6,10-dimethyldeca-5,9-dien-2-one. This ketone was converted to the labeled methyl farnesoate XIIIa as described above for XIIIb and then to the terminal epoxy farnesoate VI also as described above for V. VI had nmr δ 1.18 (s, 3 H, epoxy CH_3), 1.21 (s, 3 H, epoxy CH_3), 1.51 (m, 2 H, epoxy CH_2), 1.61 (d, 3 H, $CH_3C{=}C$), 2.14 [m, 7 H, $CH_2C{=}CHCD_2CH_2(CH_3)C{=}C$], 2.47 (t, 1 H, epoxy H), 3.59 (s, 3 H, $COOCH_3$), 5.12 (broad s, 1 H, C=CH), 5.58 (m, 1 H, $CHCOOCH_3$); M^+ 268 (>98% d_2).

Methyl *12,12,12,12',12',12'*-d_6-10,11-Epoxy-*trans,trans*-farnesoate (IV).—d_6-Acetone was reduced with lithium aluminum hydride and the resulting alcohol was converted to *1,1,1,3,3,3*-d_6-2-bromopropane according to the conditions of Wiley.[33] The bromide was mixed with an equimolar amount of triphenylphosphine and heated at 130° for 2 days to give (*1,1,1,3,3,3*-d_6-isopropropyl)triphenylphosphonium bromide (XXVI) in 30% yield. Methyl 10,11-epoxy-*trans,trans*-farnesoate[34] was converted to methyl *trans,trans*-3,7-dimethyl-9-oxonona-3,6-dienoate (XX) as described above for XVI. The aldehydo ester XX (0.5 g) was added to the dark red ylide (1 equiv), derived from the phosphonium salt XXVI in ether, and the mixture was stirred at reflux overnight. Work-up in the usual manner and bulb-to-bulb distillation (0.5 Torr) gave the d_6-labeled methyl farnesoate. After purification by preparative vpc, this *trans,trans*-farnesoate was converted to the 10,11-epoxy compound IV (as described above for V): nmr same as for VI except for absence of the methyl singlets at δ 1.18 and 1.21, and the presence of nine allylic protons at δ 2.14; M^+ 272 (91% d_6), 271 (9% d_5).

(33) G. A. Wiley, R. L. Hershkowitz, B. M. Rein, and B. C. Chang, *J. Amer. Chem. Soc.*, **86**, 964 (1964).

(34) A generous sample was provided by Dr. Clive Henrick, Zoecon Corp.

Registry No.—III, 5299-11-6; IV, 34603-22-0; V, 34635-39-7; VI, 34603-23-1; IXa, 34603-24-2; IXb, 34603-32-2; Xa, 34603-25-3; Xb, 34603-24-2; XI, 34603-26-4; XIb, 34603-27-5; XII, 34603-28-6; XIIb, 34635-40-0; XIII, 3675-00-1; XIIIb, 34603-30-0; XVI, 24603-31-1.

Acknowledgment.—We wish to thank Drs. John Siddall, John Diekman, Clive Henrick, and Loren Dunham of the Zoecon Corporation, not only for helpful discussion but for providing details of several synthetic procedures as well as several very valuable synthetic intermediates.

Stereospecific Synthesis of (20*S*,22*R*)-17α,20,22-Trihydroxycholesterol and (20*S*,22*S*)-17α,20,22-Trihydroxycholesterol[1]

R. C. NICKOLSON[2] AND MARCEL GUT*

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Received December 22, 1971

Addition of vinyl Grignard to the known 16α,17α-oxidopregnenolone acetate followed by reduction of the epoxide, conversion of the product to a 3,5-cyclo steroid, and epoxidation of the remaining double bond yields a C-22 epimeric mixture of epoxides which, when condensed with *sec*-butyllithium and reconverted to the 3β-hydroxy-Δ^5-sterols, yield the title compounds.

Interest in the preparation of compounds which are postulated intermediates in the catabolism of cholesterol to C_{21} and C_{19} hormones has led numerous investigators to synthesize cholesterol derivatives possessing hydroxyl groups at C-17, C-20, and C-22. Specifically, the syntheses of (22*R*)-22-hydroxycholesterol and its C-22 epimer,[3-5] 20α-hydroxycholesterol,[6] 20β-hydroxycholesterol,[7] and (20*R*,22*R*)- and (20*R*,22*S*)-20,22-dihydroxycholesterol[8,9] have been described previously.

In recent years, the suggestion that cholesterol can be enzymatically cleaved between C-17 and C-20 to yield dehydroepiandrosterone[10-12] has prompted the synthesis of side-chain hydroxylated cholesterols which could serve as substrates for this transformation. Compounds of importance in this series include 17α,-20α-dihydroxycholesterol, its C-20 epimer,[13] and 17α-hydroxycholesterol.[14] We now describe the synthesis of (20*S*,22*R*)-17α,20,22-trihydroxycholesterol (**21**) and (20*S*,22*S*)-17α,20,22-trihydroxycholesterol (**23**), sterols which could conceivably undergo desmolytic cleavage between C-20 and C-22 to yield 17α-hydroxypregnenolone. Alternatively, oxidative cleavage between C-17 and C-20 could occur to yield dehydroepiandrosterone, as suggested for a direct biosynthetic pathway from cholesterol to the C_{19} hormones.[10]

The stereospecific introduction of hydroxyl groups at C-17, C-20, and C-22 of the cholesterol side chain presents a problem of some complexity. Of immediate interest was the preparation of a 17,20-glycol possessing a two-carbon, unsaturated side chain which, after epoxidation, can be treated with a suitable alkyllithium to produce the desired 17,20,22-hydroxylation pattern (see Scheme I). The preparation of 17α,20-dihydroxysterols can be easily accomplished by the addition of Grignard reagents to 17α-hydroxypregnenolone acetate **29**. However, this method of preparation is unsuitable for our purposes, as the only alcohol obtained has been

(1) This work was supported by the U. S. Public Health Service Grant AM-03419 from the Institute of Arthritis and Metabolic Diseases and a contract from the Atomic Energy Commission AT(30-1)918.

(2) Taken in part from a dissertation by R. C. Nickolson in partial fulfillment of the requirements for the Ph.D. degree in organic chemistry, Clark University, Worcester, Mass. 01610.

(3) K. Tsuda and R. Hayatsu, *Chem. Pharm. Bull.*, **6**, 680 (1958).

(4) K. Tsuda and R. Hayatsu, *J. Amer. Chem. Soc.*, **81**, 5987 (1959).

(5) H. Mori, K. Shibata, K. Tsuneda, M. Sawai, and K. Tsuda, *Chem. Pharm. Bull.*, **16**, 1407 (1968); E. P. Burrows, G. M. Hornby, and E. Caspi, *J. Org. Chem.*, **34**, 103 (1969).

(6) V. Petrow and I. A. Stewart-Webb, *J. Chem. Soc.*, **46**, 75 (1956).

(7) A. Mijares, P. I. Cargill, J. A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).

(8) K. Shimizu, M. Gut, and R. I. Dorfman, *J. Biol. Chem.*, **237**, 699 (1962).

(9) N. K. Chaudhuri, R. Nickolson, H. Kimball, and M. Gut, *Steroids*, **15**, 525 (1970).

(10) R. A. Jungmann, *Biochim. Biophys. Acta*, **164**, 110 (1968).

(11) S. Burstein, H. Zamoscianyk, N. Co, M. Adelson, D. S. M. Prasad, A. Greenberg, and M. Gut, *ibid.*, **231**, 223 (1971).

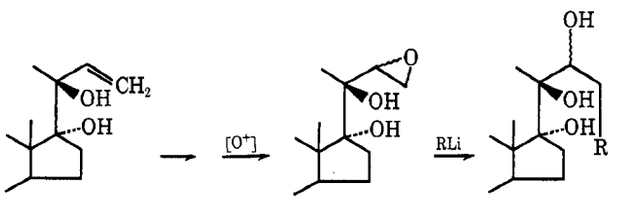
(12) R. B. Hochberg, H. Mickan, and S. Lieberman, *ibid.*, **231**, 208 (1971).

(13) N. K. Chaudhuri, J. G. Williams, R. C. Nickolson, and M. Gut, *J. Org. Chem.*, **34**, 3759 (1969); for information concerning the metabolism of these 17,20-dihydroxycholesterols see S. Burstein, H. L. Kimball, N. K. Chaudhuri, and M. Gut, *J. Biol. Chem.*, **243**, 4417 (1968).

(14) N. K. Chaudhuri, R. C. Nickolson, and M. Gut, *Steroids*, **16**, 495 (1970).

SCHEME I

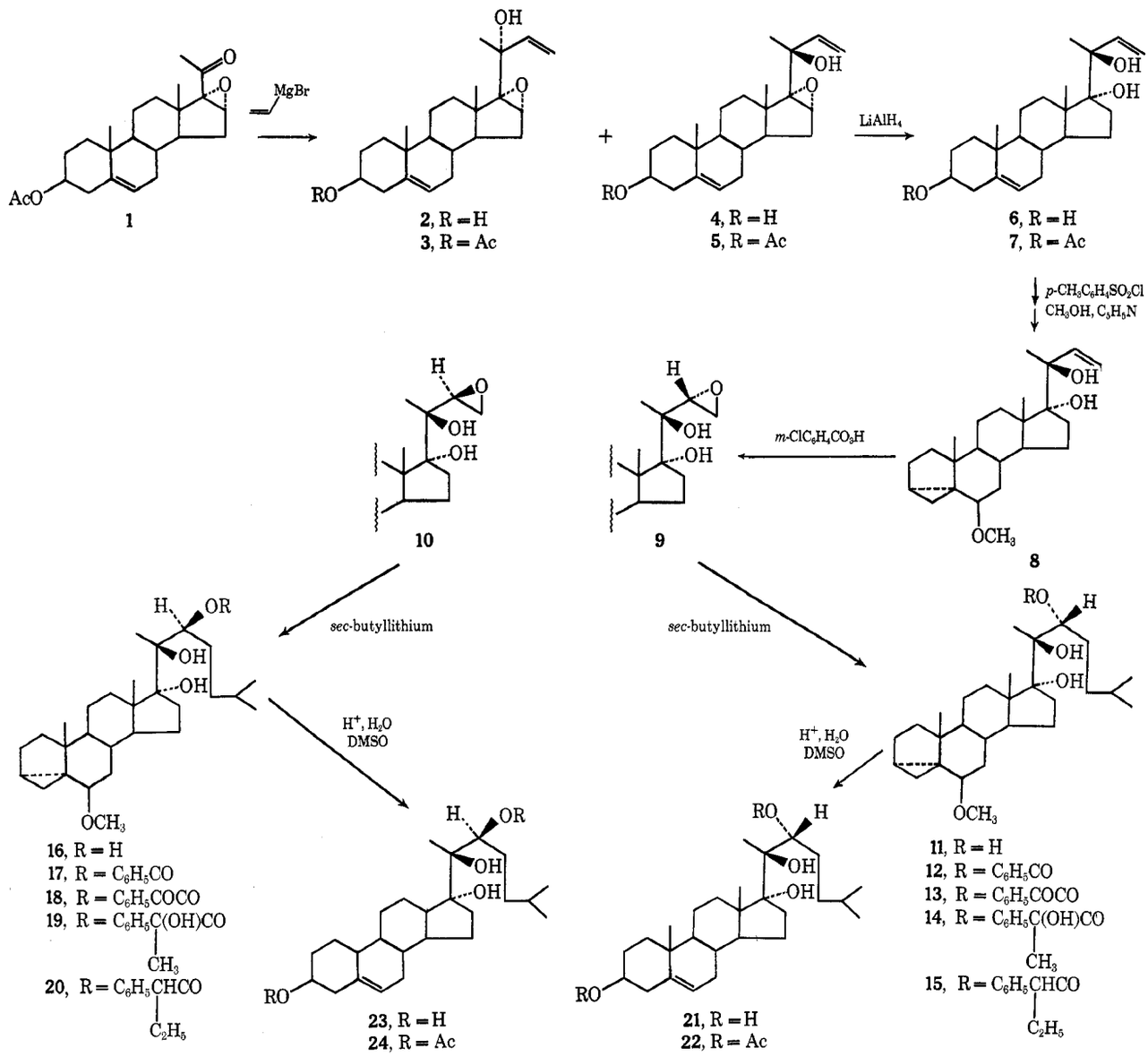
PLAN FOR THE CONSTRUCTION OF THE 17,20,22-GLYCEROL SIDE CHAIN



20α-triol **6**, involving initially the condensation of vinylmagnesium bromide with 16α,17α-oxidopregnenolone acetate (**1**), was attempted (Scheme II). Previous work has shown[13] that a mixture of C-20 epimeric alcohols is formed when the epoxy ketone is reacted with Grignard reagents. The ratio of 20α- to 20β-alcohol which results depends markedly on the size and reactivity of the particular alkylmagnesium halide used. For instance, addition of isohexyl Grignard results in predominant formation of the 20β-alcohol

SCHEME II

SYNTHESIS OF C-22 EPIMERIC 17α,20α,22-TRIHYDROXYCHOLESTEROLS

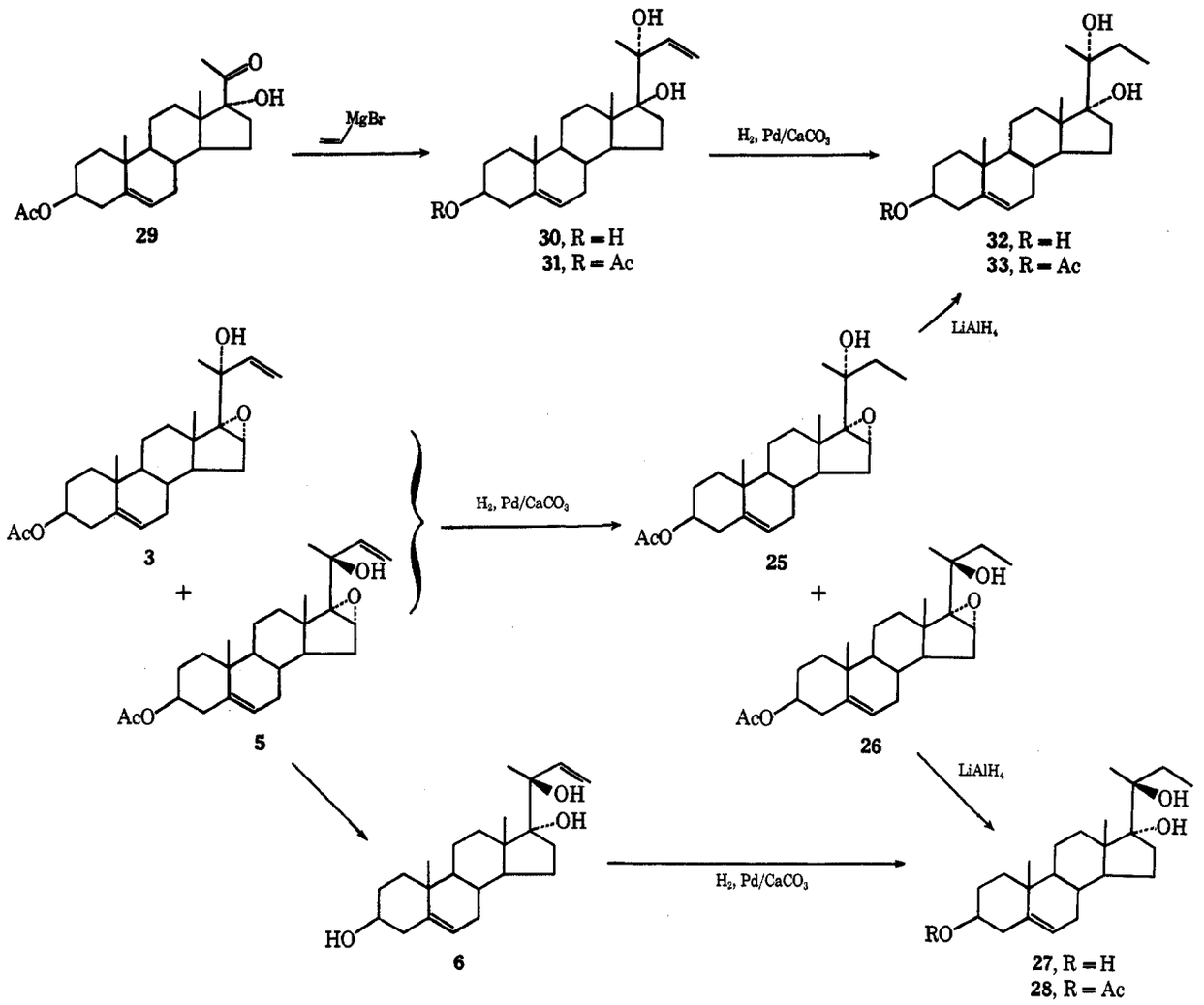


previously shown[13] to possess the unnatural 20β stereochemistry.[15]

In view of this unfortunate stereochemical consequence, an alternate synthesis of the "natural" 3β,17α,- (20α/β = 1/3), whereas use of ethyl Grignard produces a preponderance of the 20α-alcohol (20α/β = 3/2). It was expected that addition of vinyl Grignard would produce a substantial yield of the 20α-alcohol **4** which, after separation from its 20β epimer **2**, could be treated with lithium aluminum hydride to produce the desired 17α,20α-glycol **6** (see Scheme III). Fortunately, the addition of vinylmagnesium bromide to the epoxy ketone **1** was found to proceed with greater than 90% stereoselectivity to give the epoxy diol **4**, possessing the

(15) Sterols possessing a 20α-hydroxy group belong to the natural series and correspond in configuration to that of cholesterol in which the 20 H is α oriented. Both the α,β nomenclature (L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 344) and the *R*,*S* sequence rule (E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 92) will be used to designate the configuration of the side chain carbons.

SCHEME III
CORRELATION OF CONFIGURATION AT C-20



20α-hydroxyl group, which could be isolated in pure form by crystallization in 70% overall yield. Reduction of the epoxy diol **4** or the epoxy diol acetate **5** with lithium aluminum hydride gave the 3β,17α,20α-triol **6**, which was compared with an authentic sample of the 3β,17α,20β-triol[16] **30**, the C-20 epimer of **6**. The two triols **6** and **30** exhibited similar nmr spectra and could not be separated on thin layer chromatography. The melting points, infrared spectra, and crystalline properties are, however, different and a clean separation of these epimeric triols could be effected by partition chromatography (see Experimental Section). The triol **6** possessing the 20α-hydroxyl group was found to be homogeneous and free from contamination with the 20β epimer.

Selective hydrogenation of the terminal olefinic bond converted the triols **6** and **30** into their respective 22,23-dihydro derivatives, **27** and **32** (see Scheme III), whose nmr spectra are readily distinguishable. In particular, there is noted a 2 and a 4 cps difference in the chemical shifts of the 18- and 21-methyl resonances, respectively. A simple diagnostic procedure based on these nmr characteristics was used to routinely monitor the approximate purity of the triol **6**.

(16) A sample of this triol was prepared by addition of vinyl Grignard to 17α-hydroxypregnenolone acetate (**29**).

The addition of vinyl Grignard to epoxypregnenolone acetate (**1**) was not totally stereospecific as there was obtained from the mother liquors some material which is an evident mixture of C-20 epimeric epoxy diols **2** and **4**. The mother liquors from the crystallization of the epoxy diol **4** were acetylated and the acetate, after chromatography, gave a solid. The nmr spectrum of this material exhibited two 18-methyl resonances appearing at 58 and 54 cps attributable to the 20α- and 20β-hydroxy epimers, respectively. The mixture of epoxy diol acetates **3** and **5** was converted to known compounds by selective hydrogenation of the 22,23 double bond to produce the dihydro derivatives **25** and **26**, followed by reduction of the epoxides with lithium aluminum hydride and reacetylation, yielding the triol acetates **33** and **28**. All nmr data were found to be consistent with that reported by Chaudhuri, *et al.*,[13] for a similar mixture of dihydroepoxy diol acetates and triol acetates prepared by a different route.

On the basis of the above evidence, there can be no question that the epoxy diol **4** isolated in predominant yield from the vinyl Grignard condensation is a single pure isomer possessing the desired stereochemistry at C-20 (20S). Reduction of this epoxy diol with lithium aluminum hydride gave, in nearly quantitative yield, the unsaturated triol **6** which is converted to the 3,5-

cyclo steroid **8**, in order to protect the 5,6 double bond from the subsequent epoxidation.

The nmr spectra of all compounds containing the 22,23 double bond exhibited a characteristic ABX pattern in the olefinic region very similar in appearance to the vinyl proton resonances of 17α-vinylestradiol.[17]

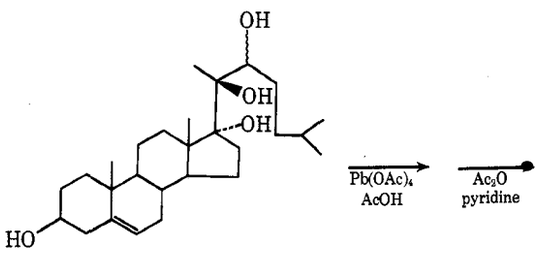
Completion of the synthesis follows the scheme outlined in Scheme II. Epoxidation of the 22,23 olefin produces a new center of asymmetry at C-22. Treatment of a methylene chloride solution of the unsaturated cyclo steroid **8** with 3 molar equiv of *m*-chloroperbenzoic acid converted the olefin quantitatively to the epoxides **9** and **10**, which were purified by chromatography over alumina. Elution with 15% ethyl acetate in benzene gave an oil which appeared as a homogeneous single spot on thin layer chromatography. The nmr spectrum, however, exhibited two widely separated 21-methyl resonances (at 79.5 and 86 cps) and a similar, but less pronounced splitting of the 18-methyl resonances (appearing at 59 and 57 cps) indicative of a C-22 epimeric mixture. The mixture was chromatographed on a Bush A partition system yielding the 22*R*- and 22*S*-epoxides **9** and **10** in a 2:3 ratio, respectively.

Condensation of the epoxides with *sec*-butyllithium gave the C-22 epimeric alcohols **11** and **16**, the configurations of which were determined by comparing the CD spectra of the respective C-22 benzoates **12** and **17** with C-22 benzoyloxy steroids of known configuration (see Discussion under "Determination of Configuration at C-22"). Each epoxide isolated in pure form by partition chromatography was condensed separately with *sec*-butyllithium. From the less abundant epoxide **9** there was isolated the 22*R*-alcohol **11** (mp 160°), whereas from the more abundant epoxide **10** there was isolated a higher melting alcohol **16** (mp 169°) possessing the 22*S* configuration.

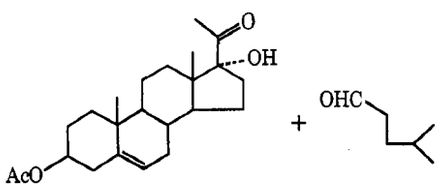
The separation of the C-22 epimers can be deferred to this stage of the synthesis. The mixture of C-22 epimeric epoxides can be treated directly with *sec*-butyllithium and the epimeric alcohols which result can be easily purified by chromatography over alumina.

Completion of the synthesis involves solvolysis of the 6-methoxy-3,5-cyclo steroid moiety to regenerate the 3β-hydroxy-Δ^5-sterols **21** and **23**. Treatment with acid[18] is known to effect this conversion; however, mild conditions had to be developed which would leave the 17,20,22-glycerol side chain intact. After some experimentation, it was found that aqueous dimethyl sulfoxide containing 0.3% perchloric acid was an ideal reagent to effect the retro cyclo steroid reaction. In separate experiments both methoxy triols **11** and **16** were converted to their corresponding cholesterol derivatives **21** and **23** possessing the indicated stereochemistry at C-22.

Support for the assigned structures was obtained from degradation experiments and spectral data (mass and nmr). Each of the tetrols, when oxidized with lead tetraacetate in acetic acid, gave a high yield of 17α-hydroxypregnenolone (isolated as the 3-acetate) and isocaproaldehyde,[19] thereby confirming the presence of a normal steroid skeleton and hydroxy groups at C-17, C-20, and C-22. No trace of dehydroepiandrosterone acetate, the product which would result if glycol cleavage had occurred between C-17 and C-20, was found.

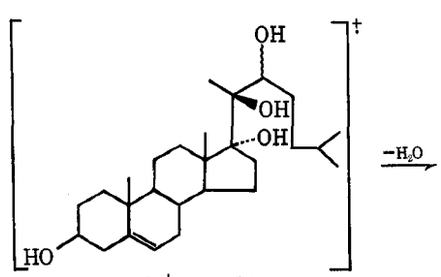


21,23

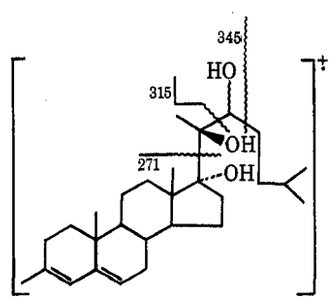


29

The mass spectra of the C-22 epimeric alcohols are qualitatively identical. Both exhibited peaks of high relative abundance resulting from cleavage of carbon–carbon bonds adjacent to hydroxyl groups. The base peak, appearing at *m/e* 271, is characteristic not only of the tetrols but of all compounds prepared in this series containing hydroxyl groups at C-17 and C-20.



M^+, *m/e* 434



m/e 416

The chemical shift data for the 18,19 and 21-methyl protons are shown below (Table I). The 18- and 21-methyl resonances are deshielded (relative to cholesterol) and their low field positions are indicative of a hydroxylated side chain.

The tetrols **21** and **23** are highly crystalline solids which give variable elemental analyses depending on the degree of solvation. The corresponding 3,22-diacetates **22** and **24** are crystallized from ether–hexane or acetone–hexane mixtures and show no tendency to cocrystallize with these solvents. Accordingly, their

(17) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, p 85.

(18) S. Winstein and R. Adams, *J. Amer. Chem. Soc.*, **70**, 838 (1948).

(19) We are indebted to Dr. Shlomo Burstein of the Institute for Muscle Disease, New York, for performing the gas chromatographic analysis of the isocaproaldehyde.

TABLE I
NMR DATA FOR QUATERNARY METHYL RESONANCES (IN CYCLES PER SECOND DOWNFIELD FROM TMS) OF THE TETROLS AND METHOXYTRIOLS

Compd	Solvent	18-CH_3	19-CH_3	21-CH_3
16	$CDCl_3$	62	62	80
	C_6D_5N	83	72	108
23	C_6D_5N	82	66	110
11	$CDCl_3$	51	62	74
	C_6D_5N	65	72	93
21	C_6D_5N	62	65	91.5

elemental analyses are in excellent agreement with the calculated composition.

The metabolism of these tetrols is now under study and will be the subject of subsequent communications.

Determination of Configuration at C-22.—Experiments to determine the configuration at C-22 were performed using the cyclo steroid alcohols **11** and **16** rather than the free tetrols **21** and **23** in order to avoid complications arising from the presence of an additional asymmetric secondary hydroxyl group at C-3. Of the methods available for the determination of configuration of secondary alcohols, two approaches were tried, namely, the Prelog atrolactate synthesis[20,21] and the Horeau α-phenylbutyric acid method[22,23] but both failed to give significant optical data which would permit an unequivocal assignment.

Application of the Prelog method to the C-22 epimeric alcohols was performed following well-standardized procedures. The benzoyl formate esters **13** and **18** were formed in quantitative yield and treated with 4.5 equiv of methylmagnesium iodide to give the atrolactates **14** and **19**. Hydrolysis of the esters resulted in recovery of 95% of the theoretical amount of atrolactic acid (based on starting alcohol). The atrolactic acid formed from the lower melting alcohol **11** was devoid of optical activity (*c* 2.2, ethanol, *l* = 2) whereas the acid isolated from the C-22 epimeric alcohol **16** exhibited a slightly positive rotation, $[\alpha]_D$ +1.4° (*c* 5.4, ethanol, *l* = 2) which corresponds to a 4% asymmetric synthesis.[24]

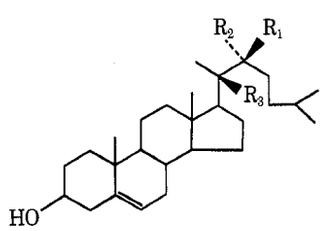
A priori, it was expected that difficulties would be encountered using the Prelog method to determine the configuration of these particular secondary alcohols. Even if the atrolactic acid isolated from these determinations had possessed significant activity, the capability of the method to yield reliable information concerning the configuration of a secondary alcohol which is in the proximity of two tertiary alcohols (at C-17 and C-20) is in serious doubt. The C-22 benzoylformyloxy group is expected to be severely constrained and the transition state leading to attack of methyl Grignard to a preferred orientation of the α-keto ester moiety is probably influenced by factors other than the relative steric arrangement of groups around the chiral carbon.

Formally, the Prelog generalization applies only to those secondary alcohols in which the chiral carbon is is attached to hydrocarbon residues. Extension of the Prelog method to more complex systems, *e.g.*, dihydrocodeine,[25] has been made with reservations.

Application of the method developed by Horeau to determine the configuration of these alcohols was also unsuccessful. Briefly, the method involves reaction of an optically active secondary alcohol with racemic α-phenylbutyric anhydride to form the α-phenyl butyrate esters **15** and **20**. Alcohols with the *R* configuration react preferentially with the *R*-(−) antipode of α-phenylbutyric acid, resulting in the accumulation of the (*S*)-(+)-α-phenylbutyric acid. If the acid isolated after esterification exhibits a positive rotation, the original alcohol possesses the *R* configuration. Conversely, if the acid isolated is laevorotatory, the original alcohol possesses the *S* configuration.

The alcohols **11** and **16** were dissolved in pyridine containing a 3-mol excess of α-phenylbutyric anhydride and allowed to stand for 10 hr. The excess anhydride is hydrolyzed and the extent of ester formation was determined by titration of the liberated acid. In neither instance did the yield of α-phenyl butyrate esters **15** and **20** exceed 10%. The recovered α-phenylbutyric acid from both determinations exhibited no significant rotation. The poor chemical yield coupled with a small optical yield was the obvious cause of failure.

Determination of the C-22 configuration was secured by examination of the ORD/CD spectra[26] of the 22-benzoate esters **12** and **17**. From previous investigations, there were available a number of C-22 epimeric alcohols of known configuration; specifically, (22*R*)- and (22*S*)-22-hydroxycholesterol and the pair of 20α,-22-dihydroxycholesterols. The 22-monobenzoate esters of each of these compounds were prepared, and the CD and ORD spectra were obtained and compared to the CD spectra of the benzoates **12** and **17** of unknown configuration.



	R_1	R_2	R_3
(22*R*)-22-Hydroxycholesterol	H	OH	H
(22*S*)-22-Hydroxycholesterol	OH	H	H
(20*R*,22*R*)-20,22-Dihydroxycholesterol	H	OH	OH
(20*R*,22*S*)-20,22-Dihydroxycholesterol	OH	H	OH

Analysis of the CD spectra obtained from the compounds of known stereochemistry showed a positive Cotton effect in the 226–233-mμ region followed by a negative Cotton effect at 218 mμ to be indicative of the 22S configuration, whereas mirror image Cotton curves were exhibited by the benzoates of opposite (22*R*) chirality.

The 22-benzoate prepared from the lower melting (mp 160°) methoxy triol **11** exhibited a trough at 238 mμ followed by a pronounced peak at 218 mμ, thus establishing its configuration as belonging to the 22*R* series, whereas the benzoate of the higher melting (169°) cyclo steroid **16** exhibited a peak at 239 mμ fol-

(20) V. Prelog, *Bull. Soc. Chim. Fr.*, 987 (1956).
(21) V. Prelog, E. Philbrin, E. Watanabe, and M. Wilhelm, *Helv. Chim. Acta*, **39**, 1086 (1956).
(22) A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).
(23) A. Horeau and J. K. Sutherland, *J. Chem. Soc. C*, 247 (1966).
(24) Based on (*R*)-(−)-atrolactic acid, $[\alpha]^{18.5}D$ −37.7° (*cf.* J. D. Morrison and H. Mosher, "Asymmetric Organic Reactions," Prentice-Hall, Englewood Cliffs, N. J., 1971, p 58).
(25) K. W. Bentley and H. M. E. Cardwell, *J. Chem. Soc.*, 3252 (1955).
(26) The full details of this method and its extension to other alcohols will be presented in a subsequent paper.

lowed by a trough at 220 mμ indicating the 22*S* configuration.

Experimental Section

Melting points are uncorrected. Nmr spectra were obtained in deuteriochloroform solution (unless otherwise stated) on a 60-Mc Varian Associates DA-60 spectrometer using tetramethylsilane as an internal reference. Mass spectra were determined on a Varian Associates M-66 mass spectrometer.

(20*S*)-16α,17α,Oxido-24-norchola-5,22-diene-3β,20-diol (4).—A solution of vinyl Grignard was prepared by the dropwise addition of a solution of 77.0 g of vinyl bromide in 500 ml of tetrahydrofuran to 17.3 g of magnesium shavings. The Grignard solution was refluxed for 0.5 hr and then cooled to −10°. A solution of 50.0 g of 16α,17α-oxidopregnenolone acetate (1) in 900 ml of tetrahydrofuran (precooled to −10°) was added to the Grignard reagent, care being taken to maintain the temperature of the solution below 5°. After addition was complete, the mixture was stirred vigorously for 10 min while cooling, after which time the Grignard complex was decomposed by the cautious addition of iced, saturated ammonium chloride solution. The contents of the reaction flask were transferred to a separatory funnel and the tetrahydrofuran layer was removed. The aqueous layer was extracted with two 500-ml portions of ethyl acetate, and the organic extracts were combined, washed with water and saturated brine, and dried over anhydrous sodium sulfate. Excess solvent was removed by vacuum distillation until the residue amounted to *ca.* 600 ml. The solid material which precipitated during evaporation of the solvent was redissolved by addition of hot benzene and the epoxy diol **4** is allowed to crystallize from this solvent mixture. Concentration of the mother liquors and subsequent crystallizations afforded two additional fractions of suitable purity for further transformation (total yield 35.2 g). An analytical sample was prepared by crystallization from benzene: s 190°, mp 199–202°; nmr 58 (18 CH_3), 61 (19 CH_3), 84 (21 CH_3), 206 (16β H), 208 (3α H), 320 (6 H), 315 (23 H) and 367 cps (22 H); mass spectrum *m/e* (rel intensity) 358 (M^+, 60), 343 (M − CH_3, 40), 340 (M − H_2O, 15), 325 (340 − CH_3, 45), 297 (325 − C_2H_4, 30), 287 (M − C_4H_7O, base peak).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.76; H, 9.41.

The 3-acetate **5** was prepared by dissolving the epoxy diol **4** in pyridine and adding an excess of acetic anhydride. The solution was allowed to stand overnight, after which time water was added and the mixture was extracted with ethyl acetate. After the pyridine was removed by extraction with iced dilute acetic acid, the ethyl acetate layer was washed two times with water, once with saturated bicarbonate solution, and once with saturated brine. The organic solution was dried over anhydrous sodium sulfate and evaporated *in vacuo*, yielding a solid residue which was dissolved in benzene and chromatographed over alumina. Elution with 10–15% ethyl acetate in benzene yielded the desired acetate **5**. An analytical sample was prepared by crystallization from methanol: mp 180–181°; nmr 58 (18 CH_3), 62 (19 CH_3), 85 (21 CH_3), 122 (acetate methyl), 207 (16β H), 276 (3α H), 314 (23 H), 324 (6 H), and 366 cps (22 H); mass spectrum *m/e* (rel intensity) 340 (M − CH_3CO_2H, base peak), 325 (340 − CH_3, 22), 307 (325 − H_2O, 11), 269 (340 − C_4H_7O, 28).

Anal. Calcd for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.85; H, 9.13.

The epoxy diol **4** obtained in the manner described above was a single C-20 epimer as shown by its nmr spectrum and thin layer and paper chromatogram (see Experimental Section under "Correlation of Configuration at C-20;" physical constants and other pertinent data are reproduced therein).

Evidence for a mixture of C-20 epimeric alcohols **2** and **4** was found by analyzing the residue remaining after isolation of the major crystalline product. Acetylation of the mother liquors followed by chromatography over alumina yielded 3.3 g of epoxy acetates **3** and **5**, the nmr of which exhibited two distinct 18-methyl resonances (58 cps, 18 CH_3 of 20*S* epimer; 54 cps, 18 CH_3 of 20*R* epimer), a broadened 21-methyl resonance, and two resonances appearing at 207 and 211 cps attributable to the 16β proton of the 20*S* and 20*R* hydroxy epimers, respectively. Selective hydrogenation of the 22,23 double bond converted the initial mixture of epoxy acetates **3** and **5** into the corresponding side chain saturated derivatives **25** and **26**, whose nmr spectrum was identical with that obtained for a similar mixture prepared previously[13] by the reaction of an ethyl Grignard with 16α,17α-oxidopregnenolone acetate (**1**) followed by acetylation: nmr 57 (18 CH_3 of 20S epimer), 58 (18 CH_3 of 20*R* epimer), 62 (19 CH_3), 78 (21 CH_3), 121 (acetate methyl), 203 (16β H of 20S epimer), 208 (16β H of 20R epimer), 275 (3α H), and 324 cps (6 H).

Further correlation of the dihydro epoxides **25** and **26** with compounds of known stereochemistry was achieved by treatment of the mixture with lithium aluminum hydride, followed by acetylation, to produce a mixture of the respective 17α,20-diol acetates **28** and **33**: nmr 52 (18 CH_3 of 20*S* epimer), 54 (18 CH_3 of 20*R* epimer), 62 (19 CH_3), 73 (21 CH_2 of 20*R* epimer), 77 (21 CH_3 of 20*S* epimer), 122 (acetate methyl), 275 (3α H), and 324 cps (6 H).

(20*S*)-24-Norchola-5,22-diene-3β,17α,20-triol (6).—To the solution of 26 g of epoxy diol **4** in 1.6 l. of tetrahydrofuran was added 7.5 g of lithium aluminum hydride and the suspension was allowed to reflux for 8 hr. Excess lithium aluminum hydride was then decomposed by the dropwise addition of 2 *N* sodium hydroxide. The white, granular lithium salts were filtered and washed with hot ethyl acetate. Evaporation of the filtrate yielded 26 g of crude triol **6**, which was recrystallized from ethyl acetate, yielding 23 g of material. An analytical sample was prepared by two more recrystallizations from the same solvents: mp 193–195°; nmr 52 (18 CH_3), 61 (19 CH_3), 83 (21 CH_3), 210 (3α H), 313 (23 H), 324 (6 H) and 371 cps (22 H); mass spectrum *m/e* (rel intensity) 342 (M − H_2O, 8), 327 (342 − CH_3, 2), 324 (342 − H_2O, 2), 309 (324 − CH_3, 4), 289 (M − C_4H_7O, 45), 271 (289 − H_2O, base peak), 253 (271 − H_2O, 70).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.83; H, 10.23.

The 3-acetate **7** was made in the usual manner. Crystallization from hexane gave an analytical sample: mp 167–168°; nmr 52.5 (18 CH_3), 62 (19 CH_3), 83.5 (21 CH_3), 270 (3α H), 313 (23 H), 324 (6 H), and 370 cps (22 H); mass spectrum *m/e* (rel intensity) 384 (M − H_2O, 1), 342 (M − CH_3CO_2H, 1), 324 (342 − H_2O, 4), 271 (342 − C_4H_7O, base peak), 253 (271 − H_2O, 75).

Correlation of Configuration of C-20. (20*S*)-24-Norchol-5-ene-3β,17α,20-triol (27) by Selective Catalytic Reduction of (20*S*)-24-Norchol-5,22-diene-3β,17α-20-triol (6).—To the solution of 361 mg of triol **6** in 20 ml of 95% ethanol was added 40 mg of 5% palladium on calcium carbonate and the suspension was stirred under an atmosphere of hydrogen. Uptake of hydrogen was rapid but ceased abruptly after 1 mol was absorbed (*ca.* 15 min). The ethanol suspension was filtered through Celite, the filtrate was evaporated, and the crude dihydrotriol **27** was crystallized from acetone: mp 229–236°; nmr 52 (18 CH_3), 61 (19 CH_3), 77 (21 CH_3), 210 (3α H), and 324 cps (6 H); mass spectrum *m/e* (rel intensity) 344 (M − H_2O, 6), 326 (M − $2H_2O$, 3), 315 (344 − C_2H_5, 5), 311 (326 − CH_3, 4), 297 (326 − C_2H_5, 4), 289 (M − C_4H_9O, 50), 271 (344 − C_4H_9O, base peak), 253 (271 − H_2O, 65%).

Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.19; H, 10.57. Found: C, 76.10; H, 10.58.

The 3-acetate **28** was prepared in the usual manner. Crystallization from acetone–hexane gave an analytical sample: mp 183–186°; nmr 52 (18 CH_3), 62 (19 CH_3), 77 (21 CH_3), 275 (3α H), and 324 cps (6 H); mass spectrum *m/e* (rel intensity) 386 (M − H_2O, 1), 344 (M − CH_3CO_2H, 1), 331 (M − C_4H_9O, 6), 326 (386 − CH_3CO_2H, 10), 297 (326 − C_2H_5, 14), 271 (344 − C_4H_9O, base peak), 253 (326 − C_4H_9O, 66).

Anal. Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.28; H, 9.97.

For comparison purposes it was desirable to synthesize the triols **30** and **32**, which are C-20 epimers of the triols **6** and **27** described above. Synthesis of the C-20 epimeric triols can be readily accomplished by reaction of 17α-hydroxypregnenolone acetate (**29**) with vinyl Grignard followed by selective reduction of the 22,23 double bond, as described below.

(20*R*)-24-Norchola-5,22-diene-3β,17α,20-triol (30) and Its Conversion to (20*R*)-24-Norchol-5-ene-3β,17α,20-triol (32).—To a solution of vinyl Grignard (prepared from 10.7 g of vinyl bromide in 60 ml of tetrahydrofuran and 2.40 g of magnesium shavings) was added 7.48 g of 17α-hydroxypregnenolone acetate in 200 ml of tetrahydrofuran over the course of 20 min. The solution was allowed to stir at ice temperature for 30 min and then brought to reflux for 4 hr. The solution was cooled and the Grignard complex was decomposed by addition of iced, saturated ammonium chloride solution. The contents of the flask were transferred to

a separatory funnel, the tetrahydrofuran layer was removed, and the aqueous solution was extracted two times with ethyl acetate. The organic extracts were combined, washed with water and saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated to yield 10 g of semisolid residue. This material was triturated with boiling hexane and the resulting suspension was filtered and washed with another portion of hexane. The hexane filtrate was discarded and the solid which remains was crystallized from aqueous methanol: mp 167–169°; nmr 51 (18 CH_3), 60 (19 CH_3), 81 (21 CH_3), 210 (3α H), 315 (23 H), 324 (6 H), and 374 cps (22 H); mass spectrum *m/e* (rel intensity) 342 (M − H_2O, 32), 324 (342 − H_2O, 1), 309 (324 − CH_3, 3), 289 (M − C_4H_7O, 25), 271 (289 − H_2O, base peak), 253 (271 − H_2O, 65).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.53; H, 10.23.

The 3-acetate **31** was prepared in the usual way. An analytical sample was prepared by crystallization from acetone–hexane: mp 199–204°; nmr 51 (18 CH_3), 62 (19 CH_3), 81 (21 CH_3), 122 (acetate methyl), 275 (3α H), 314 (23 H), 324 (6 H), and 374 cps (22 H); mass spectrum *m/e* (rel intensity) 342 (M − CH_3CO_2H, 1), 324 (342 − H_2O, 7), 314 (342 − C_2H_4, 18), 297 (324 − C_2H_3, 8), 279 (297 − H_2O, 21), 271 (342 − C_4H_7O, base peak), 253 (271 − H_2O, 66).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.67; H, 9.67.

The nmr spectra of the C-20 epimeric triols **6** and **30** were almost identical. There is noted a 3-cps difference in the chemical shift of the vinyl protons associated with the 22,23 unsaturation; however this shift is only of qualitative significance and cannot be used to estimate the relative amounts of each isomer which may be present in a mixture. These triols behave similarly on thin layer chromatography (R_f 0.32) in a benzene (65)–ethyl acetate (33)–methanol (2) solution. However, a clean separation was achieved using paper partition chromatography (20*S* epimer, R_f 0.91; 20*R* epimer, R_f 0.96) in a hexane (60)–benzene (40)–methanol (80)–water (20) system.

Conversion of the triol **30** to the 22,23-dihydro derivative **32** was accomplished by catalytic hydrogenation using the same procedure as was used to reduce its C-20 epimer **6**. An analytical sample was prepared by crystallization from acetone: mp 197–201°; nmr 54 (18 CH_3), 61 (19 CH_3), 72 (21 CH_3), 210 (3α H), and 324 cps (6 H); mass spectrum *m/e* (rel intensity) 344 (M − H_2O, 9), 326 (M − 2 H_2O, 90), 311 (326 − CH_3, base peak), 293 (311 − H_2O, 25), 271 (344 − C_4H_9O, 31), 253 (326 − C_4H_9O, 12).

Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.19; H, 10.57. Found: C, 76.40; H, 10.67.

The 3-acetate **33** was prepared in the usual manner. An analytical sample was prepared by crystallization from acetone–hexane: mp 183–186°; nmr 54 (18 CH_3), 62 (19 CH_3), 72 (21 CH_3), 122 (acetate methyl), 275 (3α H), and 324 cps (6 H); mass spectrum *m/e* (rel intensity) 386 (M − H_2O, 1), 344 (M − CH_3CO_2H, 1), 331 (M − C_4H_9O, 4), 326 (344 − H_2O, 10), 311 (326 − CH_3, 1), 308 (326 − H_2O, 2), 297 (326 − C_2H_5, 14), 271 (344 − C_4H_7O, base peak), 253 (271 − H_2O, 65).

Anal. Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.28; H, 9.97.

As stated in the text, the nmr spectra of the two C-20 isomeric dihydrotriols **27** and **32** are quite distinctive (in regard to the chemical shifts of their respective 18- and 21-methyl resonances) and can be used to routinely analyze each C-20 isomer for contamination with the other (see ref 13).

The unsaturated triol **6** possessing the natural stereochemistry at C-20 (20*S*) was subjected to partition analysis in the system described previously before being converted to the cyclo steroid **8**. Only that material which was demonstrably free of the 20*R* isomer was used in succeeding steps of the synthesis.

(20*S*)-3α,5-Cyclo-24-nor-5α-chol-22-ene-6β,17α,20-triol 6-Methyl Ether (8).—To a solution of 22.1 g of triol **6** in 400 ml of pyridine was added 20 g of *p*-toluenesulfonyl chloride. The solution was allowed to stand for 24 hr, after which time it was cooled to ice temperature and excess tosyl chloride was decomposed by slowly adding ice water. The crude tosylate crystallized from the aqueous pyridine solution and was filtered. After the residue was washed with water, the material was dried at 50° for 12 hr. The weight of crude tosylate amounted to 28 g and was dissolved in 145 ml of pyridine and 1960 ml of methanol. The solution was refluxed for 3 hr, after which time the excess methanol was removed by distillation *in vacuo*. Water was then added and the solution was extracted two times with ethyl acetate. The organic extracts were combined and washed with sufficient dilute, iced acetic acid to remove all pyridine. The ethyl acetate layer was washed two times with water and once with saturated bicarbonate and saturated brine. After drying over anhydrous sodium sulfate and evaporation of solvent, there was obtained 24 g of oil which was dissolved in benzene and chromatographed on 400 g of alumina. The desired cyclo steroid was eluted with 8–10% ethyl acetate in benzene and was crystallized from hexane, giving 17.5 g of needles: mp 90–91°; nmr 54 (18 CH_3), 61 (19 CH_3), 83 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), 167 (6α H), 200 (6β OCH_3), 312 (23 H) and 372 cps (22 H); mass spectrum *m/e* (rel intensity) 342 (M − CH_3OH, 3), 324 (342 − H_2O, 2), 309 (324 − CH_3, 6), 271 (342 − C_4H_7O, base peak), 253 (271 − H_2O, 65).

Anal. Calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.81; H, 10.08.

(20*S*,22*R*)-3α,5-Cyclo-22,23-oxido-24-nor-5α-cholane-6β,17α,-20-triol 6-Methyl Ether (9) and (20*S*,22*S*)-3α,5-Cyclo-22,23-oxido-24-nor-5α-cholane-6β,17α,20-triol 6-Methyl Ether (10).—To a solution of 9.1 g of unsaturated cyclo steroid **8** in 180 ml of methylene chloride cooled to ice temperature was added 10.0 g of *m*-chloroperbenzoic acid and the solution was allowed to stand overnight at 5°. The methylene chloride solution was extracted with 150 ml of iced 2 *N* sodium hydroxide solution. The aqueous layer was back-extracted with two 50-ml portions of methylene chloride, and the organic extracts were combined and washed with water (to neutrality) and saturated brine. After drying over anhydrous sodium sulfate and evaporation of solvent there was obtained 14 g of oily residue which was dissolved in benzene and chromatographed on alumina. The desired diol epoxides **9** and **10** are eluted with 12–15% ethyl acetate in benzene. Combination of appropriate fractions and evaporation of solvent yielded 6.7 g of oil, whose thin layer chromatogram showed only one spot (R_f 0.40, 20% ethyl acetate in benzene). The nmr spectrum, however, exhibited two 18-methyl resonances (at 57 and 59 cps) and two 21-methyl resonances (at 79.5 and 86 cps) indicative of a 3:2 mixture of C-22 epimeric epoxides. The epoxide mixture (120 mg) was partition chromatographed using a 4 × 110 cm Celite column on a Bush A (heptane–methanol–water, 10:8:2) system. Separation in this manner gave 40 mg of the (22*R*)-epoxy diol **9** (fractions 70–79) and 61 mg of the (22*S*)-epoxy diol **10** (fractions 83–94).

The oxides, either as the mixture or in pure form, are noncrystalline oils. Data for (22*R*)-epoxide **9** follow: nmr 59 (18 CH_3), 62 (19 CH_3), 80 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), 167 (6α H), 170 (23 H), 195 (22 H) and 200 cps (6β OCH_3); mass spectrum *m/e* (rel intensity) 358 (M − CH_3OH, 11), 343 (358 − CH_3, 5), 335 (M − C_4H_7, 8), 271 (358 − $C_4H_7O_2$, base peak), 253 (271 − H_2O, 66). Data for (22*S*)-epoxide **10** follow: nmr 57 (18 CH_3), 62 (19 CH_3), 86 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), 167 (6α H), 170 (23 H), 195 (22 H) and 200 cps (6β OCH_3); mass spectrum *m/e* (rel intensity) 390 (M^+, 4), 375 (M − CH_3, 9), 372 (M − H_2O, 2) 358 (M − CH_3OH, 40), 343 (358 − CH_3, 16), 335 (M − C_4H_7, 20), 297 (372 − C_2H_3O − CH_3OH, 7), 271 (358 − $C_4H_7O_2$, base peak), 253 (271 − H_2O, 60).

Anal. Calcd for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 74.02; H, 9.76.

(20*S*,22*R*)-3α,5-Cyclo-5α-cholestane-6β,17α,20,22-tetrol 6-Methyl Ether (11) and (20*S*,22*S*)-3α,5-Cyclo-5α-cholestane-6β,17α,20,22-tetrol 6-Methyl Ether (16).—*sec*-Butyllithium[27] was prepared by direct metalation of the corresponding bromide (Eastman reagent grade). The *sec*-butyl bromide was washed three times with concentrated sulfuric acid, two times with water, and once with saturated bicarbonate. The bicarbonate-washed bromide was shaken with water and saturated brine and dried for 24 hr over anhydrous calcium chloride. The bromide was decanted, distilled two times (bp 91–93°), and stored over anhydrous calcium chloride. *sec*-Butyl bromide, purified in

(27) Commercial *sec*-butyllithium purchased from two sources (Alfa Inorganics and Foote Mineral Corp.) was found to be unsatisfactory for completion of the synthesis of the cholesterol side chain. The nmr spectrum of the product resulting from condensation of the commercial lithium reagent with the epoxides did not exhibit the characteristic signals for the 26,27-methyl groups (doublet at 52 cps, J = 6 Hz). The lack of secondary methyl resonances would seem to indicate the attachment of a straight chain alkyl residue to C-23 of the epoxide. The structure of the commercial reagent was not investigated further, as it was more convenient to prepare *sec*-butyllithium in the manner described above.

this manner, can be used without any further treatment for preparation of the *sec*-butyllithium.

In a flask flushed with argon and protected from atmospheric moisture was placed 15 ml of ether and 70 mg of lithium ribbon. The ether suspension of lithium was cooled to −15° in a Dry Ice–acetone bath and 725 mg of *sec*-butyl bromide was added over 1.5 hr. After addition of all bromide, the solution was stirred at −10° for 30 min, after which the temperature of the solution was increased to 10° and the mixture was stirred for an additional 30 min. A solution of 390 mg of 22*R* epoxide **9** in 30 ml of anhydrous ether was added at once to the solution of *sec*-butyllithium (precooled to −20°). The temperature of the solution was maintained between −10 and −15° for 30 min and gradually raised to room temperature over 15 min. The lithium complex was decomposed by cautious addition of iced saturated ammonium chloride solution. The contents of the flask were transferred to a separatory funnel and the aqueous layer was extracted two times with ethyl acetate. The organic extracts were combined and washed with saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was dissolved in benzene and chromatographed over alumina and the desired methoxy triol **11** was eluted with 30–40% ethyl acetate in benzene. An analytical sample was prepared by crystallization from methylene chloride–hexane, giving 140 mg of pure compound: mp 160–162°; nmr 51 (18 CH_3), 62 (19 CH_3), 74 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), doublet centered at 54 (J = 6 Hz, 26, 27 CH_3), 167 (6α H), 200 (6β OCH_3), and 225 cps (22 H); nmr (pyridine-d_5) 65 (18 CH_3), 72 (19 CH_3), 93 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), doublet centered at 54 (J = 6 Hz, 26, 27 CH_3), 165 (6α H), 200 (6β OCH_3), and cps 242 (22 H); mass spectrum m/e 416 (M − CH_3OH, 7), 315 (416 − $C_6H_{13}O$, 39), 297 (315 − H_2O, 68), 271 (416 − $C_8H_{17}O_2$, base peak), 253 (271 − H_2O, 50).

Anal. Calcd for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78. Found: C, 75.04; H, 10.70.

The synthesis of the methoxy triol **16**, possessing the 22*S* configuration, is performed in the same manner as described above using the epimeric epoxy triol **10** as starting material. The crude product was dissolved in benzene and chromatographed on alumina. The desired methoxy triol was eluted with 12% ethyl acetate in benzene. After combination of the appropriate chromatographic fractions, the solvent was evaporated and the residue was recrystallized from methylene chloride–hexane, giving plates: mp 169–173°; nmr 62 (18 CH_3, 19 CH_3), 80 (21 CH_3), broad multiplet at 20–40 (cyclopropyl hydrogens), doublet centered at 53 (J = 6 Hz, 26, 27 CH_3), 165 (6α H), 200 (6β OCH_3), and 225 (22 H) cps; nmr (pyridine-d_5) 83 (18 CH_3), 72 (19 CH_3), 108 (21 CH_3), multiplet at 20–40 (cyclopropyl hydrogens), doublet centered at 53 (J = 6 Hz, 26, 27 CH_3), 165 (6α H), 198 (6β OCH_3), and 225 cps (22 H); mass spectrum m/e (rel intensity) 416 (M − CH_3OH, 12), 401 (416 − CH_3, 6), 330 (401 − C_5H_{11}, 20), 315 (416 − $C_6H_{13}O$, 70), 297 (315 − H_2O, 95), 271 (416 − $C_8H_{17}O_2$, base peak), 253 (271 − H_2O, 55).

Anal. Calcd for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78. Found: C, 74.80; H, 10.94.

Because of the remarkable difference in polarity between the methoxy triol **11** and **16**, the separation of the C-22 epimers does not have to be performed by partition chromatography of the epoxides **9** and **10**. Alternatively, the C-22 epoxide mixture can be condensed with *sec*-butyllithium to yield the mixture of methoxy triol which can be easily separated by chromatography over alumina.

(20*S*,22*R*)-17α,20,22-Trihydroxycholesterol (21) and (20*S*,22*S*)-17α,20,22-Trihydroxycholesterol (23).—To 70 ml of dimethyl sulfoxide were added 10.0 ml of water and 4.0 ml of 7% perchloric acid. The resulting solution was cooled to 0° and 500 mg of (22*R*)-methoxy triol **11** was added with stirring to the dimethyl sulfoxide solution. If, after 1 hr, the steroid had not completely dissolved, 3–4 ml of tetrahydrofuran was added to the solution. The mixture was allowed to stand at room temperature for 3 days, after which time it was poured onto ice and extracted three times with ethyl acetate. These extracts were washed thoroughly with water and once with saturated bicarbonate and saturated brine. After drying the organic extracts over anhydrous sodium sulfate, decanting the solvent, and evaporation, there was obtained a crystalline residue which was washed quickly with 5 ml of cold acetone. Filtration of the acetone suspension followed by recrystallization of the solid from aqueous acetone gave fine needles: mp 168–170°; nmr (pyridine-d_5) 62 (18 CH_3), 65 (19 CH_3), 91.5 (21 CH_3), doublet centered at 53 (J = 6 Hz, 26, 27 CH_3), 220 (3α H), 240 (22 H), and 320 cps (6 H); mass spectrum m/e (rel intensity) 416 (M − H_2O, 1), 398 (M − 2 H_2O, 1), 383 (398 − CH_3, 1), 365 (383 − H_2O, 1), 345 (416 − C_5H_{11}, 10), 333 (M − $C_6H_{13}O$, 7), 316 (416 − $C_6H_{12}O$, 37), 315 (416 − $C_6H_{13}O$, 74), 301 (316 − CH_3, 43), 297 (398 − $C_8H_{13}O$, 71), 271 (416 − $C_8H_{17}O_2$, base peak), 253 (271 − H_2O, 44).

The (22*R*)-tetrol analyzed for an acetone of crystallization and is reported as such.

Anal. Calcd for $C_{27}H_{46}O_4 \cdot C_3H_6O$: C, 73.12; H, 10.64. Found: C, 73.58; H, 10.40.

The 3,22-diacetate **22** was prepared by allowing a pyridine solution of the free tetrol to stand at room temperature for 48 hr in the presence of excess acetic anhydride. Isolation in the usual manner gave a solid residue which was dissolved in benzene and chromatographed on alumina. The diacetate is eluted with 10% ethyl acetate in benzene and recrystallized from methylene chloride–hexane: mp 223–226°; nmr 56 (18 CH_3), 61 (19 CH_3), 78 (21 CH_3), doublet centered at 52 (J = 6 Hz, 26, 27 CH_3), 122 (3β-acetate methyl), 127 (22-acetate methyl), 275 (3α H), 308 (22 H), and 323 cps (6 H); mass spectrum m/e (rel intensity) 500 (M − H_2O, 2), 458 (M − CH_3CO_2H, 1), 440 (500 − CH_3CO_2H, 23), 429 (500 − C_5H_{11}, 6), 380 (440 − CH_3CO_2H, 22), 357 (500 − $C_8H_{15}O_2$, 25), 313 (500 − $C_{10}H_{19}O_3$, 16), 298 (357 − CH_3CO_2, 36), 297 (357 − CH_3CO_2H, 25), 271 (458 − $C_{10}H_{19}O_3$, 74), 270 (458 − $C_{10}H_{20}O_3$, 78), 253 (271 − H_2O, 43), 226 (298 − H_2O − C_4H_6, base peak).

Anal. Calcd for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.93; H, 9.71.

The (22*S*)-methoxy triol **16** was converted to the corresponding 3β-hydroxy-Δ^5-sterol following the same procedure as was used to solvolyze its 22*R* epimer. The residue remaining after evaporation of the ethyl acetate extracts was washed with 2–3 ml of methylene chloride before being crystallized from aqueous methanol. An analytical sample was prepared by two more crystallizations from the same solvent, giving needles: mp 192–196°; nmr (pyridine-d_5) 82 (18 CH_3), 66 (19 CH_3), 110 (21 CH_3), doublet at 53 (J = 6 Hz, 26, 27 CH_3), 225 (3α H), 255 (22 H), and 320 cps (6 H); mass spectrum m/e (rel intensity) 434 (M^+, 0.3), 416 (M − H_2O, 0.4), 398 (416 − H_2O, 0.7), 383 (398 − CH_3, 0.7), 380 (398 − H_2O, 0.4), 365 (380 − CH_3, 0.9), 345 (416 − C_5H_{11}, 3), 333 (M − $C_6H_{13}O$, 1), 316 (333 − OH, 41), 315 (333 − H_2O, 33), 301 (316 − CH_3, 85), 297 (398 − $C_6H_{13}O$, 50), 271 (416 − $C_8H_{17}O_2$, base peak), 253 (271 − H_2O, 42).

Anal. Calcd for $C_{27}H_{46}O_4 \cdot H_2O$: C, 71.64; H, 10.69. Found: C, 71.45; H, 10.95.

The 3,22-diacetate **24** was prepared and purified following exactly the same procedure as was used to prepare the diacetate of the C-22 epimeric tetrol. Recrystallization from hexane–ether gave stout needles: mp 185–188°; nmr 55 (18 CH_3), 61 (19 CH_3), 74 (21 CH_3) doublet centered at 52 (J = 6 Hz, 26, 27 CH_3), 121 (3β-acetate methyl), 124 (22-acetate methyl), 275 (3α H), 306 (22 H), and 323 cps (6 H); mass spectrum m/e 458 (M − CH_3CO_2H, 1), 440 (458 − H_2O, 10), 380 (M − 2 CH_3CO_2H, 10), 357 (M − H_2O − $C_8H_{15}O_2$, 46), 313 (M − H_2O − $C_{10}H_{19}O_3$, 20), 297 (357 − CH_3CO_2H, 23), 271 (458 − $C_{10}H_{19}O_3$, 90), 270 (458 − $C_{10}H_{20}O_3$, base peak), 253 (271 − H_2O, 77%).

Lead Tetraacetate Oxidation of (20*S*,22*S*)-17α,20,22- and (20*S*,22*R*)-17α,20,22-Trihydroxycholesterols. Isolation and Identification of the Glycol Cleavage Products.—To a solution of 40 mg of tetrol **21** or **23** in 10 ml of glacial acetic acid was added a solution of 80 mg of lead tetraacetate in an additional 15 ml of glacial acetic acid. The solution was allowed to stand for 48 hr, after which time water was added and the resulting suspension was extracted with four aliquots of ethyl acetate. The ethyl acetate extracts were washed with bicarbonate until all acetic acid was removed, followed by two washes with water and saturated brine. After the organic extracts were dried over anhydrous sodium sulfate and the solvent was evaporated, there was obtained 26 mg of solid which was directly esterified by treatment with pyridine and acetic anhydride. Work-up in the usual fashion gave a solid residue which was crystallized from a small amount of ether–hexane and was found to be identical in all respects (melting point, ir, nmr) with 17α-hydroxypregnenolone acetate (**29**). Thin layer chromatographic analysis of the crude acetylated material showed no trace of dehydroepiandrosterone acetate.

To isolate the isocaproaldehyde which results from treatment of the tetrols with tetraacetate, the oxidation was performed as stated above. After the acetic acid solution was allowed to stand for 48 hr, water was added and the suspension was extracted two times with methylene chloride. The organic extracts were washed with bicarbonate to remove all acetic acid and then shaken with water. The methylene chloride solution was cooled to 0° and dried over anhydrous sodium sulfate. After drying, the organic extract was decanted off, cooled to 0°, and evaporated to 0.1 ml in a stream of nitrogen. Identification of the isocaproaldehyde as the only volatile product resulting from tetraacetate oxidation of the tetrol side chain was performed gas chromatographically utilizing the conditions previously described by Burstein, *et al.*[11]

Registry No.—**4,** 34578-46-6; **5,** 34578-47-7; **6,** 34578-48-8; **7,** 34578-49-9; **8,** 34578-50-2; **9,** 34578-51-3; **10,** 34578-52-4; **11,** 34578-53-5; **16,** 34578-54-6; **21,** 34578-55-7; **22,** 34578-56-8; **23,** 34578-57-9; **24,** 34625-43-9; **27,** 34578-58-0; **28,** 21902-63-6; **30,** 34578-60-4; **31,** 34578-61-5; **32,** 34578-62-6; **33,** 21902-62-5.

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Synthesis of 14β-Fluoro Steroids

JOHN PATAKI* AND GABRIEL SIADE B.

The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637

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14β-Fluoro-17α-hydroxy analogs of testosterone, estrone, and estradiol have been prepared from ring D unsaturated 17 ketones *via* perchloryl fluoride fluorination of the corresponding enol acetates. Small amounts of 14β-hydroxylated compounds were also isolated from the fluorination mixture.

For the use of steroid sex hormones in the chemotherapy of certain types of cancer, their primary hormonal activity is usually undesirable and may limit the applicable dose level or the duration of treatment. Consequently, efforts were directed toward the synthesis of such analogs of sex hormones that, ideally, would be hormonally inactive or at least have a favorable ratio of tumor inhibitory *vs.* hormonal activity. In the hope that substituting the strongly electronegative and somewhat larger fluorine atom for a hydrogen atom might suitably alter receptor site affinity of the resulting sex hormone analogs, we have synthesized compounds which are related to testosterone, estrone, and estradiol but are fluorinated in position 14β of the steroid nucleus differing from the normal steroid hormones in the cis juncture of rings C and D. This work reports the preparation and characterization of these compounds and of their synthetic intermediates.

The synthesis of the testosterone analog **10** is shown in Scheme I. Starting with dehydroepiandrosterone **1**, the bromo ketone **2** was prepared without attack upon the homoallylic group by direct bromination of **1** with cupric bromide in methanol.[1-3] Following dehydrobromination of the bromo ketone **2** with lithium bromide and lithium carbonate in dimethylacetamide,[3] compounds **3** and **4** were separated by fractional crystallization of the resulting equilibrium mixture. The nmr spectrum of the conjugated ketone **3** showed the vinylic protons at C_{15} and C_{16} as two doublets of doublets centered at 7.81 and 6.26 ppm (J = 6 and 2.5 Hz), and the vinyl proton at C_6 (unresolved) at 5.43 ppm. In the region corresponding to the n → π* transition, the ORD spectrum of **3** exhibited a positive Cotton effect. Sondheimer[4] reported the Cotton effect negative for 3β-hydroxyandrost-15-en-17-one but found it positive for the corresponding 14β-isomeric steroid. Recently, using circular dichroism measurements, Crabbé, Cruz, and Iriarte[5,6] found analogously oriented Cotton effects for a pair of epimeric 3-methoxyestra-1,3,5(10),15-tetraen-17-ones, *i.e.*, negative for the 14α and positive for the 14β isomer. The nmr spectrum of ketone **4** showed a broad peak at 5.53 ppm due to the two vinylic protons at C_6 and C_{15}. When **4** was acetylated, the product had the same melting point and opposite specific rotation of the same absolute value as the acetate described by St. André and coworkers.[7]

The enol diacetate **5** was prepared directly from the crude equilibrium mixture of **3** and **4**. Its nmr spectrum showed the vinyl proton resonances as an unresolved peak at 5.53 ppm (proton at C_6) and as a pair of doublets centered at 6 (J = 2.1 Hz) and 5.8 ppm (J = 2.1 Hz). Since the proton at C_{16} interacts with, and is also somewhat shielded by, the protons of the C_{17} acetoxy group, its signal is probably the less well defined doublet at higher field, while the sharper doublet at 6 ppm arises from the proton at C_{15} which only interacts with the proton at C_{16}. The ultraviolet absorption spectrum shows a maximum at 268 nm (ε 6700) due to the ring D chromophore of **5**.

Perchloryl fluoride treatment of the enol acetate **5** in aqueous tetrahydrofuran gave a mixture of fluorinated and hydroxylated products which were separated by column chromatography. The hydroxy ketone **7**, isolated in small amounts from the more polar fractions, was identified as androsta-5,15-diene-3β,14β-diol-17-one 3-acetate by its elementary analysis ($C_{21}H_{28}O_4$), presence of OH-stretching absorption at 2.88 μ, and by its nmr spectrum showing two doublets centered at 7.63 and 6.08 ppm (J = 6 Hz for both) indicating the C_{15} and C_{16} vinyl protons as well as the absence of a proton at C_{14} since further splitting was lacking. Nonreactivity of the product under acetylating conditions and observation of a positive Cotton

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