Acknowledgments.—We thank Mr. G. Motoasca, of S. B. Penick and Co., Inc., for supplies of plant material, and Dr. R. I. Reed, University of Glasgow, for the mass spectral measurements. We also thank Dr. J. T. Gillingham, Agricultural Chemical Services, Clemson University, for the loan of extraction apparatus.

Side-Chain Transformations and Deuterium Labeling in the Steroidal Sapogenin Series[1]

WILLIAM H. FAUL,[2] AMEDEO FAILLI,[3] AND CARL DJERASSI

Department of Chemistry, Stanford University, Stanford, California 94305

Received January 29, 1970

Synthetic transformations, notably through introduction of double bonds into rings E and F, have led to the preparation and characterization of a significant number of new derivatives of the basic nucleus of the steroidal sapogenin, (25*R*)-5α-spirostan, and to thirteen mono- or polydeuterated analogs. In the course of the work, it was possible to study the effect of acidic reagents on the spiroketal side chain, the ease of exchange proceeding in the order 23 >> 20 >>> 25. The availability of the various deuterium-labeled sapogenins proved of great value for many nmr assignments in this class of natural products.

In years past, synthetic work in the field of steroidal sapongenins has taken a number of directions. Characterization of unknown species either by direct chemical manipulation or by interconversion led Marker[4] and, more recently, others[5] to the identification of a wealth of these natural products. Degradation of the spiroketal side chain by modifications[6] of the original Marker procedure[7] afforded new and industrially important routes to such important hormones as the pregnanes,[7-9] cortisone,[8,9] and certain progestational agents.[8,9]

During the past 10 years, two groups[10,11] have reported total syntheses of members of this class; in addition, biosynthetic studies[12] and biodegradation experiments[13] have also appeared. Over the years the usual spectroscopic techniques, such as infrared,[14] ultraviolet,[15] nuclear magnetic resonance,[16] optical rotatory dispersion,[17] and mass spectrometry,[18] have been extensively applied with special reference to the spiroketal system of these sapogenins.

In this laboratory, in connection with a detailed study[18a] of the mass spectrometric behavior of steroidal sapogenins, it became necessary to introduce deuterium at numerous positions of the fundamental skeleton, namely (25*R*)-5α-spirostan or 3-deoxytigogenin (**1**).[19] We felt that such deuterium labeling, though laborious, would not only afford useful mass spectrometric information but would also aid in the interpretation of nuclear magnetic resonance spectra[16] by simplifying splitting patterns and by adding data to the Zürcher-type tables of Tori and Aono.[16e] With relatively few exceptions,[20] most of the chemical studies in this series have involved degradation of the spiroketal system rather than substitutions of the intact side chain. Consequently, our work was likely to contribute to this relatively scarcely studied aspect of sapogenin chemistry.

The problem which we faced may be stated as follows: starting with the basic sapogenin nucleus, **1**,

(1) Financial assistance from the National Institutes of Health (Grant No. AM 12758) is gratefully acknowledged.

(2) Taken, in most part, from the Ph.D. Thesis of W. H. F., National Institutes of Health Predoctoral Research Fellow, 1966–1970.

(3) Postdoctoral Research Fellow, 1965–1966.

(4) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, and C. H. Ruof, *J. Amer. Chem. Soc.*, **69**, 2167 (1947).

(5) For recent examples see (a) R. Tschesche, M. Tauscher, H. W. Fehlhaber, and G. Wulff, *Chem. Ber.*, **102**, 2072 (1969); (b) F. Yasuda, Y. Nakagawa, A. Akahori, and T. Okanishi, *Tetrahedron*, **24**, 6535 (1968); (c) H. Ripperger, H. Budzikiewicz, and K. Schreiber, *Chem. Ber.*, **100**, 1725 (1967); (d) H. Ripperger, K. Schreiber, and H. Budzikiewicz, *ibid.*, **100**, 1741 (1967); (e) H. Minato and A. Shimaoka, *Chem. Pharm. Bull. (Tokyo)*, **11**, 876 (1963); (f) K. Takeda, T. Okanishi, H. Minato, and A. Shimaoka, *Tetrahedron*, **19**, 759 (1963); (g) M. Ogata, F. Yasuda, and K. Takeda, *J. Chem. Soc. C*, 2397 (1967).

(6) (a) F. C. Uhle, *J. Org. Chem.*, **30**, 3915 (1965), and ref 2–7 therein; (b) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, and A. G. Long, *J. Chem. Soc.*, 2807 (1955); (c) another interesting method can be found in K. Morita, S. Noguchi, H. Kono, and T. Miki, *Chem. Pharm. Bull.* (Tokyo), **11**, 90 (1965).

(7) (a) R. E. Marker, *J. Amer. Chem. Soc.*, **62**, 3350 (1940); (b) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 518 (1940); **61**, 3592 (1939).

(8) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y. 1959, pp 547–554, 591–592, and 667–672.

(9) C. W. Shoppee, "Chemistry of the Steroids," 2nd ed, Butterworths, London, 1964, pp 186 and 419–424.

(10) (a) S. V. Kessar, A. L. Rampal, and Y. P. Gupta, *Tetrahedron*, **24**, 905 (1968); (b) S. V. Kessar, Y. P. Gupta, R. K. Mahajan, G. S. Joshi, and A. L. Rampal, *ibid.*, **24**, 899 (1968); (c) S. V. Kessar, Y. P. Gupta, R. K. Mahajan, and A. L. Rampal, *ibid.*, **24**, 893 (1968); (d) S. V. Kessar and A. L. Rampal, *ibid.*, **24**, 887 (1968); (e) S. V. Kessar, Y. P. Gupta, and A. L. Rampal, *Tetrahedron Lett.*, 4319 (1966); (f) S. V. Kessar and A. L. Rampal, *Chem. Ind* (London), 1957 (1963).

(11) Y. Mazur, N. Danieli, and F. Sondheimer, *J. Amer. Chem. Soc.*, **82**, 5889 (1960).

(12) (a) R. Joly and Ch. Tamm, *Tetrahedron Lett.*, 3535 (1967); (b) K. Takeda, H. Minato, and A. Shimaoka, *J. Chem. Soc. C*, 876 (1967).

(13) G. Ambrus and K. G. Büki, *Steroids*, **13**, 623 (1969).

(14) (a) J. E. Page, *Chem. Ind.* (London), 58 (1957), and references therein; (b) R. N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Amer. Chem. Soc.*, **75**, 158 (1953); (c) C. R. Eddy, M. E. Wall, and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953); (d) A. L. Hayden, P. B. Smeltzer, and I. Scheer, *ibid.*, **26**, 550 (1954).

(15) G. Diaz, A. Zaffaroni, G. Rosenkranz, and C. Djerassi, *J. Org. Chem.*, **17**, 747 (1952).

(16) (a) R. K. Callow, V. H. T. James, O. Kennard, J. E. Page, P. N. Paton, and L. R. diSanseverino, *J. Chem. Soc. C*, 288 (1966); (b) G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc. B*, 807 (1966); (c) D. H. Williams and N. S. Bhacca, *Tetrahedron*, **21**, 1641 (1965); (d) P. M. Boll and W. von Philipsborn, *Acta Chem. Scand.*, **19**, 1365 (1965); (e) K. Tori and K. Aono, *Ann. Rep. Shionogi Res. Lab.*, **14**, 136 (1964); (f) J. P. Kutney, W. Cretney, G. R. Pettit, and J. C. Knight, *Tetrahedron*, **20**, 1999 (1964); (g) J. P. Kutney, *Steroids*, **2**, 225 (1963); (h) W. E. Rosen, J. B. Ziegler, A. C. Shabica, and J. N. Shoolery, *J. Amer. Chem. Soc.*, **81**, 1687 (1959).

(17) C. Djerassi and R. Ehrlich, *ibid.*, **78**, 440 (1956).

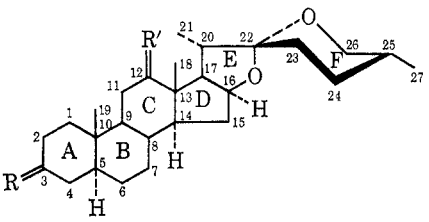
(18) (a) W. H. Faul and C. Djerassi, unpublished work; (b) C. Djerassi, *Pure Appl. Chem.*, in press; (c) H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *Monatsh. Chem.*, **93**, 1033 (1962).

(19) The sapogenin nomenclature used in this paper follows the IUPAC-IUB 1969 Revised Tentative Rules for Steroid Nomenclature, *Steroids*, **13**, 278 (1969), or *J. Org. Chem.*, **34**, 1517 (1969). In cases where a trivial name has been used in the literature for many years, it will be used (along with the proper nomenclature, in some cases) upon its first mention in the article. However, because sapogenin nomenclature has changed a number of times throughout the years, only the proper name will be used thereafter.

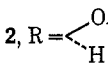
(20) (a) F. C. Uhle, *J. Org. Chem.*, **32**, 792 (1967), and references 4, 5, 8, and 11 therein; (b) L. J. Chinn, *ibid.*, **32**, 687 (1967); (c) P. Bladon, W. McMeekin, and I. A. Williams, *J. Chem. Soc.*, 5727 (1963); (d) Y. Sato, H. G. Latham, Jr., L. H. Briggs, and R. N. Seelye, *J. Amer. Chem. Soc.*, **79**, 6089 (1957); (e) references 6 and 16a.

find methods to functionalize positions, especially those in rings E and F, so that the usual deuterating reagents,[21] *e.g.* D_2, D_2O, $LiAlD_4$, $NaBD_4$, CH_3OD, CH_3COOD, DCl, etc., can be used to incorporate deuterium in a specific manner.

(25*R*)-5α-Spirostan (**1**) was prepared in good yield from hecogenin acetate (**2**) by Huang-Minlon reduction to tigogenin (**3**), followed by Jones oxidation[22] to tigogenone (**4**) and repeated Huang-Minlon reduction.



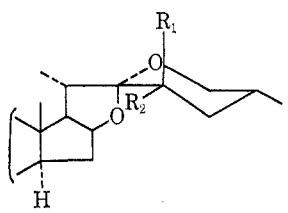
1, R = R′ = H_2

2, R =  ; R′ = O

3, R =  ; R′ = H_2

4, R = O; R′ = H_2

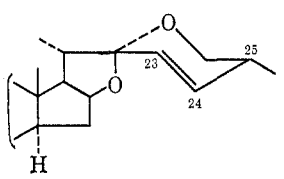
Our initial task was to introduce double bonds into the spiroketal system, a plan which apparently had not been attempted before. For this purpose, **1** was brominated by Callow's method[16a] to afford an easily separable mixture of 23,23-dibromo- (**5**), 23*R*-bromo- (**6**),[16f] and 23*S*-bromo-(25*R*)-5α-spirostan (**7**).[16f] In addition, a minor modification of the original bromination procedure[23] gave quantitative conversion to a mixture of the monobromides **6** and **7**.



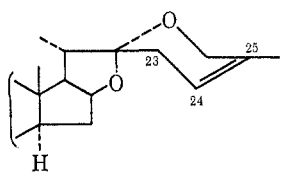
5, $R_1 = R_2 = Br$

6, $R_1 = Br$; $R_2 = H$

7, $R_1 = H$; $R_2 = Br$



8



9

In our hands, only the axial bromide **6** could be made to eliminate even under strong dehydrominating conditions[24] (potassium *t*-butoxide in DMSO–benzene). At room temperature, the reaction gave (25*R*)-5α-spirost-23-ene (**8**) while, at about 100°, the same procedure afforded the isomeric, trisubstituted olefin 5α-spirost-24-ene (**9**) through base abstraction of the tertiary, allylic proton at C-25 in the disubstituted olefin **8**.[25] The direct conversion of **8** to **9** was experimentally verified. In practice, the dehydrobromination was run on the monobromide mixture since the unreactive equatorial bromide **7** can be separated easily from the product olefin, **8** or **9**.

Since lithium aluminum deuteride reduction of tosylates is one of the most specific (position, stereochemistry, per cent incorporation) methods of deuterium introduction,[21] the isomeric olefins were converted to the respective epoxides, 23*R*,24*R*-epoxy-(25*S*)- (**10**), 23*S*,24*S*-epoxy-(25*S*)- (**11**), 24*S*,25*R*-epoxy- (**12**), and 24*R*,25*S*-epoxy-5α-spirostan (**13**), which were then opened to the alcohols **14**, **15**, and **17**–**19** (see Scheme I)[26,27] with lithium aluminum hydride.

The alcohol **17**, obtained from both epoxides **11** and **13**, is the key compound in defining the stereochemistry of essentially all of the others in Scheme I. Since it was derived from the trisubstituted epoxide **13**, it must be a 24-alcohol because the nmr spectrum exhibits a doublet for CH_3-27 in addition to the one for CH_3-21. In the infrared, the hydroxyl absorption, which did not shift upon dilution, appears at 3490 cm^{-1} indicating intramolecular hydrogen bonding, thus proving that the configuration must be *R* (as shown) since, with that stereochemistry, the ring E oxygen and the 24-hydroxyl are in a 1,3-diaxial relationship. All of the other alcohols exhibit hydroxyl absorptions in the range of 3560–3592 cm^{-1} indicating nonhydrogen bonded species. Thus, assuming no change of stereochemistry at the epoxide carbon–oxygen bond which is not opened by hydride, the stereochemistry of the epoxides **10**–**13** follows directly. Since alcohols **15** and **19** were obtained along with **17** from epoxides **11** and **13**, respectively, their configurations must be as indicated. That the alcohol **18** is the tertiary isomer of **19** and not the secondary alcohol **16** is proved by the nmr spectrum which exhibits three tertiary methyl singlets (CH_3-18,19, and 27). From models, one expects that a change of hydroxyl

(21) (a) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," "Alkaloids," Vol I, Holden-Day, San Francisco, Calif., 1964, pp 17–40. See also (b) M. Fétizon and J. C. Gramain, *Bull. Soc. Chim. Fr.*, 651 (1969).

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

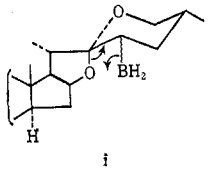
(23) C. Djerassi, H. Martinez, and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951). See also references 16a and 16f.

(24) (a) J. E. Hofmann, T. J. Wallace, and A. Schriesheim, *J. Amer. Chem. Soc.*, **86**, 1561 (1964); (b) T. J. Wallace, J. E. Hofmann, and A. Schriesheim, *ibid.*, **85**, 2739 (1963).

(25) The isomerization of olefins under strongly basic conditions is well verified: (a) C. Cerceau, M. Laroche, A. Pazdzerski, and B. Blouri, *Bull. Soc. Chim. Fr.*, 921 (1969); (b) S. Bank, C. A. Rowe, Jr., A. Schriesheim, and L. A. Naslund, *J. Amer. Chem. Soc.*, **89**, 6897 (1967); (c) S. Bank, C. A. Rowe, Jr., and A. Schriesheim, *ibid.*, **85**, 2115 (1963); (d) A. Schriesheim, C. A. Rowe, Jr., and L. Naslund, *ibid.*, **85**, 2111 (1963); (e) A. Schriesheim, R. J. Muller, and C. A. Rowe, Jr., *ibid.*, **84**, 3164 (1962); (f) A. Schriesheim and C. A. Rowe, Jr., *ibid.*, **84**, 3160 (1962); (g) A. Schriesheim and C. A. Rowe, Jr., *Tetrahedron Lett.*, 405 (1962); (h) A. Schriesheim, J. E. Hofman, and C. A. Rowe, Jr., *J. Amer. Chem. Soc.*, **83**, 3731 (1961).

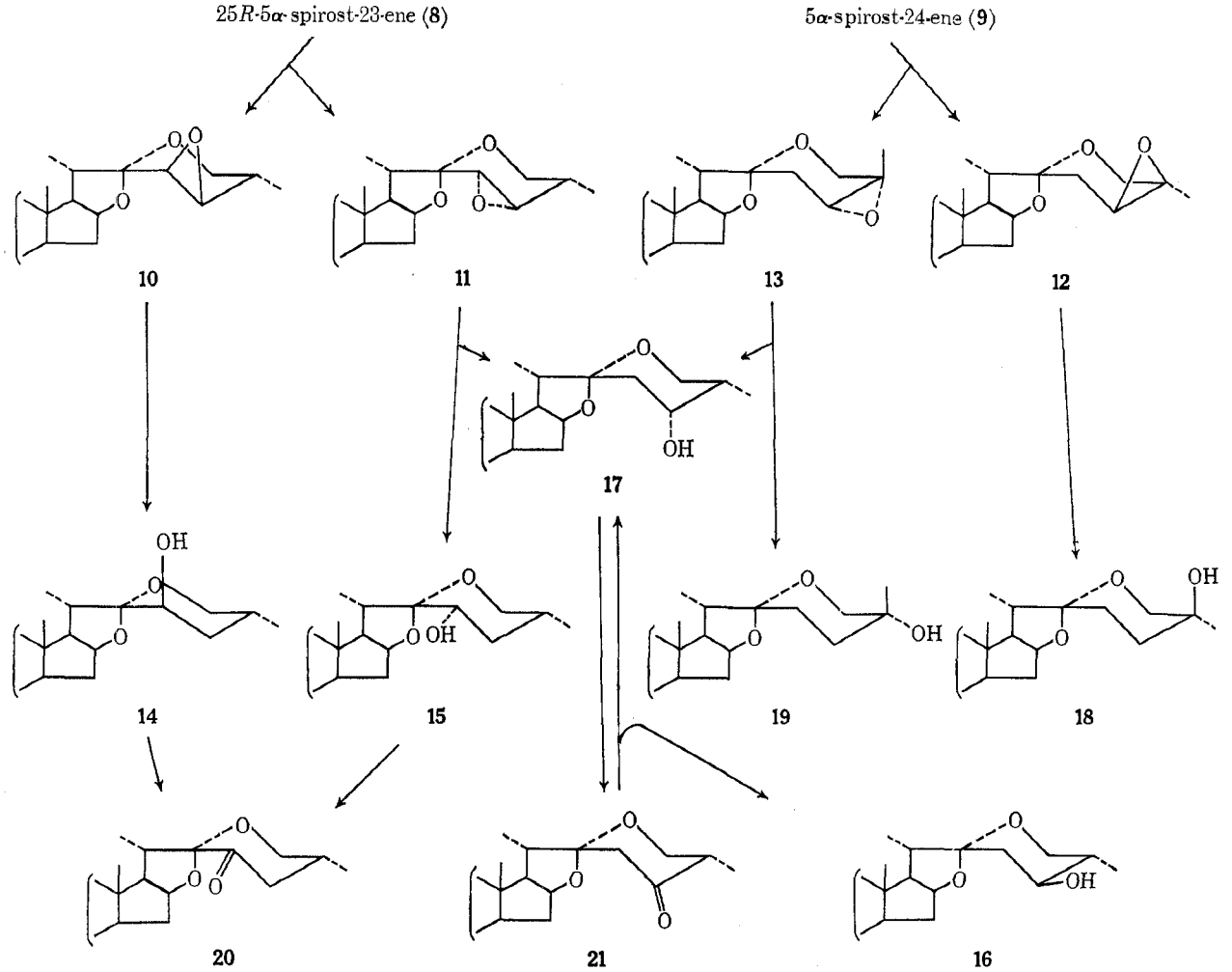
(26) Since the plane of ring F is perpendicular to the plane of the paper, the practice of designating the stereochemistry of substituents located on carbons 23–26 by dotted and solid lines and calling them "α" and "β," respectively, cannot properly be done (see reference 19). However, more clarity is obtained in Scheme I if, as one looks from the "top" of ring F, the substituents below the ring are designated by dotted lines and the ones above the ring by solid lines.

(27) One might suggest that the alcohols and even the deuterated compounds be prepared through hydroboration of the olefins [M. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, **29**, 1120 (1964)]. This procedure was, in fact, tried on the olefin **8** but gave at least twelve products owing, possibly, to an initial elimination of type i. See also G. R. Pettit and T. R. Kasturi, *ibid.*, **26**, 4553 (1961).



i

SCHEME I



configuration from 23*S* to 23*R* (**15** to **14**) would affect the nmr absorption of CH_3-21 much more than that of CH_3-27 since in **14**, the hydroxyl is situated essentially in a 1,3-diaxial relationship to CH_3-21, a situation known[28] to produce a strong downfield shift. The change 24*R* to 24*S* (**17** to **16**) would affect CH_3-27 more than CH_3-21. A 16-Hz downfield shift of CH_3-21 from **15** to **14** as well as oxidation of both to the same ketone **20** confirmed the structure of **14**. Lithium aluminum hydride reduction of the 24-ketone **21**, derived from alcohol **17**, unfortunately afforded **17** as the major product. However, the isomer **16** (24*S*) was obtained in small yield.

The nmr chemical shift data for all compounds **8** through **21** appear in Table I. We had hoped to determine the stereochemistry of both members of each epoxide pair by nmr, but results were inconclusive because, even though there are indications that ring F is in the form of a half-chair[29] and, though ample data are available for predicting chemical shifts[29a] and coupling constants,[30] the way to build a model from which to calculate is not clear owing to the possible inversion of ring F from one half-chair to another as in the case of cyclohexene,[31] a situation which greatly complicates matters. Regular steroid models[29a] do not show the same relative spacial orientations between epoxide and methyl group.

Unfortunately, tosylate formation[32] of the most readily available secondary alcohol, (25*S*)-5α-spirostan-24*R*-ol (**17**), proved abortive as did tosylhydrazone formation[33] and electrolytic reduction[34] of the derived ketone, (25*S*)-5α-spirostan-24-one (**21**).

Since ketals are known to be sensitive to acid, we considered next the possibility of acid-catalyzed exchange of the spiroketal system in a deuterium-containing medium. A number of workers[35-37] have explored this type reaction but have never applied nuclear magnetic resonance or mass spectrometry to the problem of the location and amount of incorporated deuterium. 1,4-Dioxaspiro[4.5]decane (A) shows an nmr absorption[38] for protons a at δ 1.51; so the "spike" at 1.59 in

(28) K. Tori and T. Komeno, *Tetrahedron*, **21**, 309 (1965). See especially p 318.

(29) (a) K. Tori, T. Komano, and T. Nakagawa, *J. Org. Chem.*, **29**, 1136 (1964); (b) A. McL. Mathieson, *Tetrahedron Lett.*, 81 (1963).

(30) (a) Reference 29a, pp 1139–1141; (b) K. Tori, K. Kitahonoki, Y. Takano, H. Tanida, and T. Tsuji, *ibid.*, 559 (1964).

(31) F. A. L. Anet and M. Z. Haq, *J. Amer. Chem. Soc.*, **87**, 3147 (1965).

(32) (a) Reference 5f, p 770; (b) P. S. Wharton and G. A. Hiegel, *J. Org. Chem.*, **30**, 3254 (1965) (sodium hydride method).

(33) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Chem. Ber.*, **98**, 3236 (1965).

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

(35) Reference 16a, especially pp 293 and 296.

(36) R. K. Callow and P. N. Massy-Beresford, *J. Chem. Soc.*, 2645 (1958).

(37) R. B. Woodward, F. Sondheimer, and Y. Mazur, *J. Amer. Chem. Soc.*, **80**, 6693 (1958).

(38) Sadtler Spectrum No. 430M published by Sadtler Research Laboratories, Inc., Philadelphia, Pa. 19104.

TABLE I

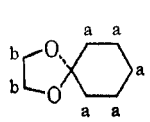
NUCLEAR MAGNETIC RESONANCE DATA OF SOME STEROIDAL SAPOGENINS[a]

Compd no.	Description[b]	δ, ppm, from TMS				
		19 H	18 H	21 H	27 H	Other H[c]
1[f]		0.794	0.760	0.956 (d, 6.6)	~0.776 (d, ~6)	23 H_2 1.59
5	23,23-dibromo-	0.800	0.994	1.229 (d, 6.9)	0.833 (d, ~6.6)	
6[g]	23*R*-bromo-	0.797	0.797	1.176 (d, 7.0)	0.803 (d, 6.2)	
7[g]	23*S*-bromo-	0.801	0.883	0.932 (d, 7.0)	0.815 (d, 6.0)	
8	Δ^{23}	0.803	0.803	0.922 (d, 6.8)	0.874 (d, 7.2)	5.83 (23 H, m, 10 and 2) 5.53 (24 H, m, 10)
9	Δ^{24}	0.791	0.781	1.003 (d, 6.7)	1.600 (s)	5.42 (24 H, m)
		0.87	0.77	1.16 (d, 6.5)	1.53 (s)	pyr (60-MHz nmr)
10	23*R*,24*R*-epoxy-(25*S*)-	0.799	0.833	0.951 (d, 7.3)	1.047 (d, 7.0)	2.92 (23 H, 24 H, m) 3.4 (26 H_2, m); 4.5 (16 H, m)
11	23*S*,24*S*-epoxy-(25*S*)-	0.803	0.850	0.992 (d, 6.8)	0.992 (d, ~6.5)	3.2 (23 H, 24 H, m) 3.3 (26 H_2, m); 4.5 (16 H, m)
12	24*S*,25*R*-epoxy-	0.796	0.761	0.960 (d, 6.4)	1.278 (s)	3.21 [24 H, m, d (?), ~5] 3.66 (26 H_a, d, 12); 3.83 (26 H_e, d, 13); 4.35 (16 H, m)
13	24*R*,25*S*-epoxy-	0.788	0.752	0.948 (d, 6.6)	1.365 (s)	3.11 (24 H, m) 3.75 (26 H_a, d, 12); 4.05 (26 H_e, d, 13) 4.3 (16 H, m)
14	23*R*-hydroxy-(25*R*)-	0.796	0.784	1.104 (d, 7.0)	0.798 (d, 6.0)	
15	23*S*-hydroxy-(25*R*)-	0.802	0.802	0.943 (d, 6.9)	0.813 (d, 6.0)	
16	24*S*-hydroxy-(25*S*)-	0.80	0.77	0.98 (d, 6.2)	0.93 (d, 6.6)	(approximate values)
17	24*R*-hydroxy-(25*S*)-	0.795	0.761	0.956 (d, 7.0)	0.870 (d, 6.9)	
18	25*S*-hydroxy-	0.790	0.768	1.018 (d, 6.2)	1.102 (s)	(60-MHz nmr) 2.4 (-OH)
19	25*R*-hydroxy-	0.790	0.758	0.967 (d, 6.2)	1.289 (s)	(60-MHz nmr) 2.1 (-OH)
20	23-oxo-(25*R*)-	0.792	0.769	0.927 (d, 6.9)	0.927 (d, 6.9)	4.60 (16 H, m); 2.85 (20 H, m); 2.42 [24 H, s (?)]; 3.7 (26 H_2, m)
21	24-oxo-(25*S*)-	0.795	0.756	0.958 (d, 6.6)	1.071 (d, 6.6)	4.3 (16 H, m); ~2.4 (23 H_a, d, 14); ~2.6 (23 H_e, d, 14); 25 H under 23; 3.6 (26 H_a, m, 11 and 11); 3.9 (26 H_e, m, 12 and 8)
24	20-deuterio-(25*R*)-	0.794	0.758	0.951 (s)	0.778 (d, 6.0)	
26	24*S*,25*R*-dideuterio-	0.790	0.758	0.952 (d, 6.6)	0.770 (s)	4.367 (16 H, m, 7.5, 6.75, and 7.6); 1.576 [23 H_2, s (?)]; 3.338 (26 H_a, d, 6.9); 3.448 (26 H_e, d, 6.6)
29	(20*S*,22ξ,25*R*)-5α-furostan-26-ol	0.792	0.792	0.980 (d, 6.8)	0.912 (d, 6.8)	C-21 and C-27 assignment could be reversed
33	26ξ-deuterio-(25*R*)-	0.796	0.765	0.960 (d, 6.6)	hidden by 18 and 19	26 HD *vs.* 16 H integrates *ca.* 0.9–1.0
37	(25*R*)-5α-furost-20(22)-en-26-yl acetate	0.796	0.661	1.573 (s)	0.930 (d, 6.8)	2.029 (-OCOCH$_3$, s); 4.71 (16 H, octet, 11, 7.5, and 6); 2.44 (17 H, d, 10); 3.85 (26 H, m, 10 and 6); 3.97 (26 H, m, 10 and 6)

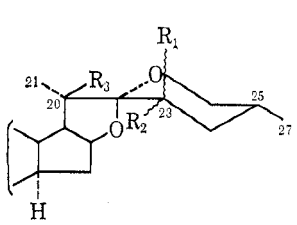
TABLE I
(*Continued*)

Compd no.	Description[b]	δ, ppm. from TMS: 19 H	18 H	21 H	27 H	Other H[c]
38	(25*R*)-5α-furost-20(22)-en-26-ol	0.79	0.67	1.58 (s)	0.93 (d, ~6)	($CDCl_3$[d]) 4.73 (16 H, octet, as in **37**); 2.46 (17 H, d, 10); ~3.40 (26 H); ~3.55 (26 H);
		0.766	0.750	1.652 (s)	1.102 (d, 6.6)	pyr C-18 and C-19 assignment could be reversed
39	(20*R*,25*R*)-5α-spirostan	0.783	0.931[e]	1.135[e] (d, 8.1)	0.778 (d, 5.7)	~2.0 (17 H, m); 2.42 (20 H, m);
40	20-acetyl-(20*R*,25*R*)-	0.776	0.994	1.343 (s)	0.774 (d, 5.6)	2.12 (-$COCH_3$, s); 2.83 (17 H, d, 6.5)
41	20-hydroxy-(20*S*,25*R*)-	0.779	0.924	1.356 (s)	0.796 (d, 6.0)	4.36 (-OH, s); 4.5 (16 H, m); 1.80 (17 H, d, 6.5); 3.6 (26 H, m)
42	$\Delta^{20(21)}$	0.785	0.672	5.07 (m, 16 and 2)	0.796 (d, 6.0)	4.58 (16 H, m); 2.65 (17 H, m, 7.5 and 2) (long range coupling to CH_2-21)
50	digallogenin (3β,15β-diol)	0.853	1.007	0.955 (d, 6.9)	0.798 (d, 5.9)	5.0 (15 H, m, 4 and 5.8)
52	3β-acetate 15-one	0.839	0.760	1.017 (d, 6.9)	0.775 (d, ~6)	2.000 (-$OCOCH_3$, s)
60	3β,12α-diol, Δ^{14}	0.867	1.112	1.002 (d, 6.5)	0.795 (d, 5.1)	(60 MHz) 2.7 (8 H, m); 5.53 (15 H, m); 4.9 (16 H, m)
61	3,12-dione, Δ^{14}	1.130	1.313	1.042 (d, 6.8)	0.798 (d, 5.7)	5.45 (15 H, m); 4.75 (16 H, m)
62	Δ^{14}	0.834	1.038	1.013 (d, 7.2)	0.788 (d, 5.5)	5.3 (15 H, m); 4.89 (16 H, m)
63	3-ethylene ketal, Δ^{14}	0.865	1.042	1.008 (d, ~7)	0.795 (d. 5.5)	5.3 (15 H, m); 3.90 ($2CH_2$ ketal, s); 4.89 (16 H, m)
64	(25*R*)-5α,14β-spirostan (66, 14β D)	0.756	0.965	0.974 (d, 6.4)	0.782 (d, 6.4)	
65	3-ethylene ketal, 14β H	0.784	0.966	0.971 (d, 6.7)	~0.78 (d, ~6)	3.90 (2 CH_2 ketal, s)
67	kryptogenin	0.803	1.038	1.053 (d, 6.6)	0.938 (d, 6.5)	5.342 (6 H, d, 4.5)

[a] All spectra were determined in deuteriochloroform (pretreated with anhydrous sodium carbonate) on a Varian Associates HA-100 nuclear magnetic resonance spectrometer (tetramethylsilane internal reference) unless otherwise noted. pyr = pyridine-d_5; s = singlet (not used in the case of 19 H and 18 H which are always singlets); d = doublet; m = multiplet. The value given after the letter "d" or "m" is the coupling constant, *J*, in hertz. Chemical shifts of all methyl resonances were determined with the counter incorporated in the instrument using an expanded scale. [b] All are derivatives of (25*R*)-5α-spirostan (1). Only the functionalities and/or changes of stereochemistry will be noted. [c] In most cases, the resonances for CH_2-26 and CH-16 are multiplets at δ 3.4 and 4.4, respectively. They will be denoted only if their chemical shifts are noteworthy. [d] Run with sodium carbonate in the tube. Immediately after an initial 250-sec sweep from which these data were obtained, a 500-sec sweep was run but showed that the compound had cyclized to **39** in the interim. Thus, even a mere *trace* of acid or even $CDCl_3$ itself catalyzes the cyclization. [e] The analagous known case of a downfield shift of a methyl resonance caused by steric crowding of one methyl group with another is the 0.076-ppm downfield shift of the C-19 resonance of cholestane upon addition of a 2β-methyl group. *Cf.* D. A. Schooley, Ph.D. Thesis, Stanford University, Stanford, Calif., 1968, p 42. [f] References 16e and 16g. [g] Reference 16f.



A, a, δ1.51
b, δ3.80



22, $R_1 = R_2 = D$; $R_3 = H$
23, $R_1 = R_2 = R_3 = D$
24, $R_1 = R_2 = H$; $R_3 = D$
25, $R_1 = D$; $R_2 = R_3 = H$

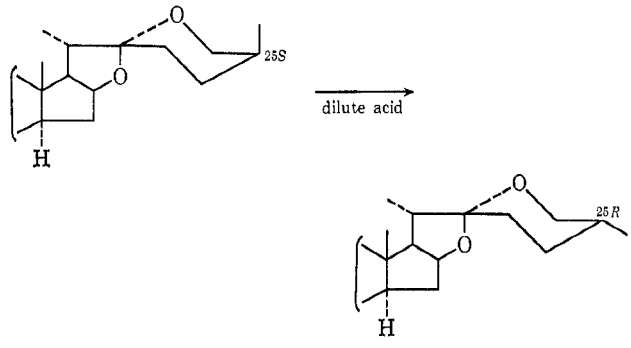
the spectrum of (25*R*)-5α-spirostan (1) has therefore been assigned[16a] (see also Table I), in part, to the protons at C-23. This signal was greatly reduced in intensity after acid-catalyzed exchange in deuterium-containing media.

Thus, when (25*R*)-5α-spirostan (1) was refluxed for 1 hr either in acetic acid-OD or in *ca.* 0.09 *N* DCl–EtOD with 1% D_2O, the 23,23-dideuterio analog **22** was obtained in 90% isotopic purity. Continued reflux gave 20,23,23-trideuterio-(25*R*)-5α-spirostan [**23** (the C-20 methyl doublet at 0.96[16g] collapsed to a singlet, δ 0.957)] which, upon mild back-exchange with acetic acid, afforded 20-deuterio-(25*R*)-5α-spirostan (**24**, 24% d_0, 75% d_1, 1% d_2).

As an additional check on the position of initial deuterium incorporation, the equatorial bromide **7** was reduced[23] with zinc in ethanol-OD to 23ξ-deuterio-(25*R*)-5α-spirostan (**25**, 93% d_1); the "spike" at δ1 .59 was found to decrease by 34%, whereas in the di-

deuterio analog 22 it decreased by 48%.[39] In a like manner, the 23,23-dibromide 5 was converted to the dideuterio derivative 22.

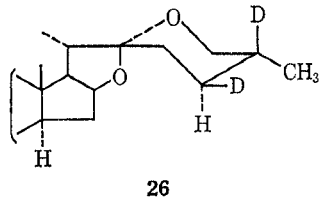
In an explanation of the "Iso-Reaction" originally discovered by Marker[40] in which a (25*S*)-spirostan is isomerized to a (25*R*)-spirostan by the action of dilute acid, Woodward proposed[41] an oxidation–reduction mechanism which, under acid-catalyzed exchange in



The Iso-Reaction[40]

deuterium-containing media, would require incorporation at C-25 as was noted by Callow.[36] Using an exchange procedure, a mass spectrometric method, and calculations all to be published elsewhere,[18a] we were able to confirm a 20% deuterium incorporation at C-25 under Callow's[36,18a] reaction procedure but, since deuterium at C-25 would collapse the C-27 doublet to a singlet, we must now prove that the acid-catalyzed exchange described above actually affords the C-20 deuterio analog 24 and not a C-25 isomer; that is, that the δ 0.96 collapsing doublet is, in fact, the resonance of C-21.

To do so, we made use of another approach to deuterium labeling of the spiroketal system, the homogeneous catalytic deuteration of the Δ^{24} olefin 9 since this procedure is known[42] not to involve isotopic scrambling in contrast to noble metal catalysts.[43] Using tris(triphenylphospho)rhodium chloride[42] in acetone solution, we obtained, in 97% isotopic purity, 24*S*,25*R*-dideuterio-(25*R*)-5α-spirostan (26), the nuclear



26

magnetic resonance spectrum of which showed a methyl doublet (J = 6.6 Hz) centered at δ 0.952 (C-21) and three methyl singlets: δ 0.758 (C-18), 0.790 (C-19), and 0.770 (C-27). This experiment provides unambiguous support for Kutney's[16g] conclusion that, of the two methyl doublets in the nmr spectrum of 1, the 0.96-ppm doublet corresponds to the C-21 methyl group and that the one at 0.77 ppm is the C-27 methyl function.

In order to establish the configuration at C-25 in the dideuterio derivative 26, use can be made of the extensive tables of Tori and Aono[16e] (Table II).

TABLE II

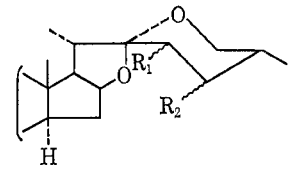
METHYL RESONANCES OF CERTAIN EXAMPLES OF THE NUCLEAR MAGNETIC RESONANCE SPECTRA OF STEROIDAL SAPOGENINS[16e]

Compd no.[a]	Substituents	C-25 stereochemistry	δ, ppm, from TMS 19 H	18 H	21 H	27 H
10	3β-acetate	*R*	0.83	0.77	0.96	0.78
11	3β-acetate	*S*	0.82	0.75	1.09	0.98
15	2α,3β-diol	*R*	0.87	0.77	0.96	0.79
16	2α,3β-diol	*S*	0.87	0.77	1.10	0.98
17	2α,3β-diacetate	*R*	0.93	0.76	0.95	0.78
18	2α,3β-diacetate	*S*	0.93	0.76	1.10	0.97

[a] Reference 16e.

Between each *R* and *S* pair, one notes that (a) C-21 is always downfield from C-27; (b) C-18 and C-19 do not shift; (c) in the *R* configuration, C-27 is always *between* C-18 and C-19 while, in the *S* configuration, it is always downfield from them; (d) in all cases, the substituents shift C-19 downfield from its position (δ 0.79) in (25*R*)-5α-spirostan (1). Thus, if the deuteration had produced a mixture or had given the 25*S* derivative, the differences would easily be discernible by a methyl singlet downfield from C-18 and C-19. No such signal was evident in the spectrum of 26. Since *cis* addition is known to occur in homogeneous hydrogenation,[44] the stereochemistry at C-24 is assigned as *S* (equatorial deuterium as ring F is drawn in 26).

(25*R*)-5α-Spirost-23-ene (8) was deuterated in the same manner giving 23ξ,24ξ-dideuterio-(25*R*)-5α-spirostan (27, 97% d_2) which, upon back-exchange in acetic acid, produced 24ξ-deuterio-(25*R*)-5α-spirostan (28, 98% d_1).



27, $R_1 = R_2 = D$
28, $R_1 = H$; $R_2 = D$

The foregoing data establish unequivocally that the monodeuterated derivative 24 is the 20-deuterio-(25*R*)-5α-spirostan and *not* the C-25 analog. Thus, the rate of acid-catalyzed exchange is C-23 >> C-20 >>> C-25.

Having effected deuterium introduction at positions 20, 23, 24, and 25, we next turned to C-26. For this

(39) A mere 13% decrease in the δ 1.59 spike in the spectrum of the C-20 deuterated analog **24** indicates that more than just the C-20 and C-23 protons have this chemical shift.

(40) R. E. Marker and E. Rohrmann, *J. Amer. Chem. Soc.*, **61**, 846 (1939). See also reference 8, p 818 ff.

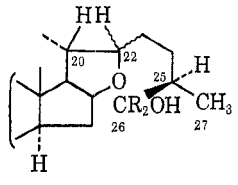
(41) See reference 37. When C-27 is substituted with an hydroxyl group, the reaction may go by a very simple mechanism (see reference 5f, p 765).

(42) W. Voelter and C. Djerassi, *Chem. Ber.*, **101**, 58 (1968), and references 2–8 therein.

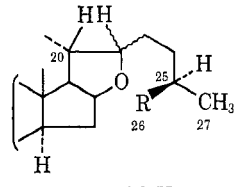
(43) C. Djerassi in "Proceedings of the Second International Congress on Hormonal Steroids," Excerpta Medica Foundation, Amsterdam, 1967, pp 261–268.

(44) J. A. Osborn, F. H. Jardine, J. F. Young, and G. Wilkinson, *J. Chem. Soc. A*, 1711 (1966).

purpose, (25*R*)-5α-spirostan (**1**) was subjected to catalytic hydrogenation in glacial acetic acid with a trace of perchloric acid as promoter to afford[45] (20*S*,22ξ,25*R*)-5α-furostan-26-ol (**29**)[46] which was oxidized[22] to (20*S*,22ξ,25*R*)-5α-furostan-26-oic acid (**30**) and then reduced with lithium aluminum deuteride to the 26,26-dideuterio alcohol **31**. Oxidation with Collins reagent[47] afforded 25-deuterio-(20*S*,22ξ,25*R*)-5α-furostan-26-al (**32**)[48] which was cyclized in acid[37,49] to 26ξ-deuterio-(25*R*)-5α-spirostan (**33**).

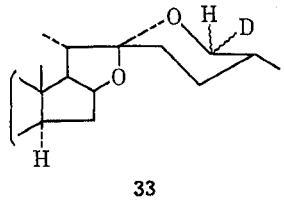


29, R = H
31, R = D



30, R = CO_2H

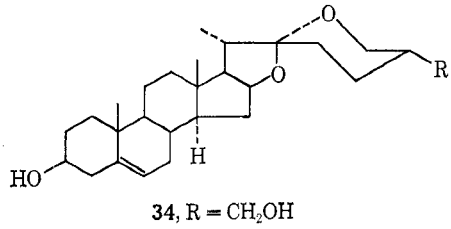
32, R = $-C(=O)D$



33

Deuterium labeling of steroidal angular methyl groups, especially of C-18[50] and C-19,[51] has, in the past, been difficult and in at least one case required total synthesis of the steroid.[50] Fortunately, the C-21 and C-27 labels have not proved so.

(25*S*)-Spirost-5-ene-3β,27-diol (**34**, "isonarthogenin")[52] was converted to the tosylate **35** which, upon displacement with lithium aluminum deuteride, gave 27-deuterio-(25*R*)-spirost-5-en-3β-ol (**36**, "27-deuteriodiosgenin"). Owing to the small amount of material



34, R = CH_2OH
35, R = CH_2OTs
36, R = CH_2D

with which we had to work, the hydroxyl group and the double bond in rings A and B were not eliminated. As such, this is the only labeled "final product" which is not a complete analog of (25*R*)-5α-spirostan (**1**).

In order to label C-21, we first converted (25*R*)-5α-spirostan (**1**) into (20*R*,25*R*)-5α-spirostan (**39**, "20-iso-3-deoxytigogenin") using Wall's[53] procedure (see Scheme II). The intermediates **37** and **38**, (25*R*)-5α-furost-20(22)-en-26-yl acetate and the corresponding alcohol, respectively, were not isolated during this procedure but were later prepared separately using acetic anhydride–pyridine with monomethylamine hydrochloride as catalyst.[54] Of the side products **40** and **41**, formation of the former has precedence in the literature.[55]

Chromium trioxide oxidation[56] of **39** to the alcohol **41**, followed by dehydration[56] to (25*R*)-5α-spirost-20-ene (**42**) and homogeneous catalytic deuteration[42] afforded 20,21-dideuterio-(20*R*,25*R*)-5α-spirostan (**43**)[57] which gave 21-deuterio-(25*R*)-5α-spirostan (**44**, 98% d_1) after gentle reflux in dilute hydrochloric acid–ethanol.[58]

The work so far described has provided a deuterium label for all nuclear positions in rings E and F peculiar to steroidal sapogenins. We now turn to rings C and D of the conventional steroid skeleton.

Desulfurization of thioketals with Raney nickel–deuterium is known[21] to be a conventional method of replacing a carbonyl function by two deuterium atoms, although the isotopic purity is frequently rather poor.[43] The sapogenin spiroketal is sensitive to some reagents used to catalyze the thioketal formation[49a] but Fieser's use[59] of perchloric acid avoids this difficulty. Any olefin formed upon reduction[60] can be eliminated with the use of silica gel containing silver nitrate in preparative thin layer chromatography.[61] It is upon these facts that the synthesis of the three deuterated analogs **47**, **49**, and **57** is based.

Thus, conversion of (25*R*)-5α-spirostan-12-one (**45**)[62] to the ethylene thioketal **46** and desulfurization with deuterio Raney nickel gave 12,12-dideuterio-(25*R*)-5α-spirostan (**47**) containing nearly equal amounts of d_1 and d_2 species. In a similar manner, after the ketone **45** was exchanged in deuteriomethanol containing sodium deuterioxide[21] to give the 11,11-dideuterio analog **48**, conversion to the thioketal and desulfurization with regular Raney nickel produced 11,11-dideuterio-(25*R*)-5α-spirostan (**49**) of high isotopic purity (91% d_2).

It is most fortunate that there are naturally occurring

(45) Reference 36, p 2648.

(46) The trivial name[19] for this system is "dihydropseudogenin," *e.g.*, "dihydro-3-deoxypseudotigogenin" (**29**).

(47) J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 3363 (1968).

(48) There is some question about the stereochemistry at C-25 in this case. Woodward[37] claims that the product is a mixture of the 25*R* and 25*S* species. Inversion of the original stereochemistry could occur under acidic[37] or basic conditions (abstraction of the proton at C-25). Thin layer chromatography did show a major and minor product but no attempt was made to separate them since the next step, acid cyclization, should give the same product or product mixture from either compound.

(49) (a) C. Djerassi, O. Halpern, G. R. Pettit, and G. H. Thomas, *J. Org. Chem.*, **24**, 1 (1959). (b) Note that III in Woodward's mechanism[37] is the protenated form of the aldehyde **32**. The mechanism for this cyclization is thus given in reference 37, starting with III, and reading to the left.

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

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

(52) References 5b,e. We wish to express our gratitude to Dr. Yuzo Nakagawa, Shionogi and Co., Osaka, Japan, for a sample of this material.

(53) M. E. Wall and H. A. Walens, *J. Amer. Chem. Soc.*, **77**, 5661 (1955).

(54) We thank Dr. Monroe E. Wall, Research Triangle Institute, Durham, N. C., for helpful discussion in this matter.

(55) W. F. Johns, *J. Org. Chem.*, **29**, 2545 (1964).

(56) (a) M. E. Wall and H. A. Walens, *J. Amer. Chem. Soc.*, **80**, 1984 (1958); (b) M. E. Wall, H. A. Walens, and F. T. Tyson, *J. Org. Chem.*, **26**, 5054 (1961).

(57) Though we could have passed directly from **37** to **41**,[56b] it is fortunate that we did not since **39** provided a mass spectrum[18a,b] both quantitatively and qualitatively different from that of **1**. In addition, micro thin layer chromatography (see the Experimental Section) gave an easy differentiation between **1** and **39** (as well as **42**) which afforced a quick solution to the stereochemistry of **43** and **44**.

(58) Reference 53, pp 5661 and 5664.

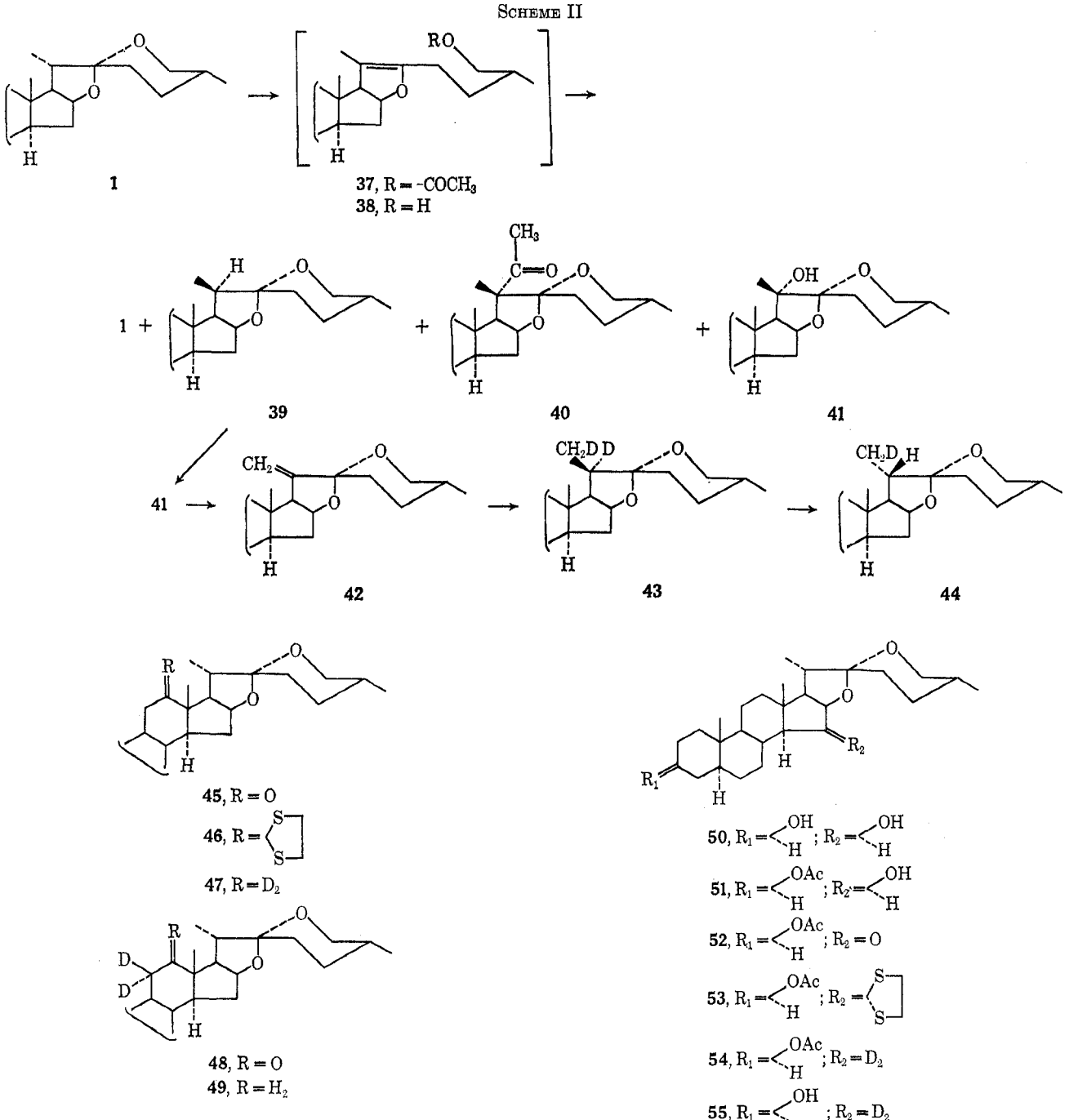
(59) D. L. Klass, M. Fieser, and L. F. Fieser, *J. Amer. Chem. Soc.*, **77**, 3829 (1955).

(60) C. Djerassi and D. H. Williams, *J. Chem. Soc.*, 4046 (1963).

(61) (a) E. Dunn and P. Robson, *J. Chromatogr.*, **17**, 501 (1965); (b) P. J. Stevens, *ibid.*, **36**, 253 (1968).

(62) M. E. Wall and S. Serota, *J. Amer. Chem. Soc.*, **78**, 1747 (1956).

SCHEME II



steroidal sapogenins with oxygen functionalities in almost all positions of the basic skeleton. Thus, 3β-acetoxy-(25*R*)-5α-spirostan-15-one (**52**), derivable[63a] from digallogenin (**50**)[63] through the acetate **51**, led, after appropriate deuterodesulfurization of its thioketal **53**, to the 15,15-dideuterio-3β-acetate **54**. Reduction of **54** with lithium aluminum hydride followed by conventional steps (**55** → **56** → **57**) gave 15,15-dideuterio-(25*R*)-5α-spirostan (**57**).

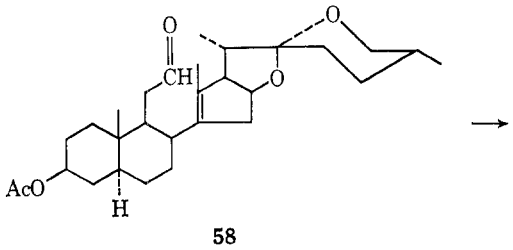
Since isotopic labeling of C-18 seemed to be accessible only through total synthesis and, hence, was not considered worthwhile, we were left with three unlabeled positions (C-14, C-16, and C-17) close to the spiroketal system. An obvious approach to C-14 and C-16, analogous to the method of preparation of the C-11 label **49**, is direct base exchange[21] of the ketone **52**. In fact, such an experiment showed (by mass spectrometric analysis)[18a] that the rate of exchange at C-14 is greater than at C-16 which would allow the selective production of either label by proper use of time and deuterated (for exchange) or nondeuterated (for back exchange) reagents. However, earlier studies in our laboratory[64] have shown that the C/D *cis* ring juncture (14βH) is more stable and the aforementioned exchange, therefore, gives a 14β-d_1 analog. The situation would not pose a problem except that attempted formation of

(63) (a) E. Bianchi, F. Girardi, F. Diaz, R. Sandoval, and M. Gonzales, *Ann. Chim.* (Rome), **53**, 1761 (1963); also see *Chem. Abstr.*, **60**, 12370*f* (1964); (b) R. Tschesche and G. Wulff, *Chem. Ber.*, **94**, 2019 (1961).

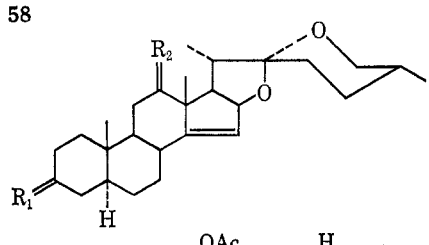
(64) C. Djerassi, T. T. Grossnickle, and L. B. High, *J. Amer. Chem. Soc.*, **78**, 3166 (1956); C. Djerassi, L. B. High, J. Fried, and E. F. Sabo, *ibid.*, **77**, 3673 (1955).

the corresponding 15-thioketal in the 14β series using the identical procedure employed in the preparation of **53** led to decomposition of the spiroketal side chain.

Labeling at C-14 was effected through the intermediacy of 12,13-seco-3β-acetoxy-(25*R*)-5α-spirost-13-en-12-one (**58**, "lumihecogenin acetate")[20b,c,65] which



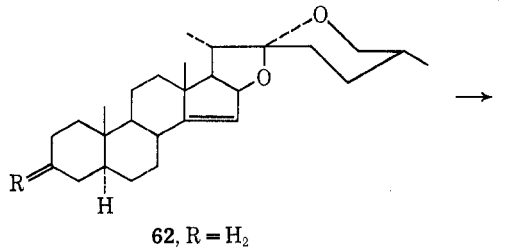
58



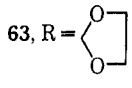
59, R_1 = 3β-OAc, 5α-H; R_2 = 12α-H, 13β-OH

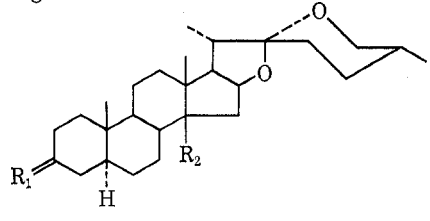
60, R_1 = 3β-OH, 5α-H; R_2 = 12α-H, 13β-OH

61, R_1 = O; R_2 = O

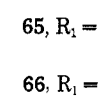


62, R = H_2

63, R = 



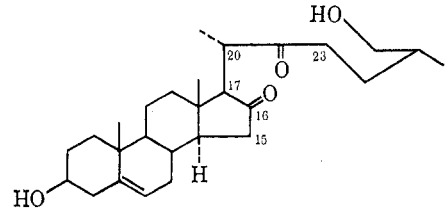
64, R_1 = H_2; R_2 = H

65, R_1 =  ; R_2 = H

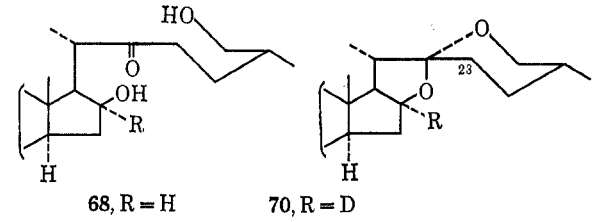
66, R_1 = H_2; R_2 = D

was transformed according to published[20b,c] procedures to 3β-acetoxy-(25*R*)-5α-spirost-14-en-12α-ol (**59**). Successive lithium aluminum hydride reduction to the diol **60**, Jones oxidation[22] to the diketone **61**, and Huang-Minlon reduction afforded (25*R*)-5α-spirost-14-ene (**62**)[66] which, upon deuteroboration with subsequent acid hydrolysis,[27,50] gave 14-deuterio-(25*R*)-5α,14β-spirostan (**66**) of acceptable isotopic purity.

The introduction of the remaining C-16 and C-17 labels starts, in both cases, from the natural product kryptogenin (**67**). Sondheimer and collaborators have already reported[11] the selective reduction of the C-16 carbonyl group to the 16β-alcohol **68** (shown to exist as the 16,22-hemiketal)[70] with sodium borohydride in 2-propanol and subsequent acid cyclization to diosgenin (**36**, R = CH_3). Repetition of this sequence using deuterated reagents (sodium borodeuteride, 2-propanol-OD) afforded **69** and **70**, which, after catalytic hydrogenation of the 5,6 double bond, Jones oxidation,[22] and Huang-Minlon reduction gave 16-deuterio-(25*R*)-5α-spirostan (**71**, 73% d_1, 26% d_2, the extra deuterium being at C-23). The corresponding 20*R* compound **72** was prepared in the same manner[53] as the unlabeled species **39**.

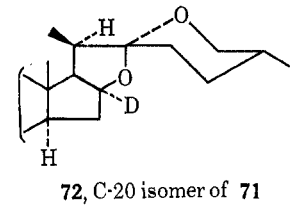


67



68, R = H **70**, R = D

69, R = D **71**, R = D, no unsaturation or oxygenation in rings A, B



72, C-20 isomer of **71**

Kryptogenin (**67**) also appeared to be the most suitable starting material for labeling C-17. Ex-

(65) We express our gratitude to Dr. Peter Bladon, University of Strathclyde, for a generous supply of this substance.

(66) Of the side products **63–65**,[67] the 3-ethylene ketals were surely formed during the first of two successive runs of the Huang-Minlon reduction in which ethylene glycol instead of diglyme was used as solvent.[68] The hydrogenated products arise due to diimide formation known[69] to occur under the strongly basic reaction conditions.

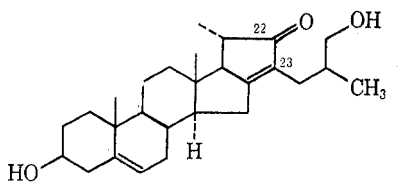
(67) The proof that the ethylene ketal of **63** and **65** is at C-3 and not at C-12 will not be detailed here. The nmr spectrum of both the C-3[18a] and C-12 ethylene ketal [Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 3722 (1964)] of (25*R*)-5α-spirostan were run and showed unequivocally that **63** and **65** are substituted as shown. In the case of **64**, compare the C-18 shift of the 14β isomer **64** and the 14α isomer **1** with the C-18 shift of 14β- and 14α-androstan (N. S. Bhacca and D. H. Williams, "Applications of nmr Spectroscopy in Organic Chemistry-Illustrations from the Steroid Field," Holden-Day, Inc., San Francisco, Calif., 1966, pp 19–24).

(68) W. H. F. performed initial small-scale studies on the sequence **59** → **60** → **61** but large-scale work on the sequence **59** → **62** was done by Dr. Erich C. Blossey in our laboratory in connection with another problem during his 1968 summer sabbatical leave from Rollins College, Winter Park, Fla.

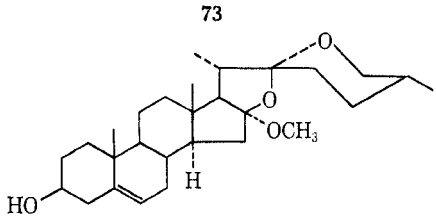
(69) E. J. Corey, W. L. Mock, and D. J. Pasto, *Tetrahedron Lett.*, 347 (1961).

(70) St. Kaufmann and G. Rosenkranz, *J. Amer. Chem. Soc.*, **70**, 3502 (1948); F. C. Uhle, *ibid.*, **83**, 1460 (1961).

change at positions 15, 17, 20, and 23 of kryptogenin (**67**) followed by cyclization, as above, with undeuterated reagents and subsequent acid back-exchange of deuterium at C-20 and C-23 should yield a 15,15,17-trideuterio analog. In point of fact, that was the accomplished scheme except that the initial exchange could be done only with acetic acid-OD[71] on the diacetate of **67**. Exchange of kryptogenin with strong base (*e.g.*, NaOH) gives fesogenin (**73**)[4] and with hydrochloric acid in methanol, bethogenin (**74**).[4]

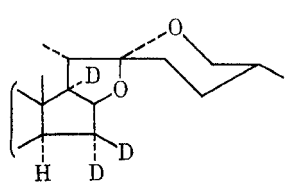


73



74

Thus, exchange of kryptogenin diacetate in refluxing acetic acid-OD followed by selective reduction[11] of the C-16 ketone to the corresponding alcohol, basic hydrolysis of the acetates, and acid cyclization gave 15,15,17-trideuterio-(25*R*)-spirost-5-en-3β-ol (**75**). The unsaturation and oxygenation in rings A and B was removed as described above in the preparation of the C-16 label to give 15,15,17-trideuterio-(25*R*)-5α-spirostan (**76**) of poor isotopic purity which, however, proved satisfactory for mass spectrometric purposes.[18a]



75, 5-en-3β-ol
76, no unsaturation or oxygenation in rings A, B

In summary, the present work describes, for the first time, procedures for extensive isotopic labeling of those positions of the steroidal sapogenin skeleton which differentiate it from the conventional steroids on which so much isotopic labeling has already been accomplished in our laboratory.[50,72] Virtually all of these procedures lend themselves to introduction not only of deuterium but also of tritium by substituting the appropriate reagents.

(71) Other reagents which we investigated were AcOD–DBr, $KHCO_3$–D_2O–EtOD, $KHCO_3$–D_2O–MeOD, D_3PO_4–DCl/D_2O, DCl–D_2O–$(CH_3)_2$CHOD.

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

Experimental Section[73]

Bromination of (25*R*)-5α-Spirostan[16a].—Bromine (324 mg) in benzene (3.5 ml) was added quickly to a solution of (25*R*)-5α-spirostan (**1**, 403 mg)[74] in benzene (50 ml) through which dry hydrogen bromide gas had been slowly bubbled for 5–10 sec both before and after addition; the reaction was kept in the dark and stirred at room temperature for 42 hr. Callow's procedure[16a] afforded 516 mg of an orange solid, separated by Rtlc twice with benzene (30)–*n*-hexane (70) to give three bands, average R_f 0.52, 0.41, 0.32 (band width *ca.* 0.08 on same scale).

The highest R_f material, recrystallized from acetone, afforded **23,23-dibromo-(25*R*)-5α-spirostan** (**5**, 99 mg): needles, mp 174–178.5° dec. A second Rtlc [three times, benzene (15%)–*n*-hexane (85%)] and two recrystallizations from acetone gave the analytical sample: needles; mp 185.5–186° (vac); ir_{max}^{KBr} practically identical with that of 23,23-dibromosapogenins in the literature;[75] $[\alpha]D$ −100° (*c* 0.755). (See Tables I and III for nmr data.)

Anal. Calcd for $C_{27}H_{42}Br_2O_2$: C, 58.07; H, 7.58; Br, 28.62; mol wt, 558.44. Found: C, 57.86; H, 7.57; Br, 28.39; mol wt, 556, 558, 560 ("triplet" from a Br_2, mass spec.).

The middle band (R_f 0.41) was identified as **23*R*-bromo-(25*R*)-5α-spirostan**[16f,76] (**6**, 69 mg): needles; mp 224–226° dec (acetone, change of form from 215–217°) (lit.[16f] mp 215–217° and mp 225–226°); ν_{max}^{KBr} 1168, 1090, 1071, 1040, 1018, 985, 971, 962, 910, 884, 854, 710, 691, 665 cm^{-1}; $[\alpha]^{26.8}D$ −95° (*c* 0.970) (lit.[16f] −87.4°).

Anal. Calcd for $C_{27}H_{43}BrO_2$: C, 67.62; H, 9.04; Br, 16.66; mol wt, 479.53. Found: C, 67.54; H, 9.01; Br, 16.48; mol wt, 478, 480 ("doublet" from Br, mass spec.).

The lower band (R_f 0.32) yielded **23*S*-bromo-(25*R*)-5α-spirostan**[16f,76] (**7**, 132 mg): needles; mp 196.5–197° with decomposition (vac) (lit.[16f] 193–194°); ν_{max}^{KBr} 1175, 1054, 1010, 1002, 944, 914, 893, 862, 763, 730, 659 cm^{-1}; $[\alpha]^{26.3}D$ −52.4° (*c* 0.667) (lit.[16f] −64.3°).

Anal. Calcd for $C_{27}H_{43}BrO_2$: C, 67.62; H, 9.04; Br, 16.66; mol wt, 479.53. Found: C, 67.61; H, 8.89; Br, 16.68; mol wt, 478, 480 ("doublet" from Br, mass spec.).

The original procedure,[23] modified to include fast addition of the bromine and ice-water quench after five min, affords a quantitative yield of a 1:1 mixture of **6** and **7**.

(25*R*)-5α-Spirost-23-ene (**8**).—To a stirred solution in a N_2 atmosphere of 23*R*-bromo-(25*R*)-5α-spirostan (**6**, 262 mg, 0.546 mmol) in dry benzene (25 ml) and DMSO (18.7 ml) was added

(73) Melting points were uncorrected and were determined on the Kofler block except for those labeled "vac" which were run in sealed, evacuated tubes. The infrared spectra were determined in chloroform, unless otherwise noted, on a Perkin-Elmer 421 grating spectrophotometer using sodium chloride cavity cells. Optical rotations, optical rotatory dispersion measurements, (Durrum-JASCO Model ORD-5 spectropolarimeter) and circular dichroism measurements (Durrum spectropolarimeter with CD attachment) were determined, unless othersise noted, in chloroform by or under direction of Mrs. R. Records. Nmr spectra (see Table I) were determined by Dr. Lois Durham and associates, notably Drs. M. Bramwell and T. Nishida. The mass spectra[18a] were measured by Mr. R. G. Ross and Dr. A. M. Duffield employing an A.E.I. MS-9 spectrometer (see ref 18a for details). Analytical thin layer chromatography [Atlc] was performed using microslides coated by dipping in chloroform/silica gel slurry; these were developed by spraying with 2% ceric sulfate in 2 *N* sulfuric acid followed by heat charring. Preparative thin layer [Ptlc] used a 1-mm-thick silica gel layer, the sample application method of Monteiro [H. J. Monteiro, *J. Chromatogr.*, **18**, 594 (1965)] and detection by "hot wire" [a simplified version of that of J. L. Bloomer and W. R. Eder, *ibid.*, **34**, 548 (1968)] and ultraviolet light. Solvent mixtures are given in parts per hundred. When repetition thin layer (Rtlc) was used, the number (N) of times the plate was run is denoted by NX. Silver nitrate plates[61] (10% of weight of silica gel) were detected by a thin strip of ceric sulfate solution which was heated by proximity to the hot wire. In all cases, silica HF-254 (E. Merck AG, Darmstadt) was used. All microanalyses were by Messrs. E. Meier and J. Consul.

(74) J. Romo, M. Romero, C. Djerassi, and G. Rosenkranz, *J. Amer. Chem. Soc.*, **73**, 1528 (1951).

(75) Compare with M. E. Wall and H. W. Jones, *ibid.*, **79**, 3222 (1957). No acetate band at 1243 cm^{-1}, of course.

(76) Because of Atlc, we feel that our two 23-monobromospirostans are the most pure yet described. A private communication from Dr. J. C. Knight (see reference 16f), now at Arizona State University, Tempe, as well as samples kindly sent by him, indicate (Atlc) that their sample of the 23*S*-bromide is contaminated with the 23*R*; the latter is less soluble and, thus, would be precipitated first in a fractional crystallization. Our experience, in opposition to literature reports,[75] has been that the 23*R* isomer will isomerize in solution, with a trace of acid, to the 23*S* isomer but that the reverse does not occur. Melting points are *not* a good criterion of purity.[16f]

potassium *t*-butoxide (245 mg, 2.18 mmol, *ca.* 0.05 *M* solution in base). After 90 min, the reaction was quenched by adding water, 1 ml 10% (aqueous) HCl, and ether.

The usual procedure gave a quantitative yield of **(25*R*)-5α-spirost-23-ene (8)**: flakes; mp 176.5–177.5° [chloroform (or ether)–methanol]; ν_{max} 1650 (C=C), 1174, 1076, 980, 921, 878, 859 cm^{-1}; $[\alpha]^{25.2°}D$ −65.9° (*c* 1.001).

Anal. Calcd for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62; mol wt, 398.61. Found: C, 81.03; H, 10.52; mol wt, 398 (mass spec.).

5α-Spirost-24-ene (9).—A solution of equal amounts of the 23-monobromides, **6** and **7**, (2.07 gm, 4.32 mmol), plus potassium *t*-butoxide (1.93 gm, 17.28 mmol, solution 0.35 *M* in base) in dry benzene (30 ml) and DMSO (20 ml) was initially stirred under nitrogen at 54° for 90 min after which the temperature was slowly raised to 90°. After 13 hr, the dark red solution was extracted with ether–10% HCl–water giving 1.5 g of a yellow oil. Column chromatography on Florisil (1:100) using increasing concentrations of benzene in *n*-hexane afforded (10–20% benzene) **5α-spirost-24-ene (9**, 762 mg, 44%): needles, spars, or fine clusters; mp 191.5–192° (vac, chloroform–methanol); ν_{max} (no C=C shows), 1175, 1159, 1068, 1009, 999, 977, 896, 887, 870 cm^{-1}; $[\alpha]D$ −88.8° (*c* 1.002).

Anal. Calcd for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62; mol wt, 398.61. Found: C, 81.14; H, 10.47; mol wt, 398 (mass spec.).

5α-Spirost-24-ene (9) from (25*R*)-5α-Spirost-23-ene (8).—(25*R*)-5α-Spirost-23-ene (**8**, 7.6 mg, 0.019 mmol) and potassium *t*-butoxide (9.2 mg, 4.3 molar excess) were slurried in dry DMSO (3 ml) and stirred at 107° in a nitrogen atmosphere. After 17 min, Atlc [benzene (70%) *n*-hexane (30%)] showed complete conversion to **5α-spirost-24-ene (9)**. Extraction with ether–very dilute HCl–water gave a quantitative yield of the trisubstituted olefin **9** whose structure was confirmed by mass spectrometry.[18a]

Epoxidation of (25*R*)-5α-Spirost-23-ene (8).—An approximately equimolar mixture of 23*S*-bromo(25*R*)-5α-spirostan (**7**) and (25*R*)-5α-spirost-23-ene (**8**, 500 mg, 1.26 mmol) was dissolved in chloroform (16.8 ml) to which was added 522 mg of *m*-chloroperbenzoic acid (min: 85% pure, 2.58 mmol; solution *ca.* 0.15 *M* in peracid). After stirring in the dark at room temperature for 79 hr, extraction with ether–carbonate afforded a semicrystalline solid (1.11 gm) separated by Rtlc [five times, benzene (70%)–*n*-hexane (30%)] to give (before crystallization) the bromide **7** (567 mg), a trace of the starting olefin **8** and the two epoxides: **23*R*,24*R*-epoxy-(25*S*)-5α-spirostan (10**, higher R_f isomer, 215 mg, 41%): needles and clusters: mp 203–206° (chloroform–methanol); ν_{max} 1157, 1068, 1040, 999, 971, 956, 880, 866, 820 cm^{-1}.

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 78.29; H, 10.04; mol wt, 414 (mass spec.).

23*S*,24*S*-Epoxy-(25*S*)-5α-spirostan (11, lower R_f isomer, 136 mg): spars; mp 187–191° (ether–methanol); ν_{max} 1161, 1124, 1065, 1031, 994, 977, 958, 933, 880, 886, 840 cm^{-1}.

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 78.22; H, 10.22; mol wt, 414 (mass spec.).

Epoxidation of 5α-Spirost-24-ene (9).—A solution of the olefin **9** (1.146 g, 2.88 mmol) and *m*-chloroperbenzoic acid (1.17 g, 2 molar excess) in chloroform (20 ml, 0.30 *M* in peracid, diluted to 40 ml, 0.15 *M*, after 1 hr) was stirred at room temperature in the dark for 24 hr and then treated as above. Careful column chromatography (activity III alumina, *n*-hexane through 50% benzene) gave the two epoxides. **24*S*,25*R*-epoxy-(25*R*)-5α-spirostan 12**, 494 mg, 41.5%, from *n*-hexane fractions only): needles; mp 242–244° (vac, chloroform–methanol); ν_{max} 1240, 1170, 1110, 1071, 1040, 1010, 970, 898, 873, 839 cm^{-1}; $[\alpha]^{25.2°}D$ −78.6° (*c* 1.005).

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 77.92; H, 10.11; mol wt, 414 (mass spec.).

24*R*,25*S*-Epoxy-(25*S*)-5α-spirostan (13, 353 mg, 29.6%, fractions 10% through 50% benzene in *n*-hexane): flakes; mp 242–245° (vac, methylene chloride–ethanol or neat methanol); ν_{max} 1240, 1170, 1125, 1058, 1044, 1003, 970, 909, 877, 860, 813 cm^{-1}; $[\alpha]^{26.7°}D$ −69.6° (*c* 1.004).

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 78.18; H, 10.12; mol wt, 414 (mass spec.).

TABLE III

EFFECTS OF SUBSTITUENTS IN RINGS D, E, AND F UPON THE POSITIONS OF THE METHYL SIGNALS IN (25*R*)-5α-SPIROSTANS

Substituent	Shift value, ppm[a] 19 H	18 H	21 H	27 H	Compd
Δ^{14} [b]	0.04	0.28	0.06	0.01	62
14βH	−0.04	0.21	0.02	0.01	64
15β-OH[b]	0.03[c]	0.25[c]	0.00	0.02	50
15 keto[b]	0.00[d]	0.00[d]	0.06	0.00	52
Δ$^{20(21)}$	−0.01	−0.09		0.02	42
20-d_1	0.00	0.02	−0.01[e]	0.02	24
20*R*-CH_3	−0.01	0.17	0.18	0.00	39
20*R*-acetyl	−0.02	0.23	0.40[e]	0.00	40
20*S*-OH	−0.02	0.16	0.40	0.02	41
Δ^{23}	0.01	0.04	−0.03	0.10	8
23*R*-Br[b]	0.00	0.04	0.22	0.03	6
23*S*-Br[b]	0.01	0.12	0.08	0.04	7
23,23-Br_2[f]	0.00	0.23	0.27	0.06	5
23*R*,24*R*-epoxy	0.01	0.07	−0.01	0.27	10
23*S*,24*S*-epoxy	0.01	0.09	0.04	0.22	11
23*R*-OH[b]	0.00	0.02	0.15	0.02	14
23*S*-OH[b]	0.01	0.04	−0.01	0.04	15
23 keto	0.00	0.01	−0.03	0.15	20
Δ^{24} [b]	0.00	0.02	0.05	0.82[e]	9
24*S*,25*R*-epoxy	0.00	0.00	0.00	0.50[e]	12
24*R*,25*S*-epoxy	−0.01	−0.01	−0.01	0.59[e]	13
24*S*-OH	0.01	0.01	0.02	0.15	16
24*R*-OH	0.00	0.00	0.00	0.09	17
24-keto	0.00	0.00	0.00	0.30	21
25*S*-OH	0.00	0.01	0.06	0.33[e]	18
25*R*-OH	0.00	0.00	0.01	0.51[e]	19
25-d_1	0.00	0.00	0.00	−0.01[e]	26

[a] Positive number indicates a downfield shift. Values are rounded to nearest hundredth ppm. Base values are the resonances of (25*R*)-5α-spirostan (**1**) (δ): 0.799 (19 H); 0.765 (18 H); 0.956 (21 H); ~0.776 (27 H). [b] Compare with the values given in reference 16e. [c] After subtracting the value due to the 3β-hydroxy group (see reference 16e). [d] After subtracting the value due to the 3β-acetoxy group (see reference 16e). [e] Singlet. [f] By direct measurement, not from addition of values from **6** and **7**.

Lithium Aluminum Hydride Reduction of the Epoxide 10.—23*R*,24*R*-Epoxy-(25*S*)-5α-spirostan (**10**, 62 mg) and lithium aluminum hydride (134 mg) were stirred for 24 hr in refluxing THF and the excess reagent decomposed with 1% (aqueous) HCl, followed by isolation of the product with ether affording 66 mg homogeneous material. Recrystallization from ether–methanol gave (25*R*)-5α-spirostan-23*R*-ol (**14**): mp 191–196°; ν_{max} 3584 (-OH), 1172, 1095, 1043, 1002, 975, 962, 939, 904, 861 cm^{-1}.

Anal. Calcd for $C_{27}H_{44}O_3$: mol wt, 416.62. Found: mol wt, 416 (mass spec.).

Lithium Aluminum Hydride Reduction of Epoxides 11, 12, and 13.—Similar reduction of epoxide **11** (26 mg) gave, after Rtlc [four times, EtOAc (10%)–*n*-hexane (90%), trace of pyridine], the following alcohols. **(25*S*)-5α-spirostan-24*R*-ol (17**, 13 mg): spars; mp 208.5–209.5° (vac, methylene chloride–methanol); ν_{max} 3490 (-OH, internal H bonding with ring-E oxygen), 1170, 1128, 1072, 1052, 1031, 1014, 990, 962, 918, 876, 850 cm^{-1}; $[\alpha]^{25.4°}D$ −79.6° (*c* 1.002).

Anal. Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65; mol wt, 416.62. Found: C, 78.12; H, 10.56; mol wt, 416 (mass spec.).

(25*R*)-5α-Spirostan-23*S*-ol (15, 4 mg before recrystallization): very fine needles; mp 163–168 (ether–methanol); ν_{max} 3560 (-OH), 1087, 1056, 1017, 996, 986, 956, 937, 909, 890, 855 cm^{-1}.

Anal. Calcd for $C_{27}H_{44}O_3$: mol wt, 416.62. Found: mol wt, 416 (mass spec.).

24*S*,25*R*-Epoxy-(25*R*)-5α-spirostan (**12**, 303 mg) gave only **(25*S*)-5α-spirostan-25-ol (18**, 264 mg crude yield, 86.8%): needles or plates; mp 172–4 (vac, methanol); ν_{max} 3575 (-OH), 1170, 1102, 1075, 1050, 1037, 973, 964, 933, 886, 844 cm^{-1}; $[\alpha]^{26.2°}D$ −73.7° (*c* 1.002).

Anal. Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65; mol wt, 416.62. Found: C, 77.69; H, 10.57; mol wt, 416 (mass spec.).

Reduction of 24*R*,25*S*-epoxy-(25*S*)-5α-spirostan (**13**, 244 mg), afforded, after column chromatography (activity II alumina 1:125, 50–50 benzene-*n*-hexane to neat benzene), **(25*S*)-5α-spirostan-24*R*-ol** (**17**, 112 mg before crystallization, 45.7%, see physical constants above) and **(25*R*)-5α-spirostan-25-ol** (**19**, 91 mg before crystallization, 38.0%): flakes; mp 174–177° (methanol); ν_{max} 3592 (–OH), 1171, 1133, 1058, 1028, 1017, 959, 917, 868, 843 cm^{-1}; $[\alpha]^{25.4}$D −74.0° (*c* 0.986).

Anal. Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65; mol wt, 416.62. Found: C, 77.42; H, 10.50; mol wt, 416 (mass spec.).

(25*R*)-5α-Spirostan-23-one (**20**).—Jones reagent[77] (0.1 ml, degassed with nitrogen) was added, in a nitrogen atmosphere, to a stirred ice-cold solution of the 23*R*-alcohol **14** (55 mg) in acetone (50 ml, $KMnO_4$ distilled). After 20 min, the reaction was quenched with 2-propanol and the product isolated with ether. Ptlc purification [benzene, trace pyridine] and two crystallizations from methylene chloride–methanol yielded (25*R*)-5α-spirostan-23-one (**20**): flakes; mp 196–198.5; ν_{max} 1730 (C=O), 1117, 1046, 1021 1001, 957, 912, 859, 850 cm^{-1}; ORD (*c* 0.070, dioxane), $[\alpha]_{589}$ −43°; $[\alpha]_{330}$ +472°; $[\alpha]_{280-282}$ −1127°; $[\alpha]_{255}$ −1042°; $[\alpha]_{240}$ −1212°; $[\alpha]^{27.3}$D −43° (*c* 0.070, dioxane, different determination from ORD).

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 78.04; H, 10.12; mol wt, 414 (mass spec.).

In the same manner, the 23*S*-alcohol **15** (4 mg) was oxidized to the ketone **20** which was identified as the material described above by an identical infrared spectrum.

(25*S*)-5α-Spirostan-24-one (**21**).—A similar Jones oxidation[77] of (25*S*)-5α-spirostan-24*R*-ol (**17**, 13 mg) gave **(25*S*)-5α-spirostan-24-one** (**21**): needles and spars; mp 209–212° (methylene chloride–methanol); ν_{max} 1716 (C=O), 1155, 1054, 990, 954, 876 cm^{-1}; ORD (*c* 0.104, dioxane), $[\alpha]_{589}$ −76.8°; $[\alpha]_{312}$ −797°, trough; $[\alpha]_{305-303}$ −701°, shoulder; $[\alpha]_{274}$ +384°, peak; $[\alpha]_{240}$ +134°; $[\alpha]^{26.8}$D −59° (*c* 0.104, dioxane, different determination from ORD); $[\alpha]^{24.8}$D −73.9° (*c* 1.001, $CHCl_3$).

Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21; mol wt, 414.61. Found: C, 78.28; H, 10.20; mol wt, 414 (mass spec.).

Lithium Aluminum Hydride Reduction of 24-Ketone (**21**).—Reduction in THF (3. 66 hr) of **(25*S*)-5α-spirostan-24-one** (**21**, *ca.* 14 mg) and purification by Ptlc [EtOAc (15%)–benzene (85%)] yielded mostly alcohol **17** (infrared and nmr comparison) together with a few mg of (25*S*)-5α-spirostan-24*S*-ol (**16**): ν_{max} 3590 (–OH), 1730 (trace of carbonyl), 1150, 1101, 1021, 978, 957, 875 cm^{-1}; for nmr see Table I. Calcd for $C_{27}H_{44}O_3$: mol wt, 416.62. Found: mol wt, 416 (mass spec.).

Acid-Catalyzed Exchange of (25*R*)-5α-Spirostan (**1**). **A. Deuterium Chloride[78]–Ethanol-OD.**[79]—A solution of (25*R*)-5α-spirostan (**1**, 100 mg)[74] and 9.2 *N* DCl–D_2O (0.5 ml) in ethanol-OD (50 ml) was refluxed for 1 hr and then allowed to stand at room temperature for 4 hr.[80] D_2O quenching of one-half the solution and extraction with ether–bicarbonate gave **23,23-d_2-(25*R*)-5α-spirostan** (**22**, 37 mg, 74%, after crystallization): mp 172–174° (ether–methanol); d_1 4%, d_2 90%, d_3 6%;[81] ν_{max} 1273, 1172, 1124, 1096, 1067, 1025, 998, 969, 906, 865, 821 cm^{-1}. The rest of the solution was refluxed for an additional 13 hr. Treatment as above gave an 88% recovery of **20,23,23-d_3-(25*R*)-5α-spirostan** (**23**), d_2 51%, d_3 49%; all "d_3" at C-20.[18a]

B. Acetic Acid-OD.[82]—(25*R*)-5α-Spirostan (**1**, 51 mg)[74] was refluxed in acetic acid-OD (6 ml) for 1 hr, cooled, and half of the solution was poured into water. Treatment as in part A (above) gave the d_2 **derivative** (**22**, d_1 9%, d_2 85%, d_3 6%).[83] A solution of (25*R*)-5α-spirostan (**1**, 303 mg)[74] in AcOD (50 ml) was refluxed for 168 hr, poured into ether, neutralized with sodium hydroxide and treated as above. Recrystallization (ether–methanol), Ptlc [EtOAc (15%)–*n*-hexane (85%)], sublimation [110°, 3 × 10^{-6} mm] and another recrystallization gave the d_3 **derivative** (**23**, 80 mg, mp 173.5–175°, d_1 14%, d_2 45%, d_3 41%) which, upon reflux in acetic acid-OH for 1 hr gave **20-d_1-(25*R*)-5α-spirostan** (**24**): d_0 24%, d_1 75%, d_2 1%; mp 172–175° (ether–methanol); ν_{max} 1257, 1235, 1151, 1055, 1010, 978, 910, 890, 860 cm^{-1}.

Reduction of 23*S*-Bromo-(25*R*)-5α-spirostan (**7**) **with Zinc and Ethanol-OD.**—A vigorously stirred mixture of the equatorial bromide **7** (201 mg) and dried zinc powder (808 mg) in ethanol-OD[79] (50 ml) was refluxed under nitrogen for 4 days and allowed to cool over a 12-hr period. The usual treatment provided **23ξ-d_1-(25*R*)-5α-spirostan** (**25**): mp 172–175° (ether–methanol); d_0 7%, d_1 93%; ν_{max} 1255, 1233, 1171, 1087, 1027, 978, 955, 892, 866, 856, 818 cm^{-1}.

Reduction of 23,23-Dibromo-(25*R*)-5α-spirostan (**5**) **with Zinc and Ethanol-OD.**—A mixture of the dibromide **5** (33 mg) and zinc dust (264 mg) in ethanol-OD[79] (16.5 ml) was refluxed for 3.5 days and treated as above. Ptlc [benzene] and then Rtlc [three times, benzene (50%)–*n*-hexane (50%)] afforded **23,23-d_2-(25*R*)-5α-spirostan** (**22**, 8 mg): mp 171–174° (ether–methanol); d_0 4%, d_1 52%, d_2 44%. Side products were not investigated.

24*S*,25*R*-d_2-(25*R*)-5α-Spirostan (**26**).—5α-Spirost-24-ene (**9**, 30.5 mg), tris(triphenylphospho)rhodium chloride[42] (72.1 mg) and a magnetic stir bar were placed in an atmospheric hydrogenation flask and the system flushed well with deuterium gas. Acetone (10 ml) was introduced and the solution stirred with intermittent bubbling of deuterium through the clear, dark red liquid. After an initial few hours of stirring, a tan precipitate appeared which eventually turned black. After 4 days, Atlc [benzene (70%)–*n*-hexane (30%)] showed quantitative conversion to the saturated nucleus. The mixture was filtered, the solvent evaporated *in vacuo*, and the residue purified by Ptlc (benzene) to give, after crystallization, **24*S*,25*R*-d_2-(25*R*)-5α-spirostan** (**26**, 12.5 mg): mp 170–172.4° (ether–methanol); d_2 97%, d_3 3%.

24ξ-d_1-(25*R*)-5α-Spirostan (**28**).—Similar homogeneous reduction of (25*R*)-5α-spirost-23-ene (**8**, 20.0 mg) gave **23ξ,24ξ-d_2-(25*R*)-5α-spirostan** (**27**, 6 mg, mp 171–173.6°; d_1 2%, d_2 97%, d_3 1%) which was refluxed in acetic acid-OH (2 ml) for 1 hr giving, after two recrystallizations from ether–methanol, **24ξ-d_1-(25*R*)-5α-spirostan** (**28**): mp 170–173°; d_1 98%, d_2 2%.

(20*S*,22ξ,25*R*)-5α-Furostan-26-ol (**29**).—(25*R*)-5α-Spirostan (**1**, 203 mg)[74] was hydrogenated in a Paar shaker at 32 psi for 71 hr over platinum dioxide (106 mg) in glacial acetic acid (40 ml) activated with 60% perchloric acid (5 drops). After filtration and extraction with ether–NaOH, the residue (224 mg) was subjected to basic hydrolysis and purified by Ptlc [EtOAc (30%)–benzene (70%), trace of pyridine] to give a 95% yield of **(20*S*,22ξ,25*R*-5α-furostan-26-ol** (**29**): mp 95–96° (sublimed); ν_{max} 3620 (–OH, free), 3415 (–OH, polymeric), 1163, 1094, 1033 (broad), 962 cm^{-1}; the normal sapogenin bands[14b,c] are entirely gone; $[\alpha]^{27.2}$D +5° (*c* 0.718 dioxane).

Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52; mol wt, 402.64. Found: C, 80.21; H, 11.27; mol wt, 402 (mass spec.).

26-d_1-(20*S*,22ξ,25*R*)-5α-Furostan-26-al (**32**).—Jones[77] oxidation of 145 mg of the alcohol **29** afforded **(20*S*,22ξ,25*R*)-5α-furostan-26-oic acid** (**30**) which was not further purified but reduced directly with lithium aluminum deuteride in THF. The resulting **26,26-d_2-(20*S*,22ξ,25*R*)-5α-furostan-26-ol** (**31**, 126 mg) was also used directly. Collins reagent (252 mg, 0.98 mmol)[47] in dry methylene chloride (10 ml) was added to a solution of the deuterated alcohol **31** (56 mg, 0.14 mmol) in the same solvent (6 ml) and stirred under nitrogen for 35 min. After filtration through silica (5% H_2O) to remove the reagent, the oxidation was repeated, as above, with 505 mg reagent giving a clear oil shown by Atlc [EtOAc (10%)–benzene (90%)] to be a two component mixture,[48] the main species of which was **26-d_1-(20*S*,22ξ,25*R*)-5α-furostan-26-al** (**32**, 33 mg), ν_{max} (mixture) 2058 (strong CD) and 1710 cm^{-1} (C=O).

(77) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(78) Deuterium chloride, 9.2 *N* in D_2O, was prepared by slow addition of D_2O to freshly distilled phosphorous oxychloride ($POCl_3$) with collection of evolved DCl in D_2O.

(79) A. Streitwieser, Jr., L. Verbit, and P. Stang, *J. Org. Chem.*, **29**, 3706 (1964).

(80) Experiments have shown that exchange at room temperature is very slow compared with that at reflux.

(81) All values are calculated from data taken directly from the mass spectrum and are corrected for natural abundance of ^{13}C, ^{2}H, and ^{18}O.

(82) Prepared in 4-mol quantity according to G. Binsch and J. D. Roberts, *J. Amer. Chem. Soc.*, **87**, 5157 (1965), by adding acetic anhydride (distilled from sodium) *to* an equimolar amount of D_2O: 99.1% AcOD; rest is unreacted anhydride with a trace of D_2O; –OD 99.9% (nmr).

(83) The other half of the solution was evaporated directly. The product showed the same isotopic composition, confirming that water does not adversely effect the label.

26ξ-d_1-(25*R*)-5α-Spirostan (33).—The deuterated aldehyde **32** (30 mg) was refluxed for 3 hr in absolute ethanol (20 ml) containing concentrated HCl (2 ml). After standing for 16 hr at room temperature, ether–carbonate extraction gave a quantitative yield of the cyclized sapogenin **33**. Further purification by Ptlc [benzene] and two crystallizations [ether–methanol] gave **26ξ-deuterio-(25*R*)-5α-spirostan (33)**: mp 160–162.5°; d_0 2%, d_1 97%, d_2 1%; ν_{max} 1200, 1152, 1063, 1044, 1021, 974, 961, 912, 879 cm^{-1}; nmr integral CH-16 *vs.* CHD-26 = 1.0/0.9.

27-d_1-(25*R*)-Spirost-5-en-3β-ol (36, 27-d_1-diosgenin).—Isonarthogenin-27-monotosylate[52] (**35**, 21 mg, prepared from 12.5 mg of the diol **34**[52]) was reduced with lithium aluminum deuteride in THF. The usual treatment and Rtlc [twice, EtOAc (20%)–benzene (80%)] to remove some diol, **34**, gave **27-d_1-(25*R*)-spirost-5-en-3β-ol (36)**: d_0 1%, d_1 90%, d_2 8%; d_3 1%.

Isomerization at C-20.[53] **(20*R*,25*R*)-5α-Spirostan (39).**—Using Wall's procedure,[53] (25*R*)-5α-spirostan (**1**, 2.3 gm)[74] was converted to the following mixture (crystallized yields), separated by Rtlc [twice, EtOAc (7%)–*n*-hexane (93%), trace of pyridine]; Starting material, **1** (15 mg, R_f 0.75). **(20*R*,25*R*)-5α-spirostan (39**, 674 mg, R_f 0.63): needles; mp 158–164°, 160–165° (ether–methanol), [lit.[62] mp 155–160°]; ν_{max} 1150, 1060, 1032, 999, 951, 914, 886, 849 cm^{-1}; $[\alpha]^{26}$D −57° (*c* 1.057) [lit.[62] −54° (dioxane, 25°)].

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07; mol wt, 400.62. Found: C, 80.90; H, 11.27; mol wt, 400 (mass spec.).

20-Acetyl-(20*R*,25*R*)-5α-spirostan (40, 307 mg, R_f 0.50): clusters; mp 220–223.5°; ν_{max} 1700 (C=O), 1154, 1140, 1059 1038, 1005, 981, 960, 920, 896, 874, 860 cm^{-1}; CD (*c* 2.78, dioxane), $[\theta]_{320}$ = 0°; $[\theta]_{280}$ = −830°; $[\theta]_{240}$ = 0°; $[\alpha]^{27.9}$D −77° (*c* 1.128).

Anal. Calcd for $C_{29}H_{46}O_3$: mol wt, 442.66. Found: mol wt, 442 (mass spec.).

(20*S*,25*R*)-5α-Spirostan-20-ol (41, 24 mg, R_f 0.35) was identified by infrared and mass spectral comparison with the same compound produced in an established way[53,56] (see below).

(25*R*)-5α-Furost-20(22)-en-26-yl acetate (37).[54]—To a refluxing solution of (25*R*)-5α-spirostan (**1**, 202 mg)[74] dissolved in a 1:1 mixture (10 ml) of pyridine–acetic anhydride was added, in a nitrogen atmosphere, methylamine hydrochloride (340 mg, recrystallized from absolute ethanol and dried) and reflux continued for 4.5 hr. The solvents were evaporated *in vacuo* and the remaining dark red sludge taken up in methylene chloride which was washed with brine and water, dried ($MgSO_4$), and evaporated *in vacuo*. Ptlc [EtOAc (5%)–benzene (95%) plus 2 drops of pyridine–100 ml solution] afforded a trace of the starting material **1** together with **(25*R*-5α-furost-20(22)-en-26-yl acetate (37)** which could not be crystallized but which was sublimed (117°, 1 × 10^{-5} mm): mp 69–71° (change of form from powder to microcrystals, mp 79°); ν_{max} 1727 (C=O), 1693 (C=C),[14d] 1243, 1032, cm^{-1}; $[\alpha]^{26.2}$D +20 (*c* 0.175, dioxane).[91]

Anal. Calcd for $C_{29}H_{46}O_3$: mol wt, 442.66. Found: mol wt, 442 (mass spec.).

Saponification of **37** gave **(25*R*)-5α-furost-20(22)-en-26-ol (38)**, ν_{max} 1685 cm^{-1} (C=C),[14d] no spiroketal peaks (**38** is very acid sensitive.)

Anal. Calcd for $C_{27}H_{44}O_2$: mol wt, 400.62. Found: mol wt, 400 [mass spec., which also shows a trace of impurity at M$^+$ − 14 (386)].

(20*S*,25*R*)-5α-Spirostan-20-ol (41).—Using Wall's procedure,[56a] (20*R*,25*R*)-5α-spirostan (**39**, 562 mg) was converted to (20*S*, 25*R*)-5α-spirostan-20-ol (**41**, 185 mg): flakes; mp 186–188° (ether–methanol); ν_{max} 3480 (–OH), 1151, 1064, 1015, 999, 988, 916, 870 cm^{-1}; $[\alpha]^{25.4}$D −80° (*c* 0.946).

Anal. Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65; mol wt, 416.62. Found: C, 77.90; H, 10.63; mol wt, 416 (mass spec.).

(25*R*)-5α-Spirost-20-ene (42).—Following known procedure,[56] the alcohol **41** (250 mg) was dehydrated to **(25*R*-5α-spirost-20-ene (42**, 107 mg, crystals): fine needles, mp 166–168° (ether–methanol); ν_{max} 3084, 1818, 1667 (C=C); 1159, 1063, 1039, 1018, 986, 966, 922, 902, 864 cm^{-1}; $[\alpha]^{24.8}$D −90° (*c* 0.992).

Anal. Calcd for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62; mol wt, 398.61. Found: C, 81.37; H, 10.60; mol wt, 398 (mass spec.).

21-d_1-(25*R*)-5α-Spirostan (44).—As in the preparation of compound **26**, the olefin **42** (20 mg) was homogeneously reduced[42] to **20,21-d_2-(20*R*,25*R*)-5α-spirostan (43**, d_1 26%, d_2 68%, d_3 6% (mp 158–162° from ether–methanol) which was isomerized[58] in refluxing 1% HCl–ethanol (3 ml) to **21-d_1-(25*R*)-5α-spirostan (44)**, 98% d_1.

(25*R*)-5α-Spirostan 12-Ethylene Thioketal (46).—The 12-ketone **45** (71 mg) was dissolved in ethanedithiol, to which was added one drop of 60% perchloric acid,[59] and stirred for 4.5 hr after which a little methanol was added. Extraction with ether–5% KOH gave, after crystallization, **(25*R*)-5α-spirostan 12-ethylene thioketal (46**, 41 mg, 49%): very fine spars; double reproducible mp 197–201.5 and 203–204 (ether–methanol); ν_{max} (no carbonyl) 1157, 1094, 1067, 1048, 1005, 979, 961, 918, 894, 857 cm^{-1}; $[\alpha]^{27.3}$D −34.3° (*c* 0.992).

Anal. Calcd for $C_{29}H_{46}O_2S_2$: mol wt, 490.79. Found: mol wt, 490 (mass spec.).

12,12-d_2-(25*R*)-5α-Spirostan (47).—Deuterio Raney nickel (prepared from 1.5 g of Raney nickel catalyst powder)[60,84] in ethanol-OD[79] (40 ml) was added to the ethylene thioketal **46** (10 mg) and the mixture was refluxed under nitrogen for 17 hr after which it was filtered and the solvent evaporated *in vacuo*. The residue, a white solid, was crystallized from ether–methanol giving crystalline material (6 mg, mp 170–172°) which was further purified on silver nitrate Ptlc[61] [benzene (50%)–*n*-hexane (50%)] giving **12,12-d_2-(25*R*)-5α-spirostan (47**, 2 mg, analytical sample); mp 171–173.5° (ether–methanol); d_0 4%, d_1 44%, d_2 49%, d_3 3%. (Infrared essentially the same as that of **1**.)

11,11-d_2-(25*R*)5α-Spirostan (49).—(25*R*)-5α-Spirostan-12-one (**45**, 10 mg),[62] dissolved in a nitrogen atmosphere in a solution composed of methanol-OD[79] (10 ml), D_2O (5 ml), and sodium (*ca.* 200 mg) was refluxed for 27 hr and extracted with ether–water. The residue, after Ptlc [EtOAc (15%)–benzene (85%)], was crystallized from acetone to give an analytical sample of **11,11-d_2-(25*R*)-5α-spirostan-12-one (48)**: mp 196–198.5° (lit.[62] 198–199°); d_1 3%, d_2 95%, d_3 2%. As in the preparation of **46**, the filtrate from this material was converted, in quantitative yield, to **11,11-d_2-(25*R*)-5α-spirostan-12-one 12-ethylene thioketal.** This residue was dissolved in ethanol (15 ml) to which was added W-2 Raney nickel (*ca.* 200 mg). After refluxing for 10.5 hr and standing overnight, the reaction was filtered, the solvent evaporated *in vacuo*, and the residue purified on silver nitrate Ptlc[60,84] [benzene], then Rtlc [twice, benzene (50%)–*n*-hexane (50%)] to give **11,11-d_2-(25*R*)-5α-spirostan (49**, ether–methanol): Atlc [benzene (70%)]–*n*-hexane (30%)], d_0 1%, d_1 8%, d_2 91%.

3β-Acetoxy-(25*R*)-5α-spirostan-15-one 15-Ethylene Thioketal (53).—The 15-oxo-3β-acetate **52** (118 mg)[63] was converted, as described for **46**, to **3β-acetoxy-(25*R*)-5α-spirostan-15-one 15-ethylene thioketal (53**, 118 mg, 86%): needles; mp 254–256° (ether–methanol); ν_{max}^{KBr} 1728 (C=O, acetate), 1178, 1145, 1129, 1108, 1054, 1024, 977, 952, 907, 893, 862; 790, 737, 721, 687, 659, 604 (C-S); $[\alpha]^{26.6}$D −92.8° (*c* 0.973).

Anal. Calcd for $C_{31}H_{48}S_2O_4$: C, 67.84; H, 8.82; S, 11.68; mol wt, 548.82. Found: C, 67.79; H, 8.79; S, 11.74; mol wt, 548 (mass spec.).

15,15-d_2-(25*R*)-5α-Spirostan (57).—The thioketal **53** (79 mg) was converted to **3β-acetoxy-15,15-d_2-(25*R*)-5α-spirostan (54**, structure confirmed by spectral comparisons[14c]) using the same procedure as for the preparation of **47**. Lithium aluminum hydride reduction of **54** (58 mg) in ether to the alcohol **55** and Jones oxidation[77] of 33 mg of this material gave the corresponding ketone **56** which showed a large amount of olefinic impurity. Wolff-Kishner reduction,[50] silver nitrate Ptlc[61,73] (benzene), and crystallization from ether–methanol gave **15,15-d_2-(25*R*)-5α-spirostan (57)**: mp 168–172.5°; d_1 34%, d_2 59%, d_3 7%.

Base-Catalyzed Exchange of 3β-Acetoxy-(25*R*)-5α-spirostan-15-one (52).—In a typical exchange, 179 mg **52** in 50 ml of methanol-OD[79] with 1.2 gm of sodium was refluxed for 39 hr and then extracted with ether–brine–water. The residue was purified by Rtlc [four times, EtOAc (20%)–benzene (80%) with 2 drops of pyridine–100 ml solvent] and sublimed 135–140° (10^{-6} mm), to give **3β-hydroxy-14,16-d_2-(25*R*)-5α,14β-spirostan-15-one** (134 mg): mp 186–189.5° (lit.[63] 181–183°). The position and amount of exchange can be obtained from consideration of several peaks in the mass spectra[18a] of the exchange products (Table IV).

(25*R*)-5α-Spirost-14-ene-3β,12α-diol (60).—The acetate **59**[20c] (1.18 gm) was reduced by refluxing 1 hr in dry ether (250 ml) with lithium aluminum hydride (1 g). The usual procedure gave **(25*R*)-5α-spirost-14-ene-3β,12α-diol (60)**: large flat prisms; no distinct melting point (two separate preparations), softens and goes clear over range 100–125°; ν_{max} 3600 (–OH), 3050 and 1640 (C=C), 1155, 1122, 1098, 1058, 1032, 1008, 975, 956, 915, 893, 858 cm^{-1}; $[\alpha]^{25.7}$D +59.2° (*c* 1.004).

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

TABLE IV

Sample	Exchange time, hr	Molecular ion[a] d_0	d_1	d_2	d_3	C-16 d_0	d_1	d_2
1	0.5 (EtOD)	18[b]	57	26		71	29	
2	12.5 (EtOD)	8	56	34	2	63	37	
3	39 (MeOD)		4	95	1	1	98	1

[a] Includes, of course, both C-14 and C-16 labels. The incorporation may actually be better than calculated. See E. Lund, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 1528 (1963). [b] 4% of "d_0-1" species.

Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83; mol wt, 430.61. Found: C, 75.00; H, 10.07; mol wt, 430 (mass spec.).

(25*R*)-5α-Spirost-14-ene-3,12-dione (61).—Jones oxidation[77] of **60** gave a quantitative yield of **(25*R*)-5α-spirost-14-ene-3,12-dione (61)**, the analytical sample from ether–*n*-hexane: very fine spars: mp 209.5–213 or 212–214.5 (both with decomposition, depends on heating rate); $\nu_{max}^{CS_2}$ 3058 and 1642 (C=C), 1710 (C=O), 1178, 1117, 1061, 1042, 1010, 978, 958, 948, 919, 898, 860, 851 cm^{-1}; ORD (*c* 0.125, dioxane), $[\alpha]_{600}$ +64°; $[\alpha]_{589}$ +64°; $[\alpha]_{316}$ +1890° (peak); $[\alpha]_{310}$ +1695°; $[\alpha]_{306}$ +1780° (peak); $[\alpha]_{300}$ +1056° (shoulder); $[\alpha]_{296}$ +992° (shoulder); $[\alpha]_{275}$ +40°; $[\alpha]_{250}$ +1728°; $[\alpha]^{27}$°D +100° (*c* 1.040).

Anal. Calcd for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98; mol wt, 426.57. Found: C, 76.11; H, 9.27; mol wt, 426 (mass spec).

Huang-Minlon Reduction of (25*R*)-5α-Spirost-14-ene-3,12-dione (61).—Initially,[68] the dione **61** (467 mg) was dissolved in ethylene glycol (20 ml), anhydrous hydrazine (2 ml), and *n*-butyl alcohol (10 ml) and distilled for 1 hr, removing material up to a boiling point of 120°. The mixture was cooled and potassium hydroxide (2 g) added. Distillation was again started until reaching 130° with subsequent reflux at that temperature for 4 hr. Acid treatment gave a yellow oil (500 mg) containing three major components, none of whose R_f matched the starting material. The residue was dissolved in absolute ethanol (20 ml), diethylene glycol (50 ml), and anhydrous hydrazine (5 ml) and refluxed (103°) for 3 hr in an argon atmosphere. Upon cooling, potassium hydroxide (5.55 g) was added and the solution distilled until the temperature reached 210°. Reflux (4 hr), acid work-up (pH 1), and Ptlc (EtOAc (5%)-benzene (95%) trace pyridine] gave two 2-component mixtures. The one of greater R_f, after Rtlc [seven times, benzene (30%)–*n*-hexane (70%)] and silver nitrate Ptlc[61,73] [benzene (50%)–*n*-hexane (50%)], gave the following analytical samples.

(25*R*)-5α-Spirost-14-ene (62, R_f 0.40): plates; mp 116–117.5° (ether–methanol); ν_{max} 3055 and 1645 (C=C), 1174, 1156, 1133, 1056, 1004, 975, 957, 918, 894, 861 cm^{-1}; $[\alpha]^{26.4}$°D *ca.* −30° (*c* 0.069).

Anal. Calcd for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62; mol wt, 398.61. Found: C, 81.31; H, 10.57; mol wt, 398 (mass spec.).

(25*R*)-5α,14β-Spirostan (64, R_f 0.54): needles; mp 131–132° (ether–methanol); ν_{max} 1170, 1076, 1054, 1013, 980, 958, 942, 919, 902, 862 cm^{-1} (different from the 14α analog, **1**, by direct comparison); $[\alpha]^{26.4}$°D −41° (*c* 0.201).

Anal. Calcd for $C_{27}H_{44}O_2$: mol wt, 400.62. Found: mol wt, 400 (mass spec.).

The second pair, after silver nitrate Ptlc[61,73] [EtOAc (10%)–benzene (90%)] gave **(25*R*)-5α-spirost-14-ene 3-ethylene ketal (63)**: rectangular plates; mp 150.5–153.5 (ether–methanol); ν_{max} 3050 and 1640 (C=C), 1170, 1151, 1128, 1089, 1056, 1001, 970, 940, 914, 906, 887, 857 cm^{-1}; $[\alpha]^{26.9}$°D +32° (*c* 0.497).

Anal. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71; mol wt, 456.64. Found: C, 76.12; H, 9.63; mol wt, 456 (mass spec.).

(25*R*)-5α,14β-Spirostan 3-ethylene ketal (65): needles; mp 173–176.5° (ether–methanol); ν_{max} 1168, 1149, 1089, 1070, 1051, 1010, 979, 942, 916, 897, 882, 863 cm^{-1}; $[\alpha]^{26}$°D −36° (*c* 0.235).

Anal. Calcd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11; mol wt, 458.66. Found: C, 76.15; H, 10.11; mol wt, 458 (mass spec.).

14-d_1-(25*R*)-5α,14β-Spirostan (66).—Deuterioborane,[50] generated[27] by the addition of sodium borodeuteride (161 mg) in dry diglyme (10 ml) to a stirred solution of boron trifluoride etherate (1.43 gm, freshly distilled) in dry diglyme (10 ml), was bubbled, in an argon atmosphere over a period of 17 min, into a stirred and cooled solution of (25*R*)-5α-spirost-14-ene (**62**, *ca.* 24 mg) in dry THF (30 ml). After an additional 13 min, the generation was repeated. The ice bath was removed after 1 hr total time and the solution stirred at room temperature for 11 hr. The reaction was then cooled to −81° (Dry Ice–acetone), propionic acid (1.5 ml, freshly distilled) added carefully, and the solution stirred for 1 hr. Upon return to room temperature, the THF was evaporated *in vacuo*, more propionic acid (10 ml) added, and the solution heated at 108–114° for 10 hr at which time it was extracted with ether–5% NaOH to give a mixture, a small part of which, by Atlc [20% silver nitrate, benzene (50%)–*n*-hexane (50%)], appeared to be the desired 14-deuterated analog of **1**. Purification by Rtlc [twice, benzene (50%)]-*n*-hexane (50%)] and again on a plate made by addition of 0.3% (w/w) rhodamine 6-G to the silica gel HF [four times, benzene (50%)–*n*-hexane (50%), uv detection] gave **14-d_1-(25*R*)-5α,14β-spirostan (66)**, identified as the 14β isomer by comparison of the nmr and of the quantitative aspects of the mass spectrum:[18a] ν_{max} 1138, 1074, 1050, 1012, 979, 964, 918, 897, 861 cm^{-1}; d_0 14%, d_1 79%, d_2 7%.

16-d_1-(25*R*)-5α-Spirostan (71).—Under an atmosphere of argon, sodium borodeuteride (50 mg) was added to kryptogenin (**67**, 131 mg) dissolved in 2-propanol-OD (30 ml). The flask was stoppered and the solution stirred for 19 hr giving, in part, the deuterated triol **69** which was not isolated. [That the cyclized product **70** was isolated after acid treatment is evidence for the existence of **69**.] After addition of a saturated solution of HCl gas in deuterium oxide[85] (2 ml) and 20 min stirring, the product was isolated with ether[11] and purified by Rtlc [twice EtOAc (30%)–benzene (70%)] giving 74 mg (ether–methanol) of **16-d_1-(25*R*)-spirost-5-en-3β-ol (74)** d_1 71%, d_2 28%, d_3 1%. This material (73 mg) was hydrogenated (Paar) at 49 psi for 4.5 hr in absolute ethanol (100 ml) over platinum dioxide (69 mg) and the resulting 3β-ol deoxygenated in the sequence Jones oxidation,[77] Wolff–Kishner reduction (see preparation of **62**, second run) to give **16-d_1-(25*R*)-5α-spirostan (71**, 10 mg): mp 171–173.5° (ether–methanol), d_1 73%, d_2 26%, d_3 1%; ν_{max} 1237, 1154, 1068, 1044, 1000, 972, 912, 885, 857 cm^{-1}.

16-d_1-(20*R*,25*R*)-5α-Spirostan (72).—16-d_1-(25*R*)-5α-Spirostan (**71**, 15 mg) was isomerized, as in the preparation[53] of **39**, to give a small amount of starting material together with **16-d_1-(20*R*,25*R*)-5α-spirostan (72)**: mp 156–163°[62]; d_0 21%, d_1 71%, d_2 8%.

15,15,17-d_3-(25*R*)-5α-Spirostan (90).—Kryptogenin diacetate[4] (**67**, as the diacetate, 1.00 gm) was refluxed for two consecutive 113-hr periods in acetic acid-OD to give an incorporation of d_2 6%, d_3 21%, d_4 33%, d_5 25%, d_6 10%, d_7 (?) 4%, d_8 (?) 1%, mp 148–149.5° (ether) (lit.[4] 152–153). This material (475 mg) was reduced in a manner analogous to the preparation of **71**, but using sodium borohydride and 2-propanol–OH. Saponification to the 3β,16β,27-triol with 10% sodium hydroxide, cyclization[11] with 6 *N* HCl (20 ml), ether[11] isolation and Rtlc [twice, EtOAc (30%)–benzene (70%)] gave the **15,15,17-d_3-(25*R*)-5α-spirost-5-en-3β-ol (75)**: mp 192–198° (lit.[86] 204–207°); $d_0$5%, d_1 26%, d_2 29%, d_3 22%, d_4 11%, d_5, 6%, d_6 1%.

The deuterated 5-en-3β-ol **75** was converted to the saturated, deoxygenated species (82 mg) by the series 69 hr hydrogenation (Paar, 46 psi, ethanol, equal weight PtO_2), Jones oxidation,[77] Wolff-Kishner reduction (see preparation of **62**, second part). Finally, 48 mg of the resulting material were refluxed in 1% ethanolic HCl for 70 hr and isolated with ether–carbonate affording, **15,15,17-d_3-(25*R*)-5α-spirostan (76)**: d_0 10%, d_1 30%, d_2 31%, d_3 21%, d_4 6%, d_5 2%.

Registry No.—**1**, 5012-14-6; **5**, 24744-26-1; **6**, 4988-84-5; **7**, 4947-69-7; **8**, 24744-29-4; **9**, 24744-30-7; **10**, 24744-31-8; **11**, 24744-32-9; **12**, 24744-33-0; **13**, 24744-34-1; **14**, 24744-35-2; **15**, 24744-36-3; **16**, 24744-37-4; **17**, 24744-38-5; **18**, 24744-39-6; **19**, 24744-40-9; **20**, 24744-41-0; **21**, 24744-42-1; **22**, 5380-66-5; **23**, 24744-44-3; **24**, 24744-45-4; **25**, 24744-46-5; **26**, 24744-47-6; **27**, 24744-48-7; **28**, 24744-49-8; **29**, 24744-50-1; **32**, 24744-51-2; **33**, 24744-52-3; **37**, 24744-53-4; **38**, 24744-54-5; **39**, 24799-49-3; **40**, 24799-50-6; **41**,

(85) H_2O should have been used here since the acidic solution produced an incorporation of 28% deuterium at C-23. (Position determined by mass spec. fragmentation[18a].)

(86) Merck Index, 7th ed, p 378.

24742-73-2; **42**, 24742-74-3; **43**, 24742-75-4; **46**, 24742-76-5; **47**, 24742-77-6; **50**, 6877-35-6; **52**, 24742-79-8; **53**, 24742-80-1; **57**, 24742-81-2; **60**, 24742-82-3; **61**, 24742-83-4; **62**, 24742-84-5; **63**, 24799-51-7; **64**, 24742-85-6; **65**, 24742-86-7; **66**, 24742-87-8; **67**, 468-99-5; **71**, 24742-89-0.

Terpenoids. LXVII.[1] Chemical Studies of Marine Invertebrates. VII.[2] Interrelation of Seychellogenin and Lanosterol through Lanostane-3β,11β,18-triol[3]

P. ROLLER,[4a] B. TURSCH,[4b] AND CARL DJERASSI

Department of Chemistry, Stanford University, Stanford, California 94305

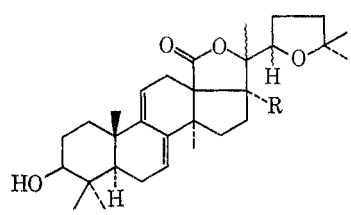
Received December 18, 1969

Seychellogenin (**9**) and lanosterol (**10**) were chemically correlated through a common intermediate, lanostane-3β,11β,18-triol (**21**). Seychellogenin was reduced to the triol **11**, whose 3,18-diacetate (**12**) was dehydrated and then hydrogenated to give a mixture of C-20 epimers of **15**. Subsequent chromium trioxide oxidation to the ene-dione **16**, followed by zinc reduction to **17** and removal of the C-7 functionality, gave 11-oxolanostane-3β,18-diol diacetate (**20**) and its C-20 epimer **19**. Reduction of **20** provided the desired triol **21**. Lead tetraacetate–iodine oxidation of 11β-hydroxylanostan-3β-yl acetate (**22**) and immediate reduction with lithium aluminum hydride yielded the 11β,18 ether **24** and the 11β,19 ether **25**. The former was oxidized to the lactone **27** and then reduced to the triol **21**, which was identical with the product of natural origin. The 11β,19 ether (**25**) was converted to lanostane-3β,11β,19-triol (**37**) which could be correlated with the known 11β,19-cyclolanostane-3β,11α-diol 3-acetate (**23**).

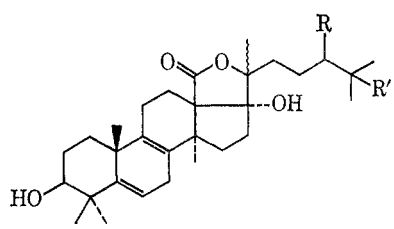
In recent years a number of triterpenoid saponins of toxic nature have been isolated from many species of sea cucumbers in the family *Holothuroidea* of the phylum *Echinodermata*. The first successful structural work was accomplished on the saponin mixture from the Cuvier glands of the Caribbean species *Actinopyga agassizi*.[5] Acid hydrolysis of the mixture yielded monosaccharides, sulfuric acid, and a mixture of triterpenoid aglycones, among them 22,25-oxidoholothurinogenin (**1**) and its deoxy analog **2**. In the saponin, an aglycone was found to be bound directly to a chain of four monosaccharides and to a sulfate ester. Enzymatic hydrolysis studies[6] have also led to some interesting speculations about the true nature of the triterpenoid portion when attached to the monosaccharide chain and the sulfate ester residue.

Chemical studies by our group established the structure of yet another aglycone, griseogenin (**3**), as an acid hydrolysis product from the body walls of the Brazilian sea cucumber *Halodeima grisea L.*[7] Structures **4** and **5** for the two sapogenins stichopogenin A_2 and stichopogenin A_4 from the Far Eastern sea cucumber *Stichopus japonicus* were assigned mainly on the basis of spectral evidence.[8]

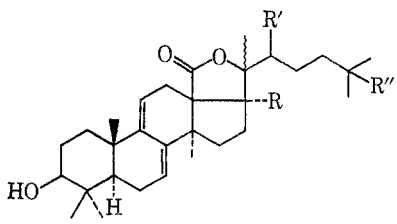
Thus all sea cucumber aglycones appear to possess a similar lanostane skeleton with structural variations in the side chain. Chemical and spectroscopic evidence all pointed to the correctness of the postulated struc-



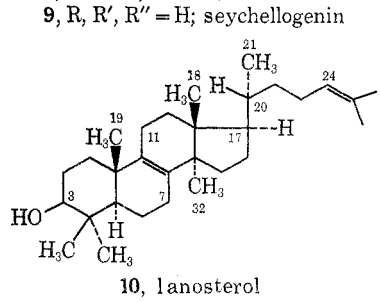
1, R = OH
2, R = H



4, R, R′ = double bond
5, R = H; R′ = OH



3, R, R′ = OH; R″ = H
6, R, R′ = H; R″ = OH
7, R, R′ = H; R″ = OMe
8, R = OH; R′ = H; R″ = OMe
9, R, R′, R″ = H; seychellogenin



10, lanosterol

(1) For part LXVI, see P. Roller and C. Djerassi, *J. Chem. Soc. C*, 1089 (1970).

(2) For part VI, see B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Lett.*, 467 (1970).

(3) Financial assistance from the National Institutes of Health Grant No. GM-06840 is gratefully acknowledged.

(4) (a) Taken in part from the Ph.D. Thesis of P. R., Stanford University, 1969; (b) Postdoctoral research associate, on leave from the Free University of Brussels, Brussels, Belgium.

(5) J. D. Chanley, T. Mezzetti, and H. Sobotka, *Tetrahedron*, **22**, 1857 (1966).

(6) J. D. Chanley and C. Rossi, *ibid.*, **25**, 1897, 1911 (1969).

(7) B. Tursch, I. S. deSouza Guimaraes, B. Gilbert, R. T. Aplin, A. M. Duffield, and C. Djerassi, *ibid.*, **23**, 761 (1967).

(8) G. B. Elyakov, T. A. Kuznetsova, A. K. Dzizenko, and Yu. N. Elkin, *Tetrahedron Lett.*, 1151 (1969).