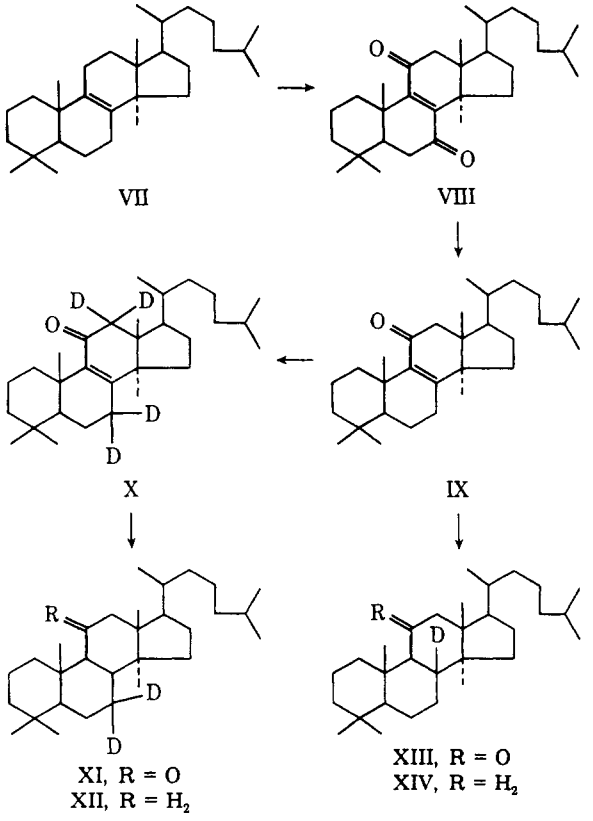
dimethyl groups of lanostane demonstrating a negligible effect upon the ring D cleavage.[9] Deuterium labeling experiments were thus carried out interchangeably on lanostane and 14α-methylcholestane to elucidate the mechanistic details of these diagnostically important processes.

Synthesis of Deuterium Labeled Lanostanes and 14α-Methylcholestanes

To follow the source of the single hydrogen transfer in the ring D cleavage, it was necessary to label rings B and C with deuterium as well as the angular methyl groups and parts of ring D. To ensure that the 4,4-dimethyl groups in lanostane did not participate in the ring D cleavage, these positions were labeled. Further labeling of ring A differentiated fragmentations occurring in this part of the molecule. By combining the data from lanostane and 14α-methylcholestane, all but the 5 and 17 positions on the steroid nucleus were labeled.[10]

Lanost-8-ene (VII)[11] served as the chief starting material for introducing deuterium into the B and C rings. Vigorous chromic acid oxidation of the Δ^8 compound (VII) to lanost-8-ene-7,11-dione (VIII)[12] followed by modified Wolff–Kishner reduction[11] furnished lanost-8-en-11-one (IX) (Scheme II). Base-

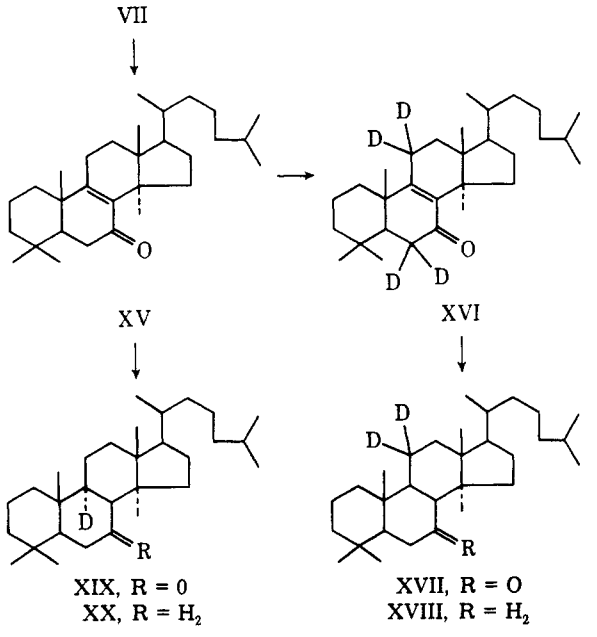
Scheme II



catalyzed equilibration of the enolizable positions of IX with methanol-*O*-*d* to the 7,7,12,12-d_4 unsaturated ketone (X) followed by lithium–ammonia reduction and back exchange of the α protons provided lanostan-11-one-*7,7*-d_2 (XI). Drastic Wolff–Kishner reduction[13] of XI yielded the desired lanostane-*7,7*-d_2 (XII) in 87% d_2 isotopic purity. Lithium–deuterioammonia reduction[5] of lanost-8-en-11-one (IX) followed by back exchange of the α protons gave lanostan-11-one-8β-d_1 (XIII) which upon drastic Wolff–Kishner reduction[13] yielded lanostane-8β-d_1 (XIV) in 82% d_1 isotopic purity.

Alternatively, mild chromic acid oxidation of lanost-8-ene (VII) furnished lanost-8-en-7-one[14] (XV) which was treated (Scheme III) in the manner described for

Scheme III



IX. Thus, alkaline equilibration of XV with methanol-*O*-*d* gave the 6,6,11,11-d_4 unsaturated ketone (XVI); lithium–ammonia reduction and back exchange yielded lanostan-7-one-*11,11*-d_2 (XVII); Wolff–Kishner reduction of XVII afforded lanostane-*11,11*-d_2 (XVIII) in 94% isotopic purity. Similarly, reduction of lanost-8-en-7-one (XV) with lithium–deuterioammonia gave lanostan-7-one-9α-d_1 (XIX), Wolff–Kishner reduction of which yielded lanostane-9α-d_1 (XX) in 94% d_1 isotopic purity.

Labeling of the 12 position was achieved in the following manner (Scheme IV). Lanostan-11-one (XXI),[15] obtained from lithium–ammonia reduction of lanost-8-en-11-one (IX), was converted to lanostan-11β-ol[15] (XXII) with lithium aluminum hydride, then dehydrated with phosphorus oxychloride to lanost-9-(11)-ene (XXIII);[15] allylic oxidation of the olefin (XXIII) with chromium trioxide in refluxing acetic acid[16] provided lanost-9(11)-en-12-one[17] (XXIV). Re-

(10) For a review of deuterium labeling procedures, see L. Tökes and L. J. Throop, "Organic Reactions in Steroid Chemistry," Vol. I, J. Fried and J. A. Edwards, Ed., Van Nostrand-Reinhold, New York, N. Y., 1972, Chapter 4.

(11) (a) J. F. McGhie, M. K. Pradhan, and J. F. Cavalla, *J. Chem. Soc.*, 3176 (1952); (b) C. Doree, J. F. McGhie, and F. Kurzer, *ibid.*, 1467 (1949).

(12) C. Doree, J. F. McGhie, and F. Kurzer, *J. Chem. Soc.*, 988 (1948).

(13) (a) D. H. R. Barton, D. A. J. Ives, and B. R. Thomas, *J. Chem. Soc.*, 2056 (1955); (b) C. Djerassi and G. H. Thomas, *J. Amer. Chem. Soc.*, **79**, 3835 (1957); (c) J. C. Knight, D. I. Wilkinson, and C. Djerassi, *J. Amer. Chem. Soc.*, **88**, 790 (1966).

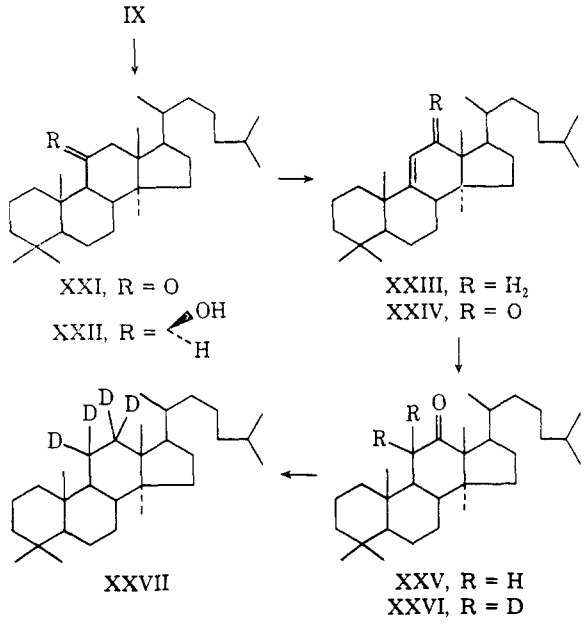
(14) J. F. McGhie, M. K. Pradhan, and W. A. Ross, *J. Chem. Soc.*, 305 (1953).

(15) D. H. R. Barton, J. E. Page, and E. W. Warnhoff, *J. Chem. Soc.*, 2715 (1954).

(16) S. Uyeo, J. Okada, S. Matsunaga, and J. W. Rowe, *Tetrahedron*, **24**, 2859 (1968).

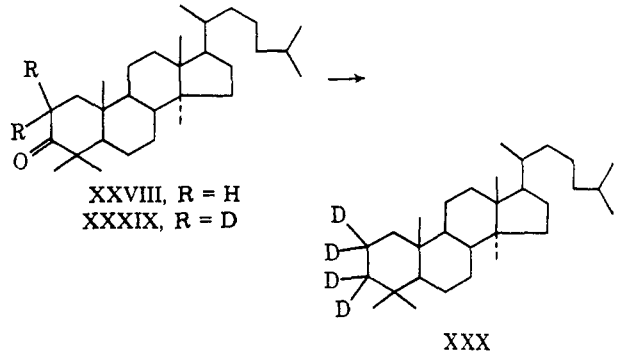
(17) Surprisingly, this compound (XXIV) does not exchange its enolizable protons (8β and 11) when treated with alkaline methanol-*O*-*d* in the usual manner. This is in sharp contrast to 5α-pregn-9(11)-en-12-one which is reported [see C. Djerassi and L. Tökes, *J. Amer. Chem.*

Scheme IV

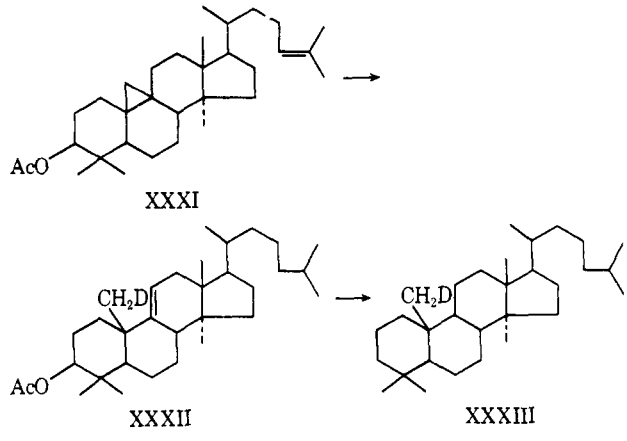


duction of the unsaturated ketone (XXIV) with lithium–ammonia gave the desired lanostan-12-one (XXV). Finding a suitable procedure for reducing the 12-ketone with efficient introduction of deuterium proved more difficult than anticipated. Reduction of the 12-ketone (XXV) with lithium aluminum deuteride, conversion of the resulting alcohol-*12-d*$_1$ to the tosylate, and cleavage of the tosylate[18] with lithium aluminum deuteride regenerated the alcohol-*12-d*$_1$. The ketone XXV failed to form the ethylene thioketal for subsequent reduction with deuterated Raney nickel,[19] as well as the tosylhydrazone, required for a Caglioti reduction.[5,20] Attempts to reduce the 12-ketone electrochemically[21] (deuterium oxide–deuteriosulfuric acid) gave back the starting material. Excellent results were obtained utilizing a deuterio-Clemmensen reduction,[22] a technique found to be very successful for the introduction of deuterium into diterpenes. One limitation of the method is the introduction of deuterium into the enolizable positions of the ketone as well as into the carbonyl position; the ketone must therefore be equilibrated with deuterium before reduction to ensure high isotopic purity. Application of this technique to the reduction of lanostan-12-one-*11,11-d*$_2$ (XXVI) gave in good yield (82%) lanostane-*11,11,12,12-d*$_4$ (XXVII) in high isotopic purity (93% d_4). Simultaneous incorporation of deuterium at C-11 presented no difficulties since lanostane-*11,11-d*$_2$ (XVIII) had already been prepared and any contribution from C-11 was thus established.

Similarly, the deuterio-Clemmensen reduction was used to label the 2 and 3 positions. Lanostan-3-one[23] (XXVIII), after equilibration with alkaline methanol-*O-d* to lanostan-3-one-*2,2-d*$_2$ (XXIX), was reduced under deuterio-Clemmensen conditions[22] to lanostane-*2,2,3,3-d*$_4$ (XXX) in 87% d_4 isotopic purity.



Utilization of cycloartenyl acetate[24] (XXXI) provided a relatively easy means for labeling the C-19 angular methyl group. Hydrogenation[25] of the side chain double bond and cleavage[26] of the cyclopropyl ring with deuterium chloride in dry chloroform gave lanost-9(11)-en-3-yl-*19-d*$_1$ acetate (XXXII).[26] Hydrogenation of the 9(11) double bond, saponification of the 3β-acetate, Jones oxidation[27] of the 3β-alcohol, and Wolff–Kishner reduction of the resulting 3-ketone gave the desired lanostane-*19-d*$_1$[15] (XXXIII) in 78% d_1 isotopic purity.



Labeling the C-32 angular methyl group required a more involved procedure which, however, proved well worthwhile since it also made ring D accessible for labeling. The synthetic route chosen was similar to that of Woodward and Barton, *et al.*[28] (Scheme V), in their synthesis of lanosterol from cholesterol with, however, two modifications; the functionality at C-3 was initially removed for simplicity and a different procedure for actually introducing the C-32 angular methyl group was devised. Thus, 7-dehydrocholesterol ben-

Soc., **88**, 536 (1966)] to exchange the two enolizable positions in high isotopic purity. The manner in which the 14α-methyl substituent affects the enolization of lanost-9(11)-en-12-one is not clear.

(18) E. J. Corey, M. G. Howell, A. Boston, R. L. Young, and R. A. Sneen, *J. Amer. Chem. Soc.*, **78**, 5036 (1956).

(19) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 2091 (1963).

(20) L. Caglioti and M. Magi, *Tetrahedron Lett.*, 1261 (1962); *Tetrahedron*, **19**, 1127 (1963); L. Caglioti and P. Graselli, *Chem. Ind. (London)*, 153 (1964).

(21) L. Throop and L. Tökes, *J. Amer. Chem. Soc.*, **89**, 4789 (1967).

(22) C. R. Enzell, *Ark. Kemi*, **26**, 87 (1966); *Tetrahedron Lett.*, 1285 (1966); C. R. Enzell and I. Wahlberg, *Acta Chem. Scand.*, **23**, 871 (1969).

(23) W. Voser, M. Montavon, Hs. H. Günthard, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **33**, 1893 (1950).

(24) Kindly provided by Professor D. H. R. Barton, Imperial College of Science and Technology, London.

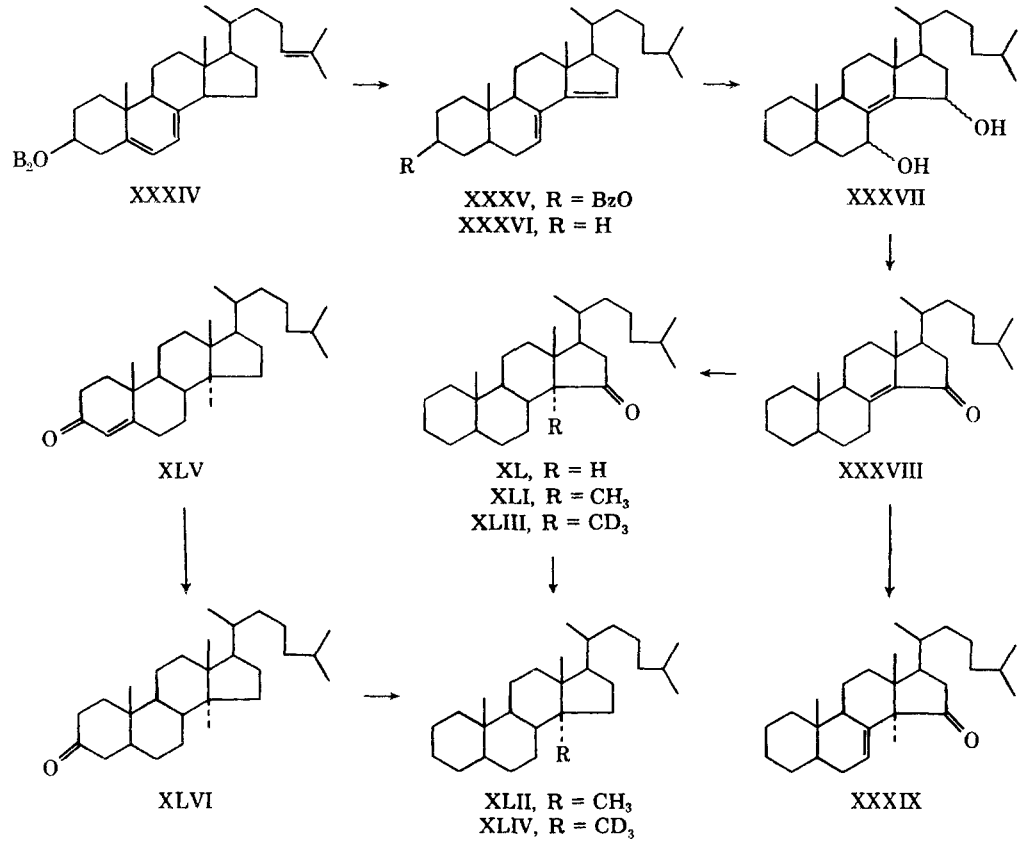
(25) D. H. R. Barton, *J. Chem. Soc.*, 1444 (1951); H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *ibid.*, 3673 (1953).

(26) D. S. Irvine, J. A. Henry, and F. S. Spring, *J. Chem. Soc.*, 1316 (1955).

(27) (a) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946); (b) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(28) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1131 (1957).

Scheme V



zoate (XXXIV) was isomerized as described[28] (Scheme V) with gaseous hydrogen chloride in chloroform to cholesta-7,14-dien-3β-yl benzoate (XXXV). Saponification of the benzoate, tosylation of the alcohol, and lithium aluminum hydride cleavage of the tosylate gave the hydrocarbon, cholesta-7,14-diene (XXXVI). Treatment[28] of the diene (XXXVI) with *m*-chloroperbenzoic acid in ether yielded cholest-8(14)-ene-7ξ,15ξ-diol (XXXVII) which upon allylic dehydration in refluxing ethanolic hydrochloric acid gave cholest-8(14)-en-15-one (XXXVIII).

The described procedure[28] for introducing the C-32 angular methyl group from the α side of the molecule involved alkylation of cholest-8(14)-en-15-one (XXXVIII) with excess methyl iodide in a solution of potassium *tert*-butoxide in *tert*-butyl alcohol, giving 14α-methylcholest-7-en-15-one (XXXIX). For our purposes, retention of the Δ^7 double bond was undesirable since it cannot be reduced by hydrogenation but requires several laborious and wasteful steps for removal. An alkylation procedure that would also reduce the double bond would better meet our objectives. For this, the reductive methylation method reported by Stork, *et al.*[29] (lithium–ammonia reduction of the unsaturated ketone followed by addition of excess methyl iodide to quench the resulting enolate), seemed well suited, especially since satisfactory results have been reported on cholest-4-en-3-one[30] and pregn-16-en-20-one.[31] Furthermore, "normal" lithium–ammonia reduction (ammonium chloride quench) of cholest-8(14)-en-15-one (XXXVIII) is reported to indeed proceed with proton addition occurring from the α side of the enolate,[32] giving cholestan-15-one (XL). When this reductive methylation procedure was applied to cholest-8(14)-en-15-one (XXXVIII) under scrupulously dry conditions, only unmethylated cholestan-15-one (XL)[32] was recovered. When lithium was replaced by sodium in the reduction reaction, a 1:1 mixture of unmethylated (XL) and methylated (XLI) cholestan-15-one was obtained. Using potassium as the reducing agent afforded completely methylated ketone, in moderate yield. Drastic Wolff–Kishner reduction of the methylated 15-ketone product provided a hydrocarbon identical with 14α-methylcholestane (XLII) prepared by lithium–ammonia reduction of 14α-methylcholest-4-en-3-one (XLV)[33] and electrolytic reduction[21] of the resulting 14α-methylcholestan-3-one (XLVI). This confirmed the structure and stereochemistry of the methylated 15-ketone as 14α-methylcholestan-15-one (XLI).

Repeating the reductive methylation procedure using methyl-d_3 iodide furnished 14α-methylcholestan-15-one-*32,32,32-d*$_3$ (XLIII) which provided upon reduction 14α-methylcholestane-*32,32,32-d*$_3$ (XLIV) in 99% d_3 isotopic purity.

The synthesis of 14α-methylcholestan-15-one (XLI) now provided an excellent means for labeling ring D.

(29) G. Stork, P. Rosen, and N. L. Goldman, *J. Amer. Chem Soc.*, **83**, 2965 (1961).

(30) R. E. Schaub and M. J. Weiss, *Chem. Ind. (London)*, 2003 (1961).

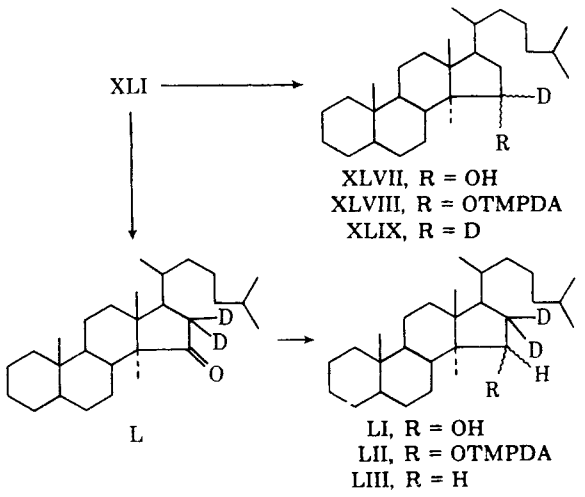
(31) R. Deghenghi and R. Gaudry, *Tetrahedron Lett.*, 489 (1962); M. J. Weiss, *Tetrahedron*, **20**, 357 (1964).

(32) I. Midgley and C. Djerassi, *J. Chem. Soc., Perkin Trans. I*, 155 (1973); for 3-substituted derivatives, see C. S. Barnes, D. H. R. Barton, and G. F. Laws, *Chem. Ind. (London)*, 616 (1953).

(33) (a) This sample was prepared by Dr. Jerry R. Dias; see G. R. Pettit and J. R. Dias, *J. Org. Chem.*, **37**, 973 (1972); *Can. J. Chem.*, **47**, 1091 (1969); (b) C. W. Shoppee, N. W. Hughes, R. E. Lack, and J. T. Pinhey, *J. Chem. Soc. C*, 1443 (1970).

The steric hindrance about the 15 position in 14α-methyl derivatives has already been noted.[28] However, a procedure for the conversion of alcohols to hydrocarbons *via* the reductive cleavage (with lithium–ethylamine) of their *N,N,N',N'*-tetramethylphosphorodiamidate (TMPDA) derivatives has been reported by Ireland, *et al.*,[34] which has given excellent yields and has been successful on hindered alcohols. In connection with other studies, we have further explored the utility of this reductive reaction as a means of deuterium labeling and found it convenient to substitute propylamine-d_2 for the reported[34] ethylamine. Thus reduction of 14α-methylcholestan-15-one (XLI) with lithium aluminum deuteride gave 14α-methylcholestan-15ξ-ol-*15*-d_1 (XLVII) (Scheme VI); treatment of the alcohol

Scheme VI

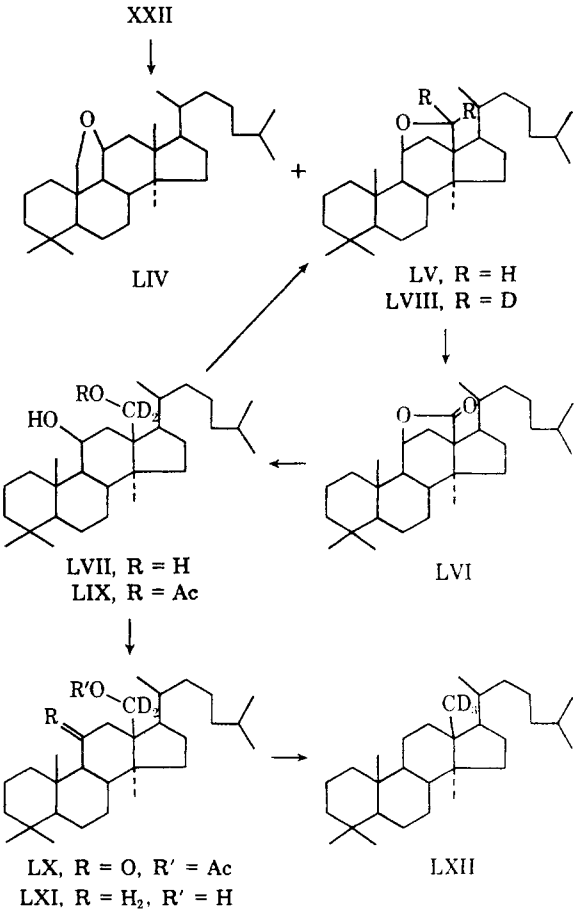


(XLVII) with 1 equiv of butyllithium in tetrahydrofuran followed by addition of excess tetramethyldiamidophosphorochloridate gave the TMPDA derivative (XLVIII). Reductive cleavage of the TMPDA derivative (XLVIII) with lithium in propylamine-d_2 gave 14α-methylcholestane-*15,15*-d_2 (XLIX) in 96% d_2 isotopic purity. Similarly, the previously exchanged 14α-methylcholestan-15-one-*16,16*-d_2 (L) was reduced with lithium aluminum hydride and the 15ξ-ol-*16,16*-d_2 (LI) converted to its TMPDA derivative (LII). Reductive cleavage of the TMPDA derivative (LII) with lithium in propylamine gave 14α-methylcholestane-*16,16*-d_2 (LIII) in 92% d_2 isotopic purity.

Access to the C-18 angular position was achieved by the photochemical remote functionalization procedure reported by Roller and Djerassi[35,36] for tetracyclic triterpenes. Treatment of lanostan-11β-ol (XXII) in the manner described[36] for the 3β-acetoxy derivative (Scheme VII) yielded a readily separable mixture of two ethers, 11β,19-epoxylanostane (LIV) and 11β,18-epoxylanostane (LV) in the respective ratio of 1:4. Chromic acid oxidation[36] of the 11β,18-ether (LV) gave the 11β,-18-lactone LVI which upon lithium aluminum deuteride reduction afforded lanostane-11β,18-diol-*18,18*-d_2 (LVII). General methods for the reduction of C-18 and C-19 functionalized carbon atoms to the angular

(34) R. E. Ireland, D. C. Muchmore, and U. Hengartner, *J. Amer. Chem. Soc.*, **94**, 5098 (1972).
(35) P. Roller and C. Djerassi, *J. Chem. Soc. C*, 1089 (1970).
(36) P. Roller, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **35**, 2585 (1970).

Scheme VII



methyl group are not readily available.[37] The encouraging results from the reductive cleavages of TMPDA derivatives, in particular, on neopentyl derivatives[34] suggested that the technique be applied here. When the phosphorylation reaction was carried out directly on the 11β,18-diol-*18,18*-d_2 (LVII), 11β,18-epoxylanostane-*18,18*-d_2 (LVIII) was recovered quantitatively. Apparently, in the derivatization of the 11β,18-diol, the primary 18 position is first phosphorylated and then suffers internal nucleophilic displacement by the 11β-alcoholate to form the *18,18*-d_2 ether (LVIII). Consequently, the 11β-hydroxy group had to be removed. Selective acetylation of the primary 18-hydroxyl function, Jones oxidation[27] of the lanostane-11β,18-diol-*18,18*-d_2 18-acetate (LIX), and drastic Wolff–Kishner reduction[13] of the resulting 18-acetoxylanostan-11-one-*18,18*-d_2 (LX) gave lanostan-18-ol-*18,18*-d_2 (LXI). Reductive cleavage of the TMPDA derivative of alcohol LXI gave in good yield lanostane-*18,18,18*-d_3 (LXII) in 96% d_3 isotopic purity.

The labeled 4,4-dimethyl groups of lanostane were introduced using the methylation procedure reported by Woodward and Barton.[28] Thus, alkylation of 14α-methylcholest-4-en-3-one (XLV)[33] with excess methyl-d_3 iodide in potassium *tert*-butoxide–*tert*-butyl alcohol solution gave lanost-5-en-3-one-*30,30,30,31,31,31*-d_6 (LXIII). Hydrogenation of the Δ^5 double bond yielded lanostan-3-one-*30,30,30,31,31,31*-d_6 (LXIV) and Wolff–Kishner reduction of LXIV afforded lanostane-*30,30,30,31,31,31*-d_6 (LXV) in 99% isotopic purity.

The facile reduction of the TMPDA derivatives

(37) C. Djerassi and M. A.Kielczewski, *Steroids*, **2**, 125 (1963).

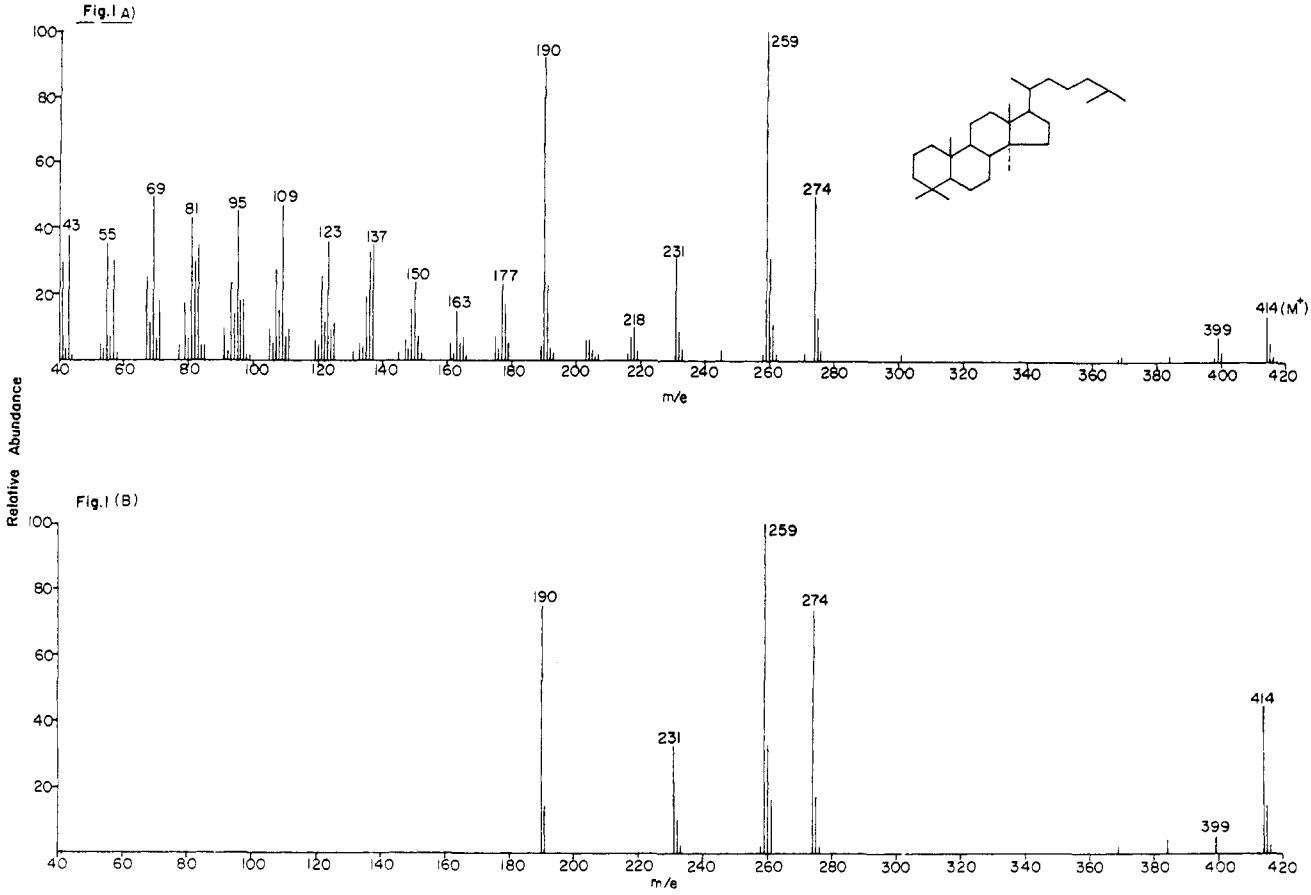
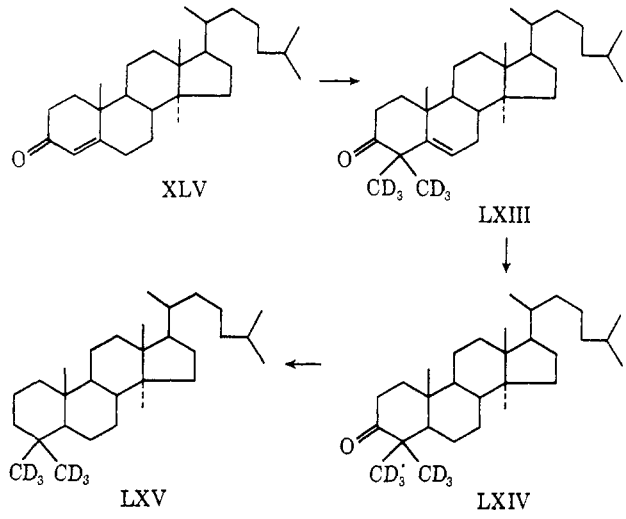
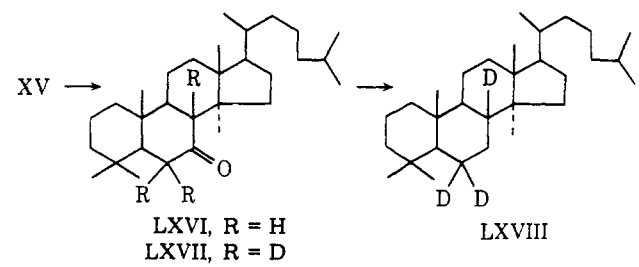


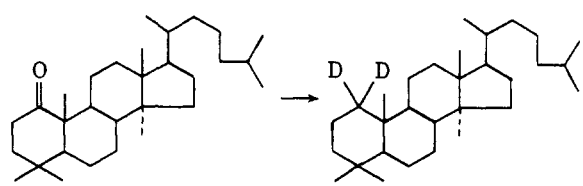
Figure 1. Mass spectra (70 eV, a, 15 eV, b) of lanostane (VI).



offered a ready means for labeling the 1 and 6 positions. Hence, lanostan-7-one (LXVI), prepared from lithium–ammonia reduction of lanost-8-en-7-one (XV) and after equilibration with methanol-*O*-*d* to the *6,6,8*-d_3 7-ketone (LXVII), was treated with lithium aluminum hydride and the TMPDA derivative of the subsequent alcohol reductively cleaved to lanostane-*6,6,8*-d_3 (LXVIII) in 92% d_3 isotopic purity. Similarly, lanostan-1-one[38] (LXIX) was treated with lithium aluminum deuteride and the TMPDA derivative of the resulting alcohol reductively cleaved with lithium–propylamine-d_2



yielding lanostane-*1,1*-d_2 (LXX) in 91% d_2 isotopic purity.



Mass Spectral Fragmentation Processes

The mass spectra of lanostane (VI) and 14α-methylcholestane (V) are reproduced in Figures 1 and 2, respectively. The base peaks in both spectra (*m/e* 259 for lanostane and *m/e* 231 for 14α-methylcholestane) correspond to loss of the side chain together with a C_3H_6 moiety, analogous to the single hydrogen transfer mechanism observed[5] for the ring D fragmentation (*m/e* 217) of cholestane. Surprisingly, the additional (reciprocal hydrogen transfer) ring D cleavage, *m/e* 260 in Figure 1 and *m/e* 232 in Figure 2, so prominent in the

(38) D. H. R. Barton, P. J. L. Daniels, J. F. McGhie, and P. J. Palmer, *J. Chem. Soc.*, 3675 (1963).

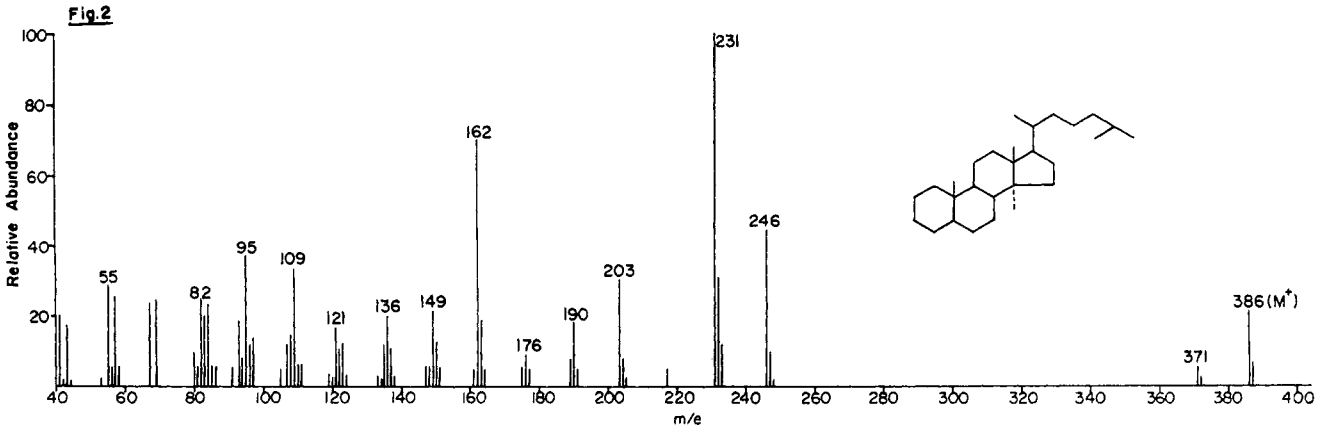


Figure 2. Mass spectrum (70 eV) of 14α-methylcholestane (V)

Table I. Shifts[a] of Mass Spectral Peaks of Deuterated Lanostane (VI) Analogs

Lanostanes	Isotopic purity	M^+	$M - CH_3$	$M - C_{10}H_{20}$	$M - C_{11}H_{22}$	$M - C_{11}H_{23}$	$M - C_{13}H_{27}$	$M - C_{16}H_{32}$
d_0 (VI)		414	399	274	260	259	231	190
1,1-d_2 (LXX)	9% d_1, 91% d_2	416	401	276	262	261	233	192
2,2,3,3-d_4 (XXX)	1% d_2, 12% d_3, 87% d_4	418	403	278	264	263	235	194
6,6,8β-d_3 (LXVIII)	1% d_1, 7% d_2, 92% d_3	417	402	277	263	262 (91%) 261 (9%)	233	193
7,7-d_2 (XII)	10% d_0, 3% d_1, 87% d_2	416	401	276	262	261 (92%) 260 (8%)	233	191
8β-d_1 (XIV)	18% d_0, 82% d_1	415	400	275	261	260 (91%) 259 (9%)	231	191
9α-d_1 (XX)	6% d_0, 94% d_1	415	400	275	261	260 (88%) 259 (12%)	231	190
11,11-d_2 (XVIII)	1% d_0, 5% d_1, 94% d_2	416	401	276	262	261	233	192
11,11,12,12-d_4 (XXVII)	1% d_2, 6% d_3, 93% d_4	418	403	278	264	263	235	192
18,18,18-d_3 (LXII)	4% d_2, 96% d_3	417	402 (75%) 399 (25%)	277	263 (10%) 262 (90%)	262 (94%) 259 (6%)	234	190
19-d_1 (XXXIII)	15% d_0, 81% d_1, 4% d_2	415	400 (55%) 399 (45%)	275	261	260 (72%) 259 (28%)	232	191
30,30,30,31,31,31-d_6 (LXV)	1% d_5, 99% d_6	420	405	280	266	265	237	196

[a] The shift values are corrected for isotopic impurity as well as for ^{13}C contributions and are reliable to ±5% for all peaks except *m/e* 260 in which the uncertainty is ±10%. All spectra were measured at 70 eV on an Atlas CH-4 with E-413 ion source.

Table II. Shifts[a] of Mass Spectral Peaks of Deuterated 14α-Methylcholestane (V) Analogs

14α-Methylcholestanes	Isotopic purity	M^+	$M - CH_3$	$M - C_{10}H_{20}$	$M - C_{11}H_{22}$	$M - C_{11}H_{23}$	$M - C_{13}H_{27}$	$M - C_{16}H_{32}$
d_0 (V)		386	371	246	232	231	203	162
15,15-d_2 (XLIX)[b]	1% d_0, 3% d_1, 96% d_2	388	373	248	232	233 (48%) 231 (52%)	203	162
16,16-d_2 (LIII)[b]	2% d_0, 6% d_1, 92% d_2	388	373	246	233 (90%) 232 (10%)	231	203	162
32,32,32-d_3 (XLIV)	1% d_2, 99% d_3	389	374 (70%) 371 (30%)	249	235	234 (62%) 233 (26%) 231 (12%)	203	162

[a] See footnote *a* in Table I. The shift values for *m/e* 232 are reliable to ±10%. [b] Atlas 711 spectrum.

spectrum of cholestane (*m/e* 218, side chain loss together with a C_3H_5 moiety) is relatively unimportant in the presence of a 14α-methyl group. Also very notable in these spectra is the diminished intensity of the parent and $M - CH_3$ ions, indicative of the increased substitution about ring D whose preferential rupture could trigger new and important fragmentation sequences. Very intense in the spectra (45–50% of the base peak) are the peaks (*m/e* 274 in lanostane and *m/e* 246 in 14α-methylcholestane) corresponding to partial ring D cleavage (loss of the side chain together with a C_2H_3 moiety). Two intense peaks in the spectra (*m/e* 231 and 190 in Figure 1 and *m/e* 203 and 162 in Figure 2) have no analogs in the cholestane spectrum and therefore owe their origin to the presence of the 14α-methyl substituent.

The results from the deuterium labeling experiments in lanostane (VI) and 14α-methylcholestane (V) are summarized in Tables I and II, respectively. The metastable defocusing data are shown in Tables III and IV; the analogous bond cleavage processes are evident here.[39]

(39) These measurements (Tables III and IV) are valid only for fragmentations occurring in the first field-free region. Although very indicative of the cleavage processes occurring at the ion source, these figures cannot necessarily be directly applied.

Table III. Parent–Daughter Relationships[a] in the Fragmentation of Lanostane (VI) Established by Defocused Measurement of Metastable Ions

Parent ions	Daughter ions						
	274	260	259	232	231	191	190
415		415 (40%)		415 (50%)		415 w	
414	414 s		414 (30%)		414 (50%)		414 w
399	399 m		399 w				
275		275 (60%)		275 (50%)		275 s	
274			274 (70%)		274 (50%)		274 s
260						260 m	
259							259 w
231							

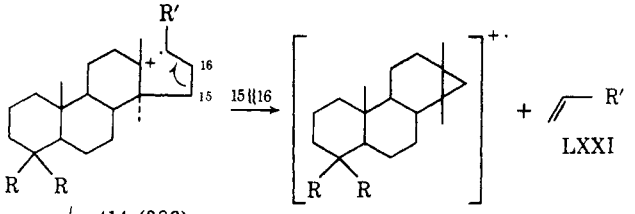
[a] Where meaningful, the value of the contribution of that parent ion to the daughter is denoted in parentheses and is accurate to ±5%. Otherwise intensities are designated as <1%, w; 1–10%, m; >10%, s. Measurements were made on an MS-9 double focusing mass spectrometer.

Table IV. Parent–Daughter Relationships[a] in the Fragmentation of 14α-Methylcholestane (V) Established by Defocused Measurements of Metastable Ions

Parent ions	Daughter ions						
	246	232	231	204	203	163	162
387		387 (40%)		387 (40%)		387 m	
386	386 s		386 (30%)		386 (50%)		386 w
371	371 m		371 w				
247		247 (60%)		247 (60%)		247 s	
246			246 (70%)		246 (50%)		246 s
232						232 m	
231							231 w
203							

[a] See footnote *a* in Table III.

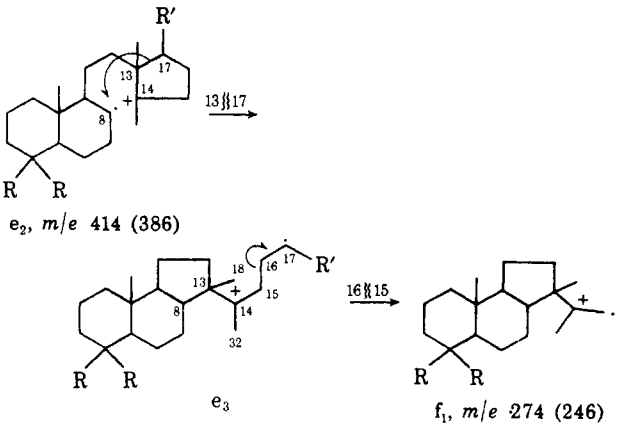
The intense peak occurring in the higher mass range, *m/e* 274 (246),[40] corresponds to a partial ring D cleavage (retention of the C-15 label, loss of the C-16 label). Homolysis of the activated 15–16 bond in molecular ion e_0 provides a neutral olefin (LXXI) and ion f_0, *m/e*



e_0, *m/e* 414 (386) f_0, *m/e* 274 (246)

274 (246), which is formally a very highly substituted ionized cyclopropane. The greater intensity of this mass 274 (246) ion (50–55% of the base peak), as compared to its analog in cholestane,[5] may be due to an additional mode of genesis *via* the molecular ion e_2, whose formation is prompted by the additional substitution at C-14. Fissure of the 13–17 bond in e_2 and formation of an 8–13 bond could give ion e_3, which would yield, after rupture of the 15–16 bond, the same neutral olefin LXXI and the ionized olefin f_1, *m/e* 274 (246) [evidence supporting the formation of both f_0 and f_1 will be presented below].

The importance of the mass 274 (246) ion in triggering the remaining fragmentations of the steroid skeleton is revealed in the metastable defocusing data (Tables III and IV). Every subsequent principal peak in the spectrum is chiefly derived from this fragment ion with



the parent ion, *m/e* 414 (386), being the minor contributor.

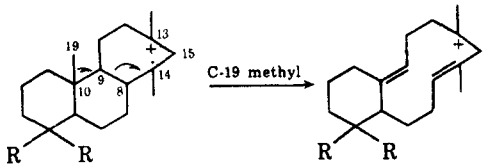
Unlike cholestane,[41] the metastable defocusing data reveal that the "conventional" ring D cleavage ions [*m/e* 259 (231)] of lanostane and 14α-methylcholestane are derived principally (~70%) through loss of a methyl group from a mass ion 274 (246) rather than directly from the molecular ion. Deuterium labeling reveals that 48% of the deuterium at C-15 is retained in the mass 259 (231) ion and that 28% of the C-19 angular methyl group, 12% of the C-32 methyl group, and 6% of the C-18 methyl group are expelled. The combined loss of the methyl groups from *m/e* 274 (246), 46%, is thus in good agreement with the retention of 48% of the C-15 label.

The predominant loss of the C-19 methyl group from ions of type f_0 has already been observed[5] and rationalized through the opening of the strained cyclopropane

(40) Hereafter, when referring to the mass of an ion, the mass of the lanostane (VI) derived ion will be given first followed, in parentheses, by the analogous ion (28 mass units lower) derived from 14α-methylcholestane (V).

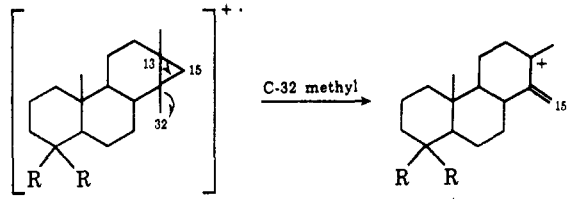
(41) C. C. Fenselau and F. D. Abramson, *Org. Mass Spectrom.*, **2**, 915 (1969).

ring with simultaneous fission of the 8–9 and 10–19 bonds. In ion f_2, the prior ionization of the very highly substituted 13–14 bond should favor the loss of the C-19 methyl group, giving ion g_1, *m/e* 259 (231).



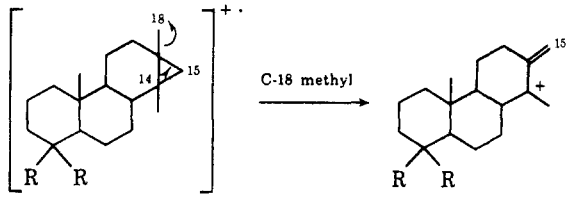
f_2, *m/e* 274 (246) g_1, *m/e* 259 (231)

Similarly, expulsion of the C-32 methyl group in f_0 with concomitant fissure of the 13–15 bond would yield the allylic carbonium ion g_2, *m/e* 259 (231). In the same



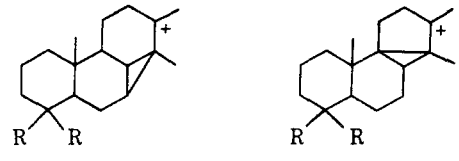
f_0, *m/e* 274 (246) g_2, *m/e* 259 (231)

fashion, the C-18 methyl group could be expelled in f_0 giving the allylic carbonium ion g_3, *m/e* 259 (231).



f_0, *m/e* 274 (246) g_3, *m/e* 259 (231)

The additional ~22–24% of the ring D cleavage ion, *m/e* 259 (231), observed by metastable defocusing measurements to be derived [70% *via* metastables, ~46–48% *via* retention of C-15 label] from the mass 274 (246) ion may originate by a two-step mechanism.[5] Abstraction of hydrogen from the C-7 or C-9 positions in an intermediate in which the cyclopropyl rings may participate in stabilizing the positive charge[42] could give rise to the ions g_4 and g_5, respectively. This would

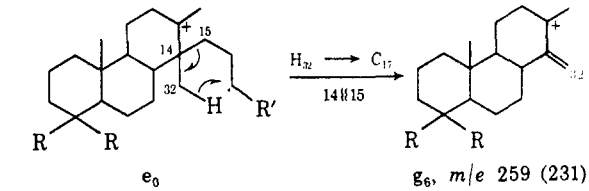


g_4, *m/e* 259 (231) g_5, *m/e* 259 (231)

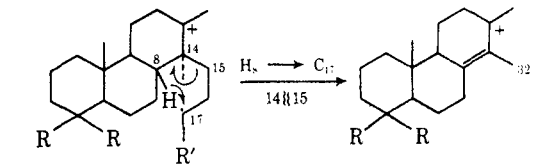
account for the observed transfer of 8% deuterium from the 7 position and 12% from the 9α position in the formation of the mass 259 (231) ion.

The remaining 34% of the ring D cleavage ion, *m/e* 259 (231), which is derived by a single hydrogen transfer followed by cleavage of the 14–15 bond in molecular ion e_0 (see Tables III and IV), is the process analogous to the "standard" ring D fission in steroids.[5] Hydrogen abstraction from the 32 position ($e_0 \rightarrow g_6$) is responsible for 26% out of the total 35% (*i.e.*, 74% of the hydrogen transfer cleavage process) with the 8β

(42) F. McLafferty, "Mass Spectrometry of Organic Ions," Academic Press, New York, N. Y., 1963, pp 517–519.



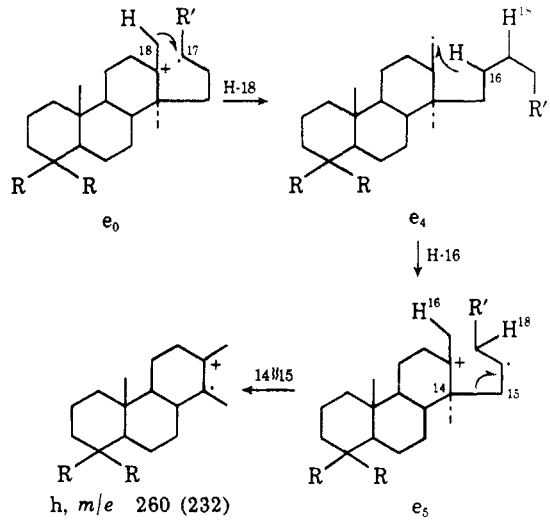
e_0 g_6, *m/e* 259 (231)



e_0 g_7, *m/e* 259 (231)

position ($e_0 \rightarrow g_7$) accounting for the remaining 9% (*i.e.*, 26% of the cleavage process). The high degree of specificity observed in this hydrogen abstraction apparently reflects the preference for six-membered rings in mass spectrometry; abstraction of any other hydrogen would involve less favorable ring sizes. The preference for the C-32 hydrogen over the C-8 hydrogen probably illustrates the greater ease of the C-17 radical site for approaching the primary hydrogen rather than the tertiary hydrogen.

The reciprocal hydrogen transfer process (*m/e* 260 (232), 8–10% of the base peak after subtracting ^{13}C contribution) is relatively unimportant in the presence of a 14α-methyl substituent as would be expected from the diminished importance of the single hydrogen transfer process in the formation of the mass 259 (231) ion. In fact, the metastable defocusing measurements (Tables III and IV) on the mass 260 (232) ion give only a slight indication of the existence of this ion in the presence of such a large ^{13}C contribution [from *m/e* 259 (231)]. The deuterium labeling results (Tables I and II) indeed confirm the high site specificity in this reciprocal hydrogen migration ($e_0 \rightarrow e_4 \rightarrow e_5 \rightarrow$ h) from

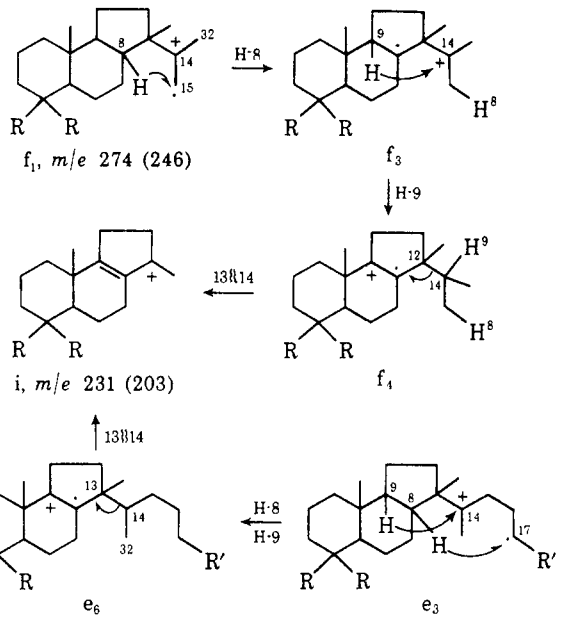


e_0 e_4 h, *m/e* 260 (232) e_5

the 16 and 18 positions (loss of 90% of a deuterium atom in the *18-d_3* labeled sample and the equivalent retention of one deuterium atom in the *16-d_2* labeled sample).

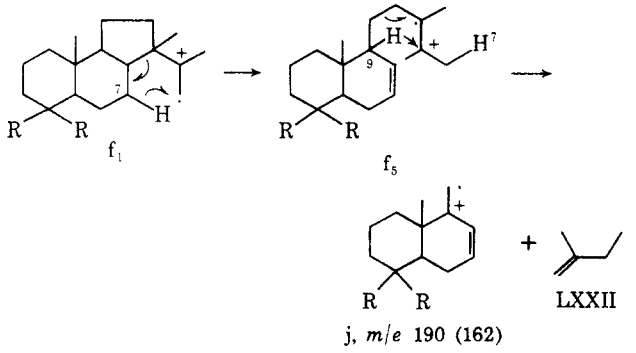
The peak at *m/e* 231 (203) (30–31% of the base peak) has no analog in the cholestane spectrum.[5] According to the metastable defocusing data (Tables III and IV), about 55% of the ion is derived from the partial ring D cleavage ion, *m/e* 274 (246), and the remaining 45%

from the molecular ion. Formation of *m/e* 231 (203) from *m/e* 274 (246) requires the loss of C_3H_7; deuterium labeling shows C-32 and C-15 to be two of the carbon atoms expelled; it follows that the third carbon must be C-14. The remaining hydrogens lost originate (Tables I and II) from C-8 and C-9. Loss of C-14, 15, and 32 can be rationalized as originating from ion f_1 through the sequence $f_1 \rightarrow f_3 \rightarrow f_4 \rightarrow$ i (the order of hydrogen

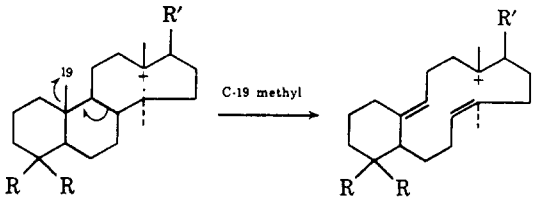


abstraction being assumed arbitrarily). Ion i can be derived with equal facility from the molecular ion e_3, *m/e* 414 (386). Abstraction of hydrogen from C-8 and C-9 (e_6) followed by allylic cleavage of the 13–14 bond would yield i, *m/e* 231 (203).

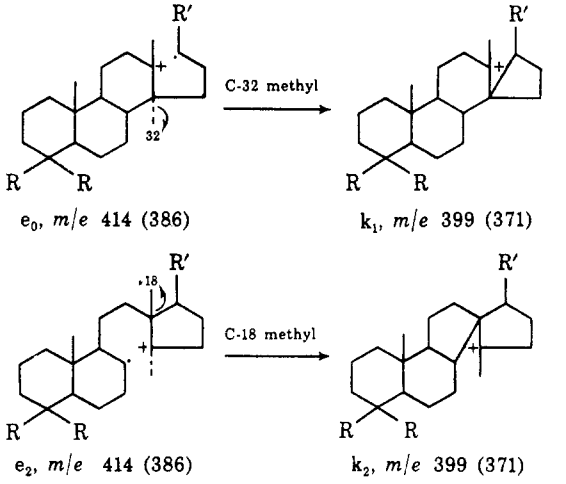
The very intense peak at *m/e* 190 (162) also has no analog in the spectrum[5] of cholestane. Metastable defocusing measurements (Tables III and IV) reveal that this ion is almost solely derived from the partial ring D cleavage ion, 274 (246), and the deuterium labeling results (Tables I and II) show that the loss of ring C (except for C-11) and of one hydrogen each from C-7 and C-9 is implicated in this process. An attractive rationale for this process involves the intermediate f_1 (which owes its formation to the presence of the 14α-methyl group and hence is absent[5] in cholestane) and proceeds through the sequence $f_1 \rightarrow f_5$ to the ionized butadiene system, j, *m/e* 190 (162).



Indicative of the increased substitution about ring D, expulsion of a methyl radical from the molecular ion is not an important process in the spectra of lanostane and 14α-methylcholestane. It is not surprising, however, that the chief source (see Tables I and II) of methyl loss is the C-19 angular methyl group (45%) triggered by



ionization of the highly substituted 13–14 bond in molecular ion e_1. The 30% loss of the C-32 methyl group can be visualized as originating from molecular ion e_0 and the 25% loss of the C-18 methyl group from



molecular ion e_2. Expulsion of one of the methyls from the 4,4-dimethyl group (30 or 31) in lanostane is not observed in this peak. The ratios of formation of the M − CH_3 ions are in excellent agreement with the ratios of methyl expulsion from the mass 274 (246) ion to form the mass 259 (231) ion. Therefore, it is tempting to consider these ratios as an indication of the preference for bond ionization in the 14α-methyl steroid system.

In summary, the present work demonstrates how extensive deuterium labeling coupled with metastable defocusing studies can lead, even in as complicated a molecule as lanostane, to a satisfactory rationalization of all important mass spectral processes. It also offers a good example of the utility of considering favored molecular ion species as the "trigger" for subsequent molecular fragmentations. By elucidating the role of the 14α-methyl group, this work provides a basis for attempting the interpretation of mass spectral fragmentations of other related tetracyclic triterpenes in which isotopic labeling is not feasible or has not been performed

Experimental Section

Melting points are uncorrected and were determined in unsealed capillaries on a Thomas-Hoover melting point apparatus. Infrared spectra were measured in chloroform solution on a Perkin-Elmer Model 421 infrared spectrophotometer using polystyrene as external reference (1601 cm^{-1}). The nmr spectra were determined in deuteriochloroform solution with tetramethylsilane as an internal reference (δ 0.00) on a Varian A-60 or HA-100 spectrometer. Mass spectra were determined by Messrs. Richard Conover and Robert Ross on an Atlas CH-4 spectrometer with an E-413 ion source using the direct inlet procedure, or on an MS-9 double focusing mass

spectrometer. All deuterium labeled hydrocarbon samples were purified by preparative vpc on a Hewlett-Packard 402 gas chromatograph using an OV25 (3%) on Gas Chrom Q 100–120 mesh column, prior to mass spectral analysis.[43]

Lanost-8-en-11-one (IX). A solution of chromium trioxide (3.0 g) in 90% acetic acid (25 ml) was added dropwise to a refluxing solution of lanost-8-ene[11] (VII, 3.0 g) in 20 ml of hexane and 100 ml of glacial acetic acid. After 2 additional hr of reflux, the product was worked up in the usual manner yielding 2.4 g (80%) of lanost-8-ene-7,11-dione (VIII).[12] Recrystallization from dichloromethane–methanol gave luminous yellow plates, mp 118–119° (lit.[12] mp 119–120°).

Modified Wolff–Kishner reduction[11] of lanost-8-ene-7,11-dione (VIII, 2.0 g) gave 1.95 g of orange oil. Chromatography of the crude product on alumina (100 g, activity 2) yielded, in the hexane fractions, 420 mg of semicrystalline compound (presumably lanost-7,9(11)-diene). Elution with 25% benzene in hexane afforded 1.32 g (66%) of lanost-8-en-11-one (IX); recrystallization from ether–methanol gave material: mp 93.5–94°; ir 1640 and 1580 cm^{-1}; nmr δ 0.79 (CH_3-18), 0.84 + 0.92 (CH_3-21, 26, 27, 30, and 31), 1.14 (CH_3-19 and 32), 2.28 (m, 2, CH_2-7), 2.44 (d, 1, J = 14 Hz, CH-12α), 2.74 (d, 1, J = 14 Hz, CH-12β), 3.00 (d, 1, J = 12 Hz, CH-1β);[44] mass spectrum (rel intensity) m/e 426 (100, M^+), 411 (16, M − CH_3), 397 (12), 313 (6), 274 (41), 245 (16), 219 (45), 205 (18), 203 (16), 191 (13).

Lanostan-11-one-7,7-d_2 (XI). Lanost-8-en-11-one (IX, 30 mg) was heated to reflux in 4 ml of methanol-*O*-*d* (under nitrogen).[45] Sodium (~50 mg), previously dissolved in methanol-*O*-*d* (1 ml), was then added and heating under reflux continued overnight. The methanol-*O*-*d* was carefully[45] distilled off, replaced with 5 ml of fresh methanol-*O*-*d* and the heating continued overnight. The methanol-*O*-*d* was again removed by distillation,[45] and the product taken up in anhydrous ether (10 ml, distilled from sodium) and washed with deuterium oxide (3 × 2 ml). Drying (magnesium sulfate) and concentrating yielded 28 mg of lanost-8-en-11-one-7,7,12,12-d_4 (X), mp 92–93°, m/e 430 (M^+).

Approximately 10 ml of ammonia was condensed (through a potassium hydroxide drying tower) and dried by vigorous stirring with 10 mg of lithium for 20 min. After a second addition of lithium (15 mg), a solution of lanost-8-en-11-one-7,7,12,12-d_4 (X, 25 mg) in 3 ml of dry ether was slowly added. The reaction mixture was stirred under reflux for 30 min, then quenched with ammonium chloride. After evaporation of the ammonia, the crude product was taken up in ether and washed with dilute hydrochloric acid, then aqueous sodium bicarbonate and finally water. Drying and evaporation of the solvent gave an off-white solid which was dissolved in methanol (15 ml) containing potassium hydroxide (~150 mg) and heated under reflux overnight to remove the deuterium introduced at C-12. Evaporation of the methanol and the usual work-up provided 21 mg (84%) of lanostan-11-one-7,7-d_2 (XI); recrystallization from ether–methanol gave mp 94–95° (lit.[15] mp 95–95.5° for unlabeled material); m/e 430 (M^+); for ir and nmr data, see unlabeled compound XXI.

Lanostane-7,7-d_2 (XII). Lanostan-11-one-7,7-d_2 (XI, 15 mg) was reduced under drastic Wolff–Kishner conditions[13] to afford 8 mg (54%) of lanostane-7,7-d_2 (XII) after removal of olefin[43] [see Table I for isotopic purity].

Lanostan-11-one-8β-d_1 (XIII). Approximately 7 ml of deuterioammonia was generated[5] by the addition of 14 ml of deuterium oxide in a stirred suspension of magnesium nitride (20 mg) in 100 ml of mineral oil. The deuterioammonia was stirred with lithium (10 mg) for 20 min, then a second piece of lithium was added (30 mg) followed by lanost-8-en-11-one (IX, 40 mg) in 5 ml of anhydrous ether. After the stirred reaction mixture was allowed to reflux 30 min, ammonium chloride was added to discharge the blue color and the ammonia was allowed to evaporate. The product was taken up in ether and washed with dilute hydrochloric acid, aqueous sodium bicarbonate, and then water. The product, after being dried and concentrated, was treated briefly with Jones reagent[27] to oxidize any over-reduced material, then heated to reflux in 5% potassium hydroxide–methanol to back exchange the α positions. The crude produdt (32 mg, 80%) was crystallized from ether–methanol to give lanostan-11-one-8β-d_1 (XIII), mp 93–95°; m/e 429 (M^+); for ir and nmr data, see unlabeled compound XXI.

Lanostane-8β-d_1 (XIV). Lanostan-11-one-8β-d_1 (XIII, 21 mg) was reduced under drastic Wolff–Kishner conditions[13] to give, after olefin removal,[43] 9 mg (44%) of lanostane-8β-d_1 (XIV) [see Table I for isotopic composition].

Lanost-8-en-7-one (XV). A solution of lanost-8-ene (VII, 3.2 g) in hexane (20 ml) and glacial acetic acid (100 ml) was immersed in an oil bath maintained at 60°. Chromium trioxide (1 g) in 10 ml of 90% acetic acid was added all at once and the reaction mixture was stirred vigorously for precisely 2 min. The mixture was then quickly poured into a cold aqueous solution of sodium bisulfite. The crude product was chromatographed on 150 g of alumina (activity 2); elution with hexane provided 1.53 g (48%) of the starting material, lanost-8-ene (VII). Further elution with 20% benzene in hexane yielded 1.04 g (34%) of lanost-8-en-7-one (XV); recrystallization from dichloromethane–methanol gave mp 118–119° (lit.[14] mp 120°); ir 1652 and 1581 cm^{-1}; nmr δ 0.67 (CH_3-18), 0.82 + 0.85 + 0.87 + 0.91 (CH_3-21, 26, 27, 30, and 31), 0.93 (CH_3-32), 1.18 (CH_3-19), 2.32 (s, 2, CH_2-6), 2.34 (q, 2, J = 2 and 14 Hz, CH_2-11); mass spectrum (rel intensity) m/e 426 (37 M^+), 411 (100, M − CH_3), 397 (6), 313 (10), 220 (10), 175 (10), 135 (10), 109 (16).

Lanostan-7-one-11,11-d_2 (XVII). Lanost-8-en-7-one (XV, 40 mg) was equilibrated[46] with methanol-*O*-*d* in the manner described for the preparation of XI. The product was lanost-8-en-7-one-6,6-11,11-d_4 (XVI, 38 mg), mp 115–117°; m/e 430 (M^+).

Lanost-8-en-7-one-6,6,11,11-d_4 (XVI, 35 mg) was reduced with lithium–ammonia as described for the preparation of XI. Back-exchange of the α protons and recrystallization from methanol afforded 19 mg of lanostan-7-one-11,11-d_2 (XVII), mp 113–115°, m/e 430 (M^+).

Lanostane-11,11-d_2 (XVIII). Wolff–Kishner reduction[11] of lanostan-7-one-11,11-d_2 (XVII, 19 mg) followed by removal[43] of olefinic contaminants provided 8 mg (42%) of lanostane-11,11-d_2 (XVIII) [see Table I for isotopic purity].

Lanostan-7-one-9α-d_1 (XIX). Lanost-8-en-7-one (XV, 45 mg) was reduced with lithium–deuterioammonia as described for the preparation of XIII. Crystallization of the crude product (36 mg, 80%) provided lanostan-7-one-9α-d_1 (XIX), mp 113–114°, m/e 429 (M^+).

Lanostane-9α-d_1 (XX). Lanostan-7-one-9α-d_1 (XIX, 20 mg) was reduced under Wolff–Kishner conditions,[11] yielding[43] 9 mg (45%) of lanostane-9α-d_1 (XX) [see Table I for isotopic purity].

Lanost-9(11)-en-12-one (XXIV). Lanost-8-en-11-one (IX, 1.0 g) in 25 ml of dry ether was reduced with 300 mg of lithium in approximately 50 ml of condensed ammonia in the manner described for XI. Recrystallization of the crude product (870 mg, 87%) from dichloromethane–methanol gave lanostan-11-one (XXI), mp 94.5–95.5° (lit.[15] mp 95–95.5°); ir 1696 cm^{-1}; nmr δ 0.71 (CH_3-18), 0.83 + 0.89 (CH_3-21, 26, 27, 30, and 31), 1.08 (CH_3-19 and 32), 2.26 (*c*, 1, J = 14 Hz, CH-12α), 2.58 (d, 1, J = 14 Hz, CH-12β), 2.78 (d, 1, J = 12 Hz, CH-1β);[44] mass spectrum (rel intensity) m/e 428 (98, M^+), 413 (8, M − CH_3), 410 (9, M − H_2O), 397 (8), 303 (78), 290 (28), 205 (100).

Lanostan-11-one (XXI, 870 mg) was reduced with 500 mg of lithium aluminum hydride in 25 ml of dry ether (heated to reflux, 2 hr). Recrystallization of the crude product (848 mg, 97%) gave lanostan-11β-ol (XXII), mp 129–131° (lit.[15] mp 132–133°); ir 3620 cm^{-1}; nmr δ 0.79 (CH_3-32), 0.82 + 0.88 (CH_3-21, 26, 27, 30, and 31), 1.00 (CH_3-18), 1.18 (CH_3-19), 4.23 (m, 1, CH-11).

Redistilled phosphorus oxychloride (2 ml) was added to lanostan-11β-ol (900 mg) in 4 ml of dry pyridine and the solution kept at 100° for 1 hr.[15] Filtration of the product through 50 g of alumina (activity 2) with hexane gave 708 mg (79%) of lanost-9(11)-ene (XXIII); recrystallization from ether–methanol gave mp 85–87° (lit.[15] mp 86–87°); ir 1601 cm^{-1} (weak); nmr δ 0.68 (CH_3-18), 0.77 (CH_3-32), 0.86 + 0.88 (CH_3-21, 26, 27, 30, and 31), 1.06 (CH_3-19), 5.22 (m, 1, CH-11).

Chromium trioxide (1.0 g) in 30 ml of 90% acetic acid was added to a vigorously stirred boiling solution of lanost-9(11)-ene (XXIII, 790 mg) in 5 ml of hexane and 30 ml of acetic acid. Heating was continued 2 hr. Chromatography of the product on 40 g of alumina (activity 2) yielded, in the fractions eluted with 25% benzene in hexane, 256 mg (32%) of lanost-9(11)-en-12-one (XXIV). Recrystallization from ether–methanol gave material with mp 149–150°; ir 1674 and 1593 cm^{-1}; nmr δ 0.64 (CH_3-32), 0.80 + 0.85

(43) Where necessary, the deuterium-labeled hydrocarbons were separated from any olefin contaminants before preparative vpc collection by (a) thin-layer chromatography on silver nitrate (10%) impregnated silica gel H or (b) brief boiling in acetic acid containing chromium trioxide and filtering the product through alumina (activity 1) with hexane.

(44) N. S. Bhacca and D. H. Williams, "Applications of NMR spectroscopy in Organic Chemistry, Illustrations from the Steroid Field," Holden-Day, San Francisco, Calif., 1964, pp 63–69.

(45) The refluxing solution must be kept scrupulously under nitrogen to prevent air oxidation to lanost-8-en-7,11-dione (VIII).

+ 0.88 (CH_3-21, 26, 27, 30, and 31), 1.01 (CH_3-19), 1.18 (CH_3-18), 5.62 (m, 1, CH-11); mass spectrum (rel intensity) *m/e* 426 (15, M^+), 411 (4, M − CH_3), 273 (40), 219 (4), 218 (5), 175 (6), 161 (8), 149 (14), 135 (100).

Attempts to exchange the enolizable positions of lanost-9(11)-en-12-one (XXIV) in alkaline methanol-*O-d* or in mixtures of alkaline dioxane–deuterium oxide yielded only unexchanged XXIV.

Lanostan-12-one (XXV). Lanost-9(11)-en-12-one (XXIV, 47 mg) was reduced with 25 mg of lithium in ammonia (~10 ml) as in the preparation of XI. Recrystallization of the crude product (42 mg, 89%) gave lanostan-12-one (XXV): mp 115–116°; ir 1697 cm^{-1}; nmr δ 0.65 (CH_3-32), 0.86 + 0.88 (CH_3-21, 26, 27, 31, and 31), 1.02 (CH_3-19), 1.09 (CH_3-18); mass spectrum (rel intensity) *m/e* 428 (6, M^+), 413 (4, M − CH_3), 315 (5), 275 (100).

Lanostan-12-one-*11,11-d*$_2$ (XXVI). Lanostan-12-one (XXV, 30 mg) was heated under reflux in 5 ml of methanol-*O-d* to which sodium (50 mg), previously dissolved in 1 ml of methanol-*O-d*, was added. Heating under reflux was continued overnight; the methanol was then distilled and 5 ml of fresh methanol-*O-d* added, and refluxing was continued another 24 hr. The product was worked up in the manner described for X to give 28 mg of lanostan-12-one-*11,11-d*$_2$ (XXVI), mp 114–116°, *m/e* 430 (M^+).

Lanostane-*11,11,12,12-d*$_4$ (XXVII). Deuterium oxide (5 ml) was cautiously added to freshly distilled acetyl chloride (10 ml) at ice bath temperature. When all the deuterium oxide was added, the solution was brought up to room temperature, then added to lanostan-12-one-*11,11-d*$_2$ (XXVI, 25 mg) previously dissolved in 1 ml of acetic acid-*O-d*. Mossy zinc (1.0 g) was stirred with mercuric chloride (100 mg) in 1.5 ml of 5% hydrochloric acid for 5 min. The solution was decanted and the zinc washed successively with water, methanol, and anhydrous ether. After being dried under vacuum, the zinc was added to the reaction solution and the whole mixture slowly brought to reflux temperature. Gas chromatographic analysis of the reaction after 2 hr revealed the reduction to be proceeding slowly, so heating was continued overnight. The cooled mixture was taken up in ether, and then washed successively with water, aqueous sodium bicarbonate, and then water again. Drying and evaporation yielded 16 mg (53%) of lanostane-*11,11,12,12-d*$_4$ (XXVII) [see Table I for isotopic composition].

Lanostan-3-one-*2,2-d*$_2$ (XXIX). Lanostan-3-one[23] (XXVIII, 30 mg) was equilibrated with deuterium as described for the preparation of XXV. The yield was 27 mg of lanostan-3-one-*2,2-d*$_2$ (XXIX), mp 125–127°, *m/e* 430 (M^+).

Lanostane-*2,2,3,3-d*$_4$ (XXX). Lanostan-3-one-*2,2-d*$_2$ (XXIX, 27 mg) was reduced under deuterio-Clemmensen conditions as described for the preparation of XXVI. Removal of olefin contaminants[43] gave 17 mg (63%) of lanostane-*2,2,3,3-d*$_4$ (XXX) [see Table I for isotopic purity].

Lanostane-*19-d*$_1$ (XXXIII). Cycloartenyl acetate[24,25] (XXXI, 100 mg) was hydrogenated to cycloartanyl acetate (using 200 mg of 10% platinum on carbon catalyst in 50 ml of glacial acetic acid), then directly cleaved in the described manner[26] in dry chloroform (10 ml) containing 2 drops of deuterium oxide under a deuterium chloride atmosphere. The crude product (87 mg), rich in lanost-9(11)-enyl-*19-d*$_1$ acetate (XXXII), was hydrogenated[15] (with 100 mg of platinum oxide in 25 ml of glacial acetic acid at 80°), saponified (5% potassium hydroxide–methanol), oxidized with Jones reagent,[27] and reduced (Wolff–Kishner) by standard procedures.[15] Brief boiling of the product with chromium trioxide in acetic acid and passage through alumina (activity 1) with hexane removed the Δ^8 contaminant and yielded 12 mg of pure lanostane-*19-d*$_1$ (XXXIII)[15] [see Table I for isotopic purity].

Cholesta-7,14-diene (XXXVI). A solution of 7-dehydrocholesteryl benzoate[46] (XXXIV, 25 g) in 700 ml of dry chloroform was isomerized with hydrogen chloride in the described manner.[28] The crude product, enriched in cholesta-7,14-dienyl benzoate (XXXV), was saponified (5% potassium hydroxide–methanol, with benzene added for solubility), tosylated (45 g of tosyl chloride in 400 ml of dry pyridine, 0° for 2 days), and reductively cleaved with lithium aluminum hydride (12 g) in anhydrous ether (450 ml, heating under reflux overnight). Passage of the crude product through 400 g of alumina (activity 2) with hexane yielded 15.1 g (82%) of clear pale yellow oil which crystallized on standing. Recrystallization from ether–methanol gave cholesta-7,14-diene (XXXVI) as white plates: mp 57–58°; nmr δ 0.79 + 0.82 + 0.87 (CH_3-18, 19, 21, 26, and 27), 5.61 (m, 2, CH-7 and 15); mass spectrum (rel intensity) *m/e* 368 (100, M^+), 353 (56, M − CH_3), 312 (11), 283 (10), 255 (63), 241 (21).

(46) Dawe's Laboratories, Chicago, Ill.

Cholest-8(14)-en-15-one (XXXVIII). A cooled solution of cholesta-7,14-diene (XXXVI, 12.2 g) in 250 ml of anhydrous ether was treated[28] with *m*-chloroperbenzoic acid (10 g, 85%). The solution was allowed to stand 2 days at room temperature, with periodic checking (potassium iodide–starch paper) to ensure an excess of oxidizing reagent. The crude product was dissolved in 350 ml of ethanol (95%) and heated to reflux with 10 g of potassium hydroxide. After removal of ethanol under vacuum, the product was taken up in ether and washed with water, dilute hydrochloric acid, and finally aqueous sodium bicarbonate. The dried and concentrated enediol (XXXVII) was dissolved in 350 ml of ethanol (95%) containing 20 ml of concentrated hydrochloric acid and heated to reflux 3 hr. The resulting dark brown oil was chromatographed on 350 g of alumina (activity 2). Elution with 40% benzene–hexane afforded 2.2 g of solid cholest-8(14)-15-one (XXXVIII), recrystallized from ether–methanol, mp 101–102° (lit.[32] 103–104°); ir, nmr, and mass spectra identical with that reported.[32]

14α-Methylcholestan-15-one (XLI). Approximately 20 ml of ammonia was condensed (using a potassium hydroxide drying tower) under a Dry Ice condensor equipped with a potassium hydroxide drying tube. Small pieces of clean potassium were vigorously stirred in the ammonia so as to maintain the blue color 30 min. To the dry refluxing ammonia was added potassium (200 mg) followed by cholest-8(14)-15-one (XXXVIII, 100 mg) in 5 ml of anhydrous ether (distilled from $LiAlH_4$). Stirring was continued 45 min, then methyl iodide (0.75 ml, distilled from calcium hydride) was added to discharge the blue color.[29] Stirring was continued an additional 15 min, then the ammonia was evaporated. Chromatography of the product on 10 g of alumina (activity 2) yielded in the 20% benzene–hexane fractions 36 mg (36%) of crystalline 14α-methylcholestan-15-one (XLI): mp 110–113°, raised to mp 125–126° after two recrystallizations from methylene chloride–methanol; ir 1727 cm^{-1}; nmr δ 0.82 + 0.86 (CH_3-18, 19, 21, 26, and 27), 1.04 (CH_3-32); mass spectrum (rel intensity) *m/e* 400 (100, M^+), 385 (16, M − CH_3), 382 (4, M − H_2O), 287 (47), 232 (97), 223 (42), 217 (54), 205 (27).

Drastic Wolff–Kishner reduction[13,28] of 14α-methylcholestan-15-one (XLI, 20 mg) gave 14α-methylcholestane (XLII, 9 mg), identical in all respects (melting point, nmr, mass spectrum, and gc retention time) with authentic material prepared as described below. This confirmed the structure and stereochemistry of ketone XLI.

14α-Methylcholestan-3-one (XLVI). 14α-Methylcholest-4-en-3-one (XLV, 35 mg)[23] was reduced in lithium–ammonia, then briefly treated with Jones reagent.[27] Chromatography of the product through alumina (5 g, activity 2) with 25% benzene–hexane yielded 26 mg (74%) of 14α-methylcholestan-3-one (XLVI): mp 107–109°; ir 1701 cm^{-1}; nmr δ 0.78 + 0.82 + 0.88 (CH_3-18, 21, 26, 27, and 32), 1.07 (CH_3-19); mass spectrum (rel intensity) *m/e* 400 (28, M^+), 260 (46), 245 (100), 218 (27), 178 (30), 176 (50).

14α-Methylcholestane (XLII). 14α-Methylcholestan-3-one (XLVI, 25 mg) was reduced electrolytically.[21] Chromatography of the product through alumina with hexane gave 14α-methylcholestane (XLII, 16 mg): mp 85–87°; nmr δ 0.78 + 0.82 + 0.88 + 0.91 (CH_3-18, 19, 21, 26, 27, and 32) [for mass spectrum, see Figure 2].

14α-Methylcholestan-15-one-*32,32,32-d*$_3$ (XLIII). The reductive methylation procedure described in the preparation of XLI was repeated using methyl-d_3 iodide. Purification in the same fashion gave 14α-methylcholestan-15-one-*32,32,32-d*$_3$ (XLIII), mp 123–125°; for ir and nmr see XLI; *m/e* 403 (M^+).

14α-Methylcholestane-*32,32,32-d*$_3$ (XLIV). Drastic Wolff–Kishner reduction[13,28] of 14α-methylcholestan-15-one-*32,32,32-d*$_3$ (XLIII, 15 mg), after purification,[43] gave 6 mg of 14α-methylcholestane-*32,32,32-d*$_3$ (XLIV) [see Table II for isotopic purity].

14α-Methylcholestane-*15,15-d*$_2$ (XLIX). 14α-Methylcholestan-15-one (XLI, 36 mg) was reduced with lithium aluminum deuteride (60 mg) in 10 ml of anhydrous ether (heating to reflux 2 hr). Chromatography of this product on alumina (activity 2, 5 g) yielded with 40% benzene–hexane elutions 23 mg of 14α-methylcholestan-15ξ-ol-*15-d*$_1$ (XLVII) as a clear oil (probably a mixture of isomers); ir 3615 cm^{-1}; *m/e* 402 (M^+).

14α-Methylcholestan-15ξ-ol-*15-d*$_1$ (XLVII, 23 mg), dissolved in dry tetrahydrofuran (5 ml, distilled from LAH) containing a crystal of triphenylmethane, was titrated with stirring with 1.9 *M* butyllithium until the pink colored persisted 5 min. *N,N,N',N'*-Tetramethyldiamidophosphorochloridate[47] (0.5 ml) in triethylamine (1 ml, distilled from potassium hydroxide) was added to discharge

(47) Commercial grade (Aldrich) must be distilled (*in vacuo*).

the pink color and stirring continued 1.5 hr.[34] The solution was taken up in ether and washed thoroughly with water, dilute hydrochloric acid, aqueous sodium bicarbonate, and water again. The dried and concentrated sample was passed through alumina (6 g), first with a hexane flush (to remove hydrocarbon impurities in butyllithium), then eluted with ethyl acetate. The latter collection, when concentrated, gave 30 mg of the semicrystalline TMPDA derivative (XLVIII); nmr δ 2.66 (d, 12, J = 10 Hz, -N(CH_3)$_2$).

The TMPDA derivative (XLVIII, 30 mg) in 6 ml of propylamine-d_2[48] was treated with 50 mg of clean lithium, then stirred overnight at room temperature under a potassium hydroxide drying tube. The lithium was removed (mechanically) and the propylamine-d_2 distilled off (bp 49°).[49] The product was taken up in ether, washed thoroughly with dilute hydrochloric acid, then aqueous sodium bicarbonate and water. Drying and concentration gave, after chromatography through alumina with hexane, 8 mg of 14α-methylcholestane-*15,15-d*$_2$ (XLIX) [see Table II for isotopic purity].

14α-Methylcholestan-15-one-*16,16-d*$_2$ (L). 14α-Methylcholestan-15-one (XLI, 43 mg) was equilibrated with methanol-*O-d* in the usual manner (see XXVI) giving 14α-methylcholestan-15-one-*16,16-d*$_2$ (L, 40 mg); mass spectrum 402 (M^+).

14α-Methylcholestane-*16,16-d*$_2$ (LIII). In the same fashion described for the unlabeled ketone (XLI), the *16,16-d*$_2$-15-one (L, 40 mg) was reduced with lithium aluminum hydride and the 15ξ-ol-*16,16-d*$_2$ (LI) converted to its TMPDA derivative (LII). Reductive cleavage of the TMPDA derivative (LII) with lithium in propylamine gave 11 mg of 14α-methylcholestane-*16,16-d*$_2$ (LIII) [see Table II for isotopic purity].

11β,19-Epoxylanostane (LIV) and 11β,18-Epoxylanostane (LV). Lanostan-11β-ol (XXII, 2.12 g) was treated essentially according to the described[35,36] photochemical procedure except that the total reflux time was curtailed to 1 hr. The product was carefully chromatographed on 100 g of alumina (activity 2). The early fractions eluted with 6% benzene–hexane provided 569 mg (27%) of 11β,18-epoxylanostane (LV): mp 118–119°; ir 1020 cm^{-1}; nmr δ 0.79 + 0.82 + 0.86 + 0.88 + 1.02 (CH_3-19, 21, 26, 27, 30, 31, and 32), 3.65 (s, 2, CH_2-18), 4.29 (d, 1, $J_{11\alpha,12\beta}$ = 6 Hz, CH-11); mass spectrum (rel intensity) m/e 428 (100, M^+), 413 (53, M − CH_3), 410 (3, M − H_2O), 395 (26), 383 (4), 343 (6), 316 (14), 249 (8).

The latter fractions eluted with 6% benzene–hexane yielded 142 mg (7%) of 11β,19-epoxylanostane (LIV): mp 108–109°; ir 1040 and 1010 cm^{-1}; nmr δ 0.67 + 0.70 + 0.81 + 0.86 + 0.91 (CH_3-18, 21, 26, 27, 30, 31, and 32), 3.72 (s, 2, CH_2-19), and 4.18 (m, 1, CH-11); mass spectrum (rel intensity) m/e 428 (60, M^+), 413 (11, M − CH_3), 397 (85), 288 (48), 275 (50), 274 (46), 257 (100), 345 (41), 243 (31), 205 (89).

The middle fractions eluted with 6% benzene–hexane contained a mixture of the ethers LV and LIV (85 mg total).

Lanostane-18,11β-lactone (LVI). 11β,18-Epoxylanostane (LV, 252 mg) was treated with chromium trioxide (300 mg) in acetic acid (3 ml) in the manner described[36] for the 3β-acetoxy derivative, with a little hexane added for solubility. Chromatography of the product on alumina (20 g, activity 2) yielded with 10% benzene–hexane elutions, 101 mg of the starting material (LV). Elution with 50% benzene–hexane gave 106 mg (42%) of lanostane-18,-11β-lactone (LVI): mp 163–164°; ir 1759 cm^{-1}; nmr δ 0.78 + 0.83 + 0.85 + 0.87 + 0.89 + 1.00 (CH_3-19, 21, 26, 27, 30, 31, and 32), 2.51 (q, 1, J = 6 and 13 Hz, CH-12), 4.72 (d, 1, $J_{11\alpha,12\beta}$ = 6.5 Hz, CH-11); mass spectrum (rel intensity) m/e 442 (58, M^+), 427 (63, M − CH_3), 397 (10), 381 (8), 313 (1M), 289 (100), 285 (78), 254 (18), 237 (15).

Lanostane-11β,18-diol-*18,18-d*$_2$ (LVII). Lanostane-18,11β-lactone (LVI, 45 mg) was reduced[36] with excess lithium aluminum deuteride in anhydrous ether (reflux 2 hr). The product, lanostane-11β,18-diol-*18,18-d*$_2$ (LVII, 45 g), upon recrystallization gave mp 187–188°; ir 3570 cm^{-1}; nmr δ 0.82 + 0.89 + 0.97 (CH_3-21, 26, 27, 30, 31, and 32), 1.17 (CH_3-19), 4.24 (m, 1, CH-11α); mass spectrum (rel intensity) m/e 430 (10, M − 18), 415 (15), 398 (32), 397 (27), 383 (19), 285 (100), 220 (23), 193 (75).

(48) Propylamine-d_2 was prepared by three consecutive distillations (bp range 71–86°) of propylamine from an equivalent volume of deuterium oxide. The final distillate was cautiously treated with a few small pieces of clean sodium, and after the initial reaction had subsided, redistilled (over the sodium). The fraction of bp 49° had an isotopic purity of 99+ % d_2.

(49) This propylamine-d_2 may be used again directly or if isotopic purity has suffered, it may be enriched by a single distillation from deuterium oxide and then distilled over sodium.

Lanostane-18,11β-lactone (LVI) was reduced in a similar manner with the lithium aluminum hydride giving the 18-unlabeled derivative; nmr same as described for LVII with the additional pair of doublets at δ 3.38 and 3.95 depicted by the nonequivalent C-18 methylene protons (J = 12 Hz).

11β,18-Epoxylanostane-*18,18-d*$_2$ (LVIII). Lanostane-11β,18-diol-*18,18-d*$_2$ (LVII, 30 mg) was treated under the phosphorylation conditions described in the preparation of XLVIII. Chromatography of the product through alumina with benzene gave 11β,18-epoxylanostane-*18,18-d*$_2$ (LVIII, 30 mg): mp 116–118°; ir 1020 cm^{-1}; nmr identical with that described for unlabeled compound LV except for the CH_2-18 absorption; mass spectrum (rel intensity) m/e 430 (100, M^+), 415 (54, M − CH_3), 412 (3, M − H_2O), 397 (35), 383 (4), 345 (6), 318 (13), 251 (8).

Lanostane-11β,18-diol-*18,18-d*$_2$ 18-Acetate (LIX). Lanostane-11β,-18-diol-*18,18-d*$_2$ (LVII, 100 mg) was dissolved in 1 ml of acetic anhydride–pyridine (1:1) and set aside overnight at room temperature. Work-up in the usual manner provided 97 mg of lanostane-11β,18-diol-*18,18-d*$_2$ 18-acetate (LIX); recrystallization gave mp 175–177°; ir 3550 and 1736 cm^{-1}; nmr δ 0.82 + 0.89 + 0.97 (CH_3-21, 26, 27, 30, 31, and 32), 1.15 (CH_3-19), 2.05 (s, 3, CH_3-OCO-18), 4.28 (m, 1, CH-11α); mass spectrum (rel intensity) 490 (1, M^+), 472 (12, M − H_2O), 471 (12, M − HDO), 430 (14), 397 (100), 299 (12), 273 (24).

In another experiment, the 18-unlabeled derivative of the diol monoacetate (LIX) was prepared (acetylation of the 18-unlabeled derivative of the diol LVIII); nmr same as described for the labeled LIX with the additional pair of doublets at δ 4.13 and 4.48 depicted by the nonequivalent C-18 methylene protons (J = 12 Hz).

18-Acetoxylanostan-11-one-*18,18-d*$_2$ (LX). Jones oxidation[27] of lanostane-11β,18-diol-*18,18-d*$_2$ 18-acetate (LIX, 95 mg) gave, after chromatography through alumina with benzene, 80 mg of 18-acetoxylanostan-11-one-*18,18-d*$_2$ (LX), crystallized from ether–methanol: mp 135–138°; ir 1692 and 1725 cm^{-1}; nmr δ 0.83 + 0.89 + 1.09 (CH_3-21, 26, 27, 30, and 31), 1.13 (CH_3-32), 1.25 (s, 3, CH_3-19), 2.04 (s, 3, CH_3OCO-18), 2.37 (d, 1, J = 14 Hz, CH-12α), 2.70 (d, 1, J = 14 Hz, CH-12β), 2.78 (d, 1, J = 12 Hz, CH-1β);[44] mass spectrum (rel intensity) 488 (100 M^+), 442 (42), 427 (53), 413 (21), 397 (19), 363 (32), 350 (10).

Jones oxidation[27] of the 18-unlabeled derivative of the diol monoacetate (LIX, see above) gave the 18-unlabeled derivative of the keto acetate LX; nmr same as described for LX with an additional absorption at δ 4.03 (broad singlet, 2, CH_2-18); mass spectrum (rel intensity) 486 (100, M^+), 440 (26), 426 (37), 413 (14), 397 (9), 361 (34), 348 (13).

Lanostan-18-ol-*18,18-d*$_2$ (LXI). Drastic Wolff–Kishner reduction[13] of 18-acetoxylanostan-11-one-*18,18-d*$_2$ (LX, 70 mg) gave, after careful chromatography through alumina (activity 2) in the 80% benzene–hexane elutions, 18 mg of semicrystalline lanostan-18-ol-*18,18-d*$_2$ (LXI); recrystallization from ether–methanol gave mp 168–170°; ir 3640 cm^{-1}; nmr δ 0.82 + 0.89 + 0.97 (CH_3-19, 21, 26, 27, 30, 31, and 32); mass spectrum (rel intensity) 399 (100, M − CH_3OD).

In a similar manner, an 18-unlabeled derivative of the alcohol (LXI) was prepared from the 18-unlabeled derivative of the keto acetate (LX); nmr same as described for LXI with an additional pair of doublets at δ 3.52 and 3.92 depicted by the nonequivalent methylene protons at C-18 (J = 13 Hz); mass spectrum (rel intensity) 398 (100, M − CH_3OH).

Lanostane-*18,18,18-d*$_3$ (LXII). Lanostan-18-ol-*18,18-d*$_2$ (LXI, 15 mg) was converted to its TMPDA derivative, then reductively cleaved with lithium–propylamine-d_2 in the manner described for the preparation of XLIX. The usual work-up and chromatography through alumina with hexane gave 12 mg of lanostane-*18,18,18-d*$_3$ (LXII) [see Table I for isotopic purity].

Lanost-5-en-3-one-*30,30,30,31,31,31-d*$_6$ (LXIII). 14α-Methylcholest-4-en-3-one (XLV, 40 mg)[33] in 0.5 ml of methyl-d_3 iodide was injected into a solution of potassium (200 mg) previously dissolved in 8 ml of *tert*-butyl alcohol (freshly distilled over sodium). The yellow creamy suspension was heated under reflux for 2 hr with vigorous stirring.[28] The usual work-up gave 37 mg of semicrystalline lanost-5-en-3-one-*30,30,30,31,31,31-d*$_6$ (LXIII); recrystallized from ether–methanol this had mp 118–120°; ir 1695 cm^{-1}; nmr δ 0.82 + 0.85 + 0.92 (CH_3-18, 19, 21, 26, 27, and 32), 5.58 (m, 1, CH-5); mass spectrum (rel intensity) 432 (35, M^+), 417 (12, M − CH_3), 399 (5), 130 (100).

Lanostan-3-one-*30,30,30,31,31,31,-d*$_6$ (LXIV). Lanost-5-en-3-one-*30,30,30,31,31,31-d*$_6$ (LXIII, 25 mg) in 10 ml of glacial acetic acid was stirred overnight at 80° with 20 mg of 10% palladium on carbon catalyst under an atmosphere of hydrogen. Work-up afforded

23 mg of **lanostan-3-one-*30,30,30,31,31,31-d*$_6$** (LXIV); recrystallization gave mp 123–124° (lit.[23] mp 127–128° for the unlabeled compound); ir 1695 cm^{-1}; nmr δ 0.81 + 0.90 + 1.09 (CH_3-18, 19, 21, 26, 27, and 32); mass spectrum (rel intensity) 434 (10, M^+), 307 (11), 294 (68), 279 (100), 251 (61), 238 (26), 210 (63).

Lanostane-*30,30,30,31,31,31-d*$_6$ (LXV). Lanostan-3-one-*30,30,30,31,31,31-d*$_6$ (LXIV, 10 mg) was reduced under Wolff–Kishner[11] conditions. Purification[43] gave 4 mg of lanostane-*30,30,30,31,31,31-d*$_6$ (LXV) [see Table I for isotopic composition].

Lanostan-7-one (LXVI). Lithium–ammonia reduction of lanost-8-en-7-one (XV, 100 mg) in the manner described for the preparation of XI gave 92 mg of lanostan-7-one (LXVI); recrystallization gave mp 116–118°; ir 1695 cm^{-1}; nmr δ 0.78 (CH_3-18, 0.82 + 0.89 + 0.93 + 0.97, (CH_3-21, 26, 27, 30, and 31) 1.10 (CH_3-32), 1.18 (CH_3-19), 2.15–3.0 (complex, 4, CH_2-6, CH-8β, CH-15);[44] mass spectrum (rel intensity) *m/e* 428 (50, M^+), 413 (11, M − CH_3), 315 (16), 304 (5), 288 (22), 275 (12), 219 (21), 206 (100), 164 (38).

Lanostan-7-one-*6,6,8β-d*$_3$ (LXVII). Lanostan-7-one (LXVI, 40 mg) was converted to lanostan-7-one-*6,6,8β-d*$_3$ (LXVII) quantitatively in the manner described for XXVI; mass spectrum 431 (M^+).

Lanostane-*6,6,8β-d*$_3$ (LXVIII). Lanostan-7-one-*6,6,8β-d*$_3$ (LXVII, 30 mg) was reduced with lithium aluminum hydride, then the crude alcohol-*d*$_3$ was directly converted to its TMPDA derivative and reductively cleaved (see XLIX and LIII). Purification[43] gave 17 mg of lanostane-*6,6,8β-d*$_3$ (LXVIII) [for isotopic purity see Table I].

Lanostane-*1,1-d*$_2$ (LXX). Lanostan-1-one[38] (LXIX, 23 mg) was treated with lithium aluminum deuteride and the TMPDA derivative of the resulting alcohol reductively cleaved (see preparation of XLIX). After separation from olefin, the product amounted to 6 mg of lanostane-*1,1-d*$_2$ (LXX) [see Table I for isotopic purity].

Carbon-13 Nuclear Magnetic Resonance Titration Shifts in Amino Acids

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Abstract: Nmr titration curves for the individual carbon atoms in a series of representative amino acids have been determined. A computer model involving multiple ionizations has been used to obtain ionization constants and titration shifts, even when ionizations overlap. CNDO/2 molecular orbital calculations suggest a rationalization of the observed changes in ^{13}C shielding on deprotonation. For carbon atoms near the site of ionization a decrease in excitation energy dominates the chemical shift expression, resulting in deshielding despite an increase in electron density; for more distant carbon atoms changes in electron density dominate, yielding shifts in either direction.

Fundamental to the application of ^{13}C nmr to peptides and proteins is knowledge of the characteristic behavior of the ^{13}C chemical shifts of the amino acids as a function of pH.[2–6] We report the result of a ^{13}C nmr study of the titration of a number of representative difunctional and trifunctional amino acids and amino acid derivatives. Computer curve fitting[7] has been used to obtain both accurate pK values and a quantitative estimate of the contribution of each ionization to the observed ^{13}C shifts in the overall titration. CNDO/2 molecular orbital calculations[8] have been carried out for each major ionic species existing during the titration sequence in an attempt to rationalize the observed shifts on deprotonation.

Results and Discussion

In the typical titration curves in Figures 1 and 2,[9] the ^{13}C chemical shift of each carbon is sensitive to more than one titration.[2–6] The solid lines in the figures indicate the final least-squares fit to the observed ^{13}C nmr titration values. The number of ionizations in each model equation (see Experimental Section) is the minimum necessary to reproduce the shape of the experimental curve; in general this is equal to the number of ionizable groups in the molecule. The dashed lines in the figures represent the best fit with the number of ionizations reduced by one. It is clear that even remote ionizations can be monitored *via* the ^{13}C chemical shifts. The value of computer curve fitting is particularly obvious when, as in the glutamic acid titration (Figure 2), there are overlapping ionizations whose separate pK values cannot be obtained by inspection.

The computer calculated pK and ^{13}C nmr titration shift values, together with the standard deviations, are given in Table I. Estimates of the pK of the same

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