was added and the mixture refluxed for 12 hr. The reaction mixture was cooled, acidified with dilute hydrochloric acid, and extracted with ether. The organic phase was washed with water, aqueous sodium bicarbonate, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in a 3:1 mixture of acetic acid and concentrated hydrochloric acid and refluxed for 5 hr. The cooled reaction mixture was made basic with aqueous sodium bicarbonate and extracted with ether. The ether layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to yield a yellow solid which was purified by column chromatography over silica gel, mp 82–84° from methanol: NMR ($CDCl_3$, 220 MHz) 145 (s, 3 H), 175 (s, 3 H), 185 (s, 3 H), 495 Hz (m, 2 H); ir (CCl_4) 1748 cm^{-1}. Anal. Calcd for $C_{27}H_{40}O$: C, 83.87; H, 11.99. Found: C, 83.90; H, 12.40.

5β-Ethylcholestan-3-one (8a). To a solution of lithium diethylcopper in ether, prepared from 3.0 g (15. 7 mmol) of cuprous iodide and 42 ml (31.2 mmol) of 0.736 *M* ethyllithium, was added a solution of 2.0 g (5.2 mmol) of **6** in ether at 0°. The enolate **7a** was hydrolyzed as described for **8b** to yield **8a** from methanol, mp 85–87°, ir (CCl_4) 1723 cm^{-1}. Anal. Calcd for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 84.13; H, 12.17.

5β-Ethyl-*A*-norcholestan-3-one (5a). A sample of 5β-ethyl-*A*-norcholestan-3-one (**5a**) was prepared from **6** using lithium diethylcopper in the same sequence as described for **5b**. The intermediates **9a**, NMR ($CDCl_3$, 220 MHz) 1240 (t of d, J = 2.5, 10 Hz), 1165 (d, J = 10 Hz), 140 Hz (s, 3 H), and **10a**, ir (CCl_4) 1733, 1747 cm^{-1}, NMR ($CDCl_3$, 220 MHz) 802 (s, 6 H), 145 Hz (s, 3 H), were colorless oils and were obtained in yields of 80 and 87%, respectively. The sample of **5a** prepared in this manner was identical in all physical properties with the dihydro derivative of the major photoproduct **3** of **1**.

Acknowledgments. We wish to express our thanks to Dr. C. A. Weeks (Medical Foundation of Buffalo, Buffalo, N.Y.) for providing the programs of the Hauptman group and also to Dr. J. W. Edmonds (Medical Foundation of Buffalo) for discussions with T. A. while attending the Summer School in Direct Methods (Sept 1973).

Registry No.—**1**, 51355-04-5; **2**, 19682-31-6; **3**, 19594-90-2; **4**, 19682-32-7; **5a**, 19682-33-8; **5b**, 39932-87-1; **6**, 601-57-0; **7a**, 56829-83-5; **7b**, 56829-84-6; **8a**, 51355-05-6; **8b**, 13163-71-8; **9a**, 51355-07-8; **9b**, 23931-38-6; **10a**, 56829-85-7; **10b**, 56829-86-8.

Supplementary Material Available. A diagram showing deviations of atoms from mean planes and a listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th Street, N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JOC-75-3675.

References and Notes

(1) (a) Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan. (b) I. C. I. Pharmaceuticals Ltd., Mereside, Alderley Park, Cheshire SK10 4TG, England. (c) Philip Morris Research Center, Richmond, Va.
(2) (a) J. I. Seeman and H. Ziffer, *Tetrahedron Lett.*, 4409 (1973); (b) J. I. Seeman and H. Ziffer, *ibid.*, 4413 (1973).
(3) M. Fischer and B. Zeeh, *Chem. Ber.*, **101**, 2360 (1968).
(4) (a) R. E. Ireland and G. Pfister, *Tetrahedron Lett.*, 2145 (1969); (b) D. C. Muchmore, *Org. Synth.*, **52**, 109 (1972).
(5) C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, **24**, 13 (1968).
(6) K. S. Pitzer and W. E. Donath, *J. Am. Chem. Soc.*, **81**, 3213 (1959).
(7) D. A. Norton, G. Kartha, and C. T. Lu, *Acta Crystallogr.*, **16**, 89 (1963).
(8) D. F. High and J. Kraut, *Acta Crystallogr.*, **21**, 88 (1966).
(9) (a) J. M. Conia, J. P. Drouet, and J. Gorr, *Tetrahedron*, **27**, 2481 (1971); (b) A. Moscowitz, K. Mislow, M. A. W. Glass, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1945 (1962); (c) R. C. Cookson and J. Hudec, *J. Chem. Soc.*, 429 (1962).
(10) D. A. Lightner and T. C. Chang, *J. Am. Chem. Soc.*, **96**, 3016 (1974).
(11) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(12) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956).
(13) H. A. Hauptman, "Crystal Structure Determination", Plenum Press, New York, N.Y., 1972.
(14) C. M. Weeks, S. Pokrywiecki, and J. W. Edmonds, PHASE—Direct Methods Programs Set, Medical Foundation of Buffalo, Buffalo, N.Y., 1973.
(15) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, "The X-Ray System", Technical Report TR-192, Computer Center, University of Maryland, 1972.
(16) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1962.
(17) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(18) C. K. Johnson, ORTEP-ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
(19) S. W. Peterson and H. A. Levy, *Acta Crystallogr.*, **10**, 70 (1957). The correction factor used was $0.0004F^2$.
(20) W. Nagata, S. Hirai, H. Itazaki, and K. Takeda, *Chem. Ber.*, **641**, 196 (1961).
(21) Listing of structure factors. See paragragh at end of paper regarding supplementary material.

Synthesis and C-25 Chirality of 26-Hydroxycholesterols

Ravi K. Varma,[1a,b] Masato Koreeda,[2a] Boris Yagen,[1a,c] Koji Nakanishi,[2a] and Eliahu Caspi*[1a]

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545,[1d] and Department of Chemistry, Columbia University, New York, New York 10027[2b]

Received June 2, 1975

Samples of cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether were prepared via hydroboration of cholest-5,25-dien-3β-ol tetrahydropyranyl ether with (a) disiamylborane, (b) (+)-diisopinocampheylboranes (DIPCB), and (c) (−)-(DIPCB). The 26-hydroxy compounds were converted first to cholest-4-en-3-on-26-ol *p*-bromobenzoates and then to cholest-4-ene-3β,26-diol 26-*p*-bromobenzoates. Authentic (25*R*)- and (25*S*)-cholest-4-ene-3β,26-diol *p*-bromobenzoates were prepared from kryptogenin and from (25*S*)-cholest-4-en-3-on-26-ol *p*-bromobenzoate, respectively. The magnitudes of the Cotton effects of the 25*R* and 25*S* samples were the same but of the opposite sign. The 25*R* compound had a negative Cotton effect while the 25*S* compound had a positive Cotton effect. Both compounds were assumed to be optically pure (100%). The CD spectra of the corresponding analogs derived from the hydroboration of the C-25 olefin were recorded. Based on the sign and amplitude of their Cotton effects, their stereochemistry and optical purity at C-25 was defined.

For studies of the stereochemistry of the reduction of the C-24 double bond of lanosterol in the course of the biosynthesis of cholesterol in the S-10 fraction of rat livers[3–5] we required samples of (25*R*)- and (25*S*)-26-hydroxycholestenone.[4–5] The attempted preparation of these compounds via the selective hydroboration of cholesta-5,25-dien-3β-ol

3-tetrahydropyranyl ether (3-THP ether)[6] with (−)- and (+)-diisopinocampheylborane[7-9] is the subject of this communication.

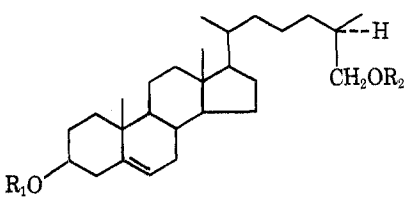
Experimental Section

Materials. Tetrahydrofuran and boron trifluoride etherate were purified by procedures previously described.[7] Sodium borohydride (minimum 98% pure) was supplied by Fisher Scientific Co. The previously used samples of (+)- and (−)-α-pinene were employed in the present study.[8]

Physical Measurements. Melting points were taken on a hot-stage apparatus and are corrected. Infrared (ir) spectra were recorded on a Perkin-Elmer 237 spectrophotometer as KBr wafers. Ultraviolet spectra were measured on a Perkin-Elmer 202 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian DA60 spectrometer at 60 MHz. Chemical shifts are quoted in parts per million downfield from internal tetramethylsilane. Coupling constants are quoted in hertz. Mass spectra were measured on a Varian Associates M-66 instrument. A Hilger MK-III instrument was used for the measurement of all optical rotations.[4] The optical rotations of the samples of cholest-4-en-3-on-26-ols were also recorded on a Rudoph and Son's photoelectric polarimeter.[4] The CD measurements were carried out on a Jasco J-40 instrument.

Chromatography. Gas chromatographic (GLC) analyses were performed on a Perkin-Elmer Model 811 instrument equipped with a flame ionization detector. A 3-ft silanized glass column of 3% XE-60 on Chromosorb at 240–250° was used for all analyses. Silica gel (Merck $HF_{254+366}$) was used for preparative and analytical TLC in the indicated solvent systems. The products were detected under ultraviolet light and by color reactions with phosphomolybdic acid. Radiochromatograms were scanned on a Vanguard automatic chromatogram scanner (Model 880).

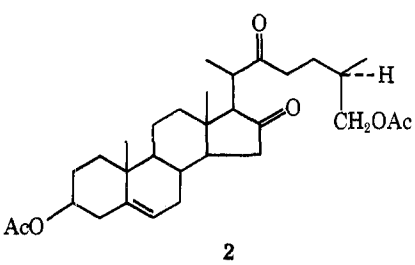
Preparation of (25*R*)-Cholest-5-ene-3β,26-diol (1a) from Kryptogenin Diacetate (2). A specimen of kryptogenin diacetate (2) was purified by preparative TLC [ethyl acetate–benzene (1:4)] and crystallized from ethyl acetate–hexane to give needles: mp 148–151°; $[\alpha]^{21}D$ −176.3 ± 0.5° (*c* 4.84, $CHCl_3$) [reported $[\alpha]^{20}D$ −167° ($CHCl_3$[10])]; $[\alpha]^{23}D$ −182° (*c* 0.08, dioxane; from ORD measurement[11]).



1

25*R*, derived from 2

a, R_1 = H, R_2 = H
b, R_1 = $C(C_6H_5)_3$; R_2 = $C(C_6H_5)_3$
c, R_1 = H; R_2 = $C(C_6H_5)_3$
d, R_1 = Ac; R_2 = $C(C_6H_5)_3$
e, R_1 = Ac; R_2 = Ac
f, R_1 = Ac; R_2 = H

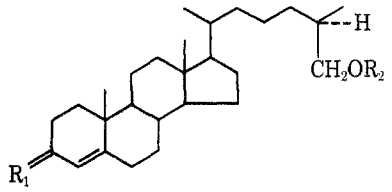


2

The diacetate (>90% pure) was subjected to Clemmensen reduction.[12] The ir spectrum of the crude product revealed complete reduction of the side-chain carbonyl (1710 cm^{-1}). The material was then further reduced by the Huang-Minlon procedure[12] and the resulting mixture was crystallized from ethyl acetate, giving needles. Trace impurities were removed by preparative TLC and the purified material was recrystallized to give (25*R*)-cholest-5-ene-3β,26-diol (**1a**): mp 172–173°; $[\alpha]^{21}D$ −27.7 ± 1.4° (*c* 1.65, DMF); $[\alpha]^{21}D$ −26.3 ± 1.6° (*c* 1.22, dioxane); $[\alpha]^{21}D$ −33.5 ± 1.3° (*c* 1.5, $CHCl_3$) [reported[12] mp 177–178°, $[\alpha]^{20}D$ −30° (*c* ~1, $CHCl_3$)].

(25*R*)-Cholest-5-ene-3β,26-diol 26-Triphenylmethyl Ether (1c) (Derived from Kryptogenin). A solution of the diol **1a** (700 mg, 1.75 mmol) and purified triphenylchloromethane (532 mg, 1.93 mmol) in dry pyridine (20 ml) was refluxed for 3 hr.[13] The reaction was terminated by pouring into water and the products were extracted with ether. The organic phase was washed several times with a solution of KH_2PO_4 and then with water. The solution was dried (Na_2SO_4) and the solvent was removed to furnish a noncrystalline product, which was purified by chromatography on a column of silica (40 g). Elution with hexane–benzene (1:4, 700 ml) furnished an amorphous powder (130 mg) considered from its spectral properties to be the ditrityl ether **1b.** Elution of the column with benzene (800 ml) gave triphenylmethylcarbinol and subsequent use of ether–benzene (1:9, 1.8 l.) yielded the 26-monotrityl ether **1c** (520 mg) as an amorphous solid [ir ν_{max} (KBr) 3380 cm^{-1} (OH) and aromatic absorption bands]. Finally, elution of the column with methanol–ether (1:49, 600 ml) furnished the unreacted diol **1a** (190 mg) having the same optical rotation as the starting material. Treatment of this diol (190 mg) as described above gave an additional 102 mg of monotrityl ether **1c.**

(25*R*)-Cholest-4-en-3-on-26-ol (3a). The ether **1c** was further purified by preparative TLC [ethyl acetate–benzene (1:1)] to give an amorphous powder, characterized by its NMR spectrum ($CDCl_3$): 0.67 (s, 3 H, 18-H), 0.93 (d, *J* = 6.5 Hz, 6 H, 21- and 27-H), 0.99 (s, 3 H, 19-H), 2.88 (d, *J* = 6 Hz, 2 H, 26-H), ca. 3.50 (broad, 1 H, 3α-H), 5.31 (1 H, 6-H), ca. 7.33 ppm (m, aromatic H).



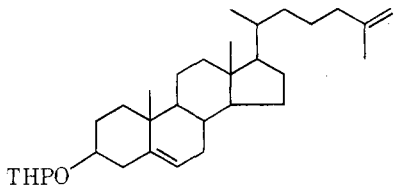
3

25*R*, derived from 1

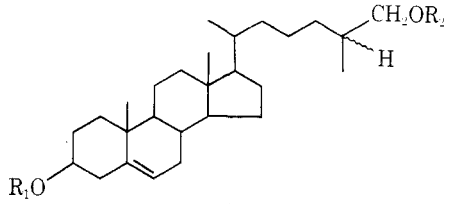
a, R_1 = O; R_2 = H;
b, R_1 = O; R_2 = *p*-BrC_6H_4CO
c, R_1 = β-OH; R_2 = *p*-BrC_6H_4CO

A mixture of the monotrityl ether **1c** (520 mg), toluene (125 ml), and cyclohexanone (2.5 ml) was stirred and distilled until 50 ml of toluene was collected. Aluminum isopropoxide (300 mg) was added and the mixture was refluxed with the exclusion of moisture (18 hr). After cooling, the solution was treated with a saturated solution of potassium hydrogen tartrate (20 ml) and the product was isolated in the conventional manner. The volatile components were distilled under reduced pressure, and the resulting oily residue was refluxed (2.5 hr) with 85% aqueous acetic acid (20 ml). The reaction mixture was poured into water and the product was extracted with ether. The extract was processed in the usual manner to yield an oily residue which was fractionated by TLC [ethyl acetate–benzene (1:3)] and (25*R*)-cholest-4-en-3-on-26-ol (**3a**) was isolated. Crystallization from ethyl acetate–hexane furnished needles (180 mg): mp 129–131°; ν_{max} (KBr) 3380 (OH), 1663 (C=O), 1615 cm^{-1} (C=C); λ_{max} (EtOH) 241 nm (ε 15800); $[\alpha]^{21}D$ +84.5 ± 0.8° (*c* 2.5, $CHCl_3$) and $[\alpha]^{22}D$ +85.6 ± 0.5° (*c* 5.3, $CHCl_3$); homogenous by GLC (250°; t_R 24 min).

(25*RS*)-Cholest-5-ene-3β,26-diol 3-Tetrahydropyranyl Ether (5a) (Hydroboration of 4 with (+)-Diisopinocampheylborane). A mixture of sodium borohydride (1.5 mmol, 57.0 mg) and (−)-α-pinene (4.4 mmol, 0.70 ml) in tetrahydrofuran (10 ml) was stirred and cooled to 0° as previously described.[7-9] Boron trifluoride etherate (2 mmol, 0.25 ml) was added dropwise from a syringe in 2 min and the mixture was stirred at 0–2° for 3.5 hr. A solution of cholest-5,25-dien-3β-ol tetrahydropyranyl ether[6a] (**4**, 240 mg) in tetrahydrofuran (5–10 ml) was then added during 5 min. The mixture was stirred for 2.5 hr at 0–2° and allowed to warm up to room temperature. Then 3 *N* NaOH (3.0 ml) and 30% H_2O_2 (3.0 ml) were added and the stirring was continued for 1.5 hr at ~40°. The product was recovered with ether and the volatile components were removed under reduced pressure at 70–80°. The residue was chromatographed on a column of silica gel (20 g). Elution with benzene (150 ml) furnished starting material (**4**, 45 mg) and elution with methanol–dichloromethane (1:9, 200 ml) gave (25*RS*)-cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether (**5a**, 180 mg). The ir spectrum of **5a** and its chromatographic properties were identical with those of the 25*S* isomer (**6a**) (23% optical purity described previously[6a,b]) (see below).



4



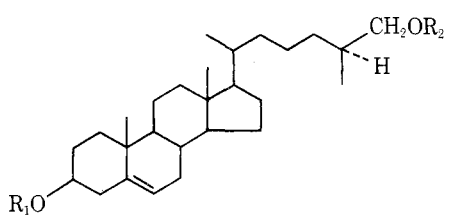
5

25*RS*

a, hydroboration of 4 with (+)-DIPCB; R_1 = THP; R_2 = H
b, from 5a; $R_1 = R_2$ = H
c, from 5b; R_1 = H; $R_2 = C(C_6H_5)_3$
d, from acid 9c; $R_1 = R_2$ = H
e, from acid 9d; $R_1 = R_2$ = H
f, [25-^{3}H]; $R_1 = R_2$ = H

(25*RS*)-Cholest-5-ene-3β,26-diol (5b) (from 5a). The above material (**5a**, 150 mg) was dissolved in methanol (15 ml), *p*-toluenesulfonic acid (5 mg) was added, and the mixture was refluxed for 1 hr. The solution was diluted with water and extracted with ether. The ether solution was washed with dilute NaOH and water and dried (Na_2SO_4) and the solvent was evaporated. Crystallization of the residue from ethyl acetate–hexane (1:1) gave needles (100 mg), mp 171.5–172.5°. A portion of the crystalline material was further purified by TLC [ethyl acetate–benzene (1:1)] and crystallized to furnish needles of (25*RS*)-cholest-5-ene-3,26-diol (**5b**), mp 171.5–172.5°, $[\alpha]^{25}D$ −35 ± 1.33° (*c* 1.51, $CHCl_3$). The product was homogeneous by GLC analysis.

(25*S*)-Cholest-5-ene-3β,26-diol (6b) (ca. 23% Optical Purity) (Hydroboration of 4 with Disiamylborane). The preparation of 4 and its selective hydroboration with disiamylborane to yield **6a** was carried out exactly as previously described.[6a] The obtained 3-THP ether **6a** was then hydrolyzed to the 3,26-diol **6b**, mp 168.5°, $[\alpha]^{25}D$ −35.9 ± 1.53° (*c* 1.31, $CHCl_3$).



6

25*S*, ca. 23% optical purity

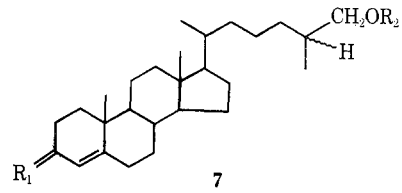
a, hydroboration of 4 with disiamylborane; R_1 = THP; R_2 = H
b, from 6a; $R_1 = R_2$ = H
c, from 6a; R_1 = THP; R_2 = Ac
d, from 6c; R_1 = H; R_2 = Ac

25*S*, ca. 83% optical purity

e, hydroboration of 4 with (−)-DIPCB; R_1 = THP; R_2 = H
f, from 6e; $R_1 = R_2$ = H
g, from 6f; R_1 = H; $R_2 = C(C_6H_5)_3$

(25*S*)-Cholest-5-ene-3β,26-diol (6f) (ca. 83% Optical Purity) (Hydroboration of 4 with (−)-Diisopinocampheylborane). The reaction was performed in the manner described for the hydroboration with (+)-diisopinocampheylborane except that (+)-α-pinene was used for the preparation of the (−)-DIPCB reagent. The 26-hydroxytetrahydropyranyl ether (**6e**) was isolated, purified, and converted as above to the (25*S*)-cholest-5-ene-3β,26-diol (**6f**). mp 171–172°, $[\alpha]^{25}D$ −38.0 ± 1.16° (*c* 1.72, $CHCl_3$).

(25*RS*)-Cholest-4-en-3-on-26-ol (7a) (from 5b). The 25*RS* diol **5b** was converted through the 26-monotrityl ether **5c** to (25*RS*)-cholest-4-en-3-on-26-ol (**7a**) by the procedure described above. The product was homogeneous by TLC and GLC and showed $[\alpha]^{22}D$ +87.4 ± 0.8° (*c* 2.7, $CHCl_3$) and $[\alpha]^{22}D$ +85.9 ± 0.5° (*c* 4.3, $CHCl_3$).



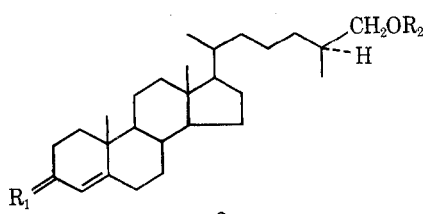
7

25*RS*, derived from 5a

a, R_1 = O; R_2 = H
b, R_1 = O; R_2 = *p*-BrC_6H_4CO
c, R_1 = β-OH; R_2 = *p*-BrC_6H_4CO

(25*S*)-Cholest-4-en-3-on-26-ol (8a) (ca. 23% Optical Purity) (from 6a). A solution of (25*S*)-cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether (**6a**, 1.35 g) in pyridine (5 ml) and acetic anhydride (0.2 ml) was kept at 25° overnight and the product was worked up in the conventional manner to give the 3-THP 26-acetate **6c** (1.45 g). This compound was dissolved in 90% ethanol (50 ml) and treated with 2 *N* HCl (0.25 ml) under reflux for 5 min. The usual work-up procedure furnished 1.21 g of material which was chromatographed on silica gel (50 g). Elution of the column with ethyl acetate–benzene (1:9, 450 ml) gave 180 mg of a mixture containing starting material and the required 26-acetoxy-3β-alcohol (**6d**). Further elution (4.0 l.) furnished (25*S*)-cholest-5-ene-3β,26-diol 26-acetate (**6d**), ν_{max} (KBr) 3415 (OH) and 1740 cm^{-1} (-$OCOCH_3$).

Oxidation of the hydroxy acetate (150 mg) with aluminum isopropoxide (170 mg) and cyclohexanone (0.75 ml) in dry toluene (20 ml) at reflux (7 hr) was performed as described for **3a**. The oily product was dissolved in a mixture of 5% aqueous Na_2CO_3 in methanol (18 ml) and stored for 16 hr at 25°. Water was added and the product was isolated with ether. The residue (129 mg) was fractionated by preparative TLC to give (25*S*)-cholest-4-en-3-on-26-ol (**8a**) (23% optical purity) (75 mg), which was crystallized from ethyl acetate–hexane: mp 140.5–145.5°; λ_{max} (EtOH) 241 nm (ε 16400); $[\alpha]^{21}D$ +80.4 ± 0.4° (*c* 2.58, $CHCl_3$) and $[\alpha]^{22}D$ +82.4 ± 0.4° (*c* 5.6, $CHCl_3$); mass spectrum *m/e* 400 (M^+), 358 (M − 42) 277, 229, 124 (base peak).



8

25*S*, ca. 23% optical purity; derived from 6a

a, R_1 = O; R_2 = H
b, R_1 = O; R_2 = *p*-BrC_6H_4CO
c, R_1 = β-OH; R_2 = *p*-BrC_6H_4CO

25*S*, ca. 83% optical purity; derived from 6e

d, R_1 = O; R_2 = H
e, R_1 = O; R_2 = *p*-BrC_6H_4CO
f, R_1 = β-OH; R_2 = *p*-BrC_6H_4CO

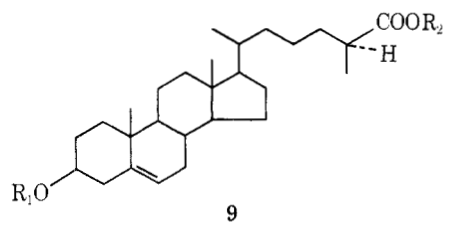
25*S*, 100% optical purity; derived from the incubation of cholesterol with *M. smegmatis*

g, R_1 = O; R_2 = *p*-BrC_6H_4CO
h, R_1 = β-OH; R_2 = *p*-BrC_6H_4CO

(25*S*)-Cholest-4-en-3-on-26-ol (ca. 83% Optical Purity) (8d) (from 6e). The 25*S* diol **6f** was converted via **6g** to the 25*S* keto alcohol **8d**, $[\alpha]^{22}D$ +74.8 ± 0.8° (*c* 2.5, $CHCl_3$).

(25*S*)-3β-Tetrahydropyranyloxycholest-5-en-26-oic Acid (ca. 23% Optical Purity) (9a). A solution of (25*S*)-cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether (**6a**) (23% optical purity) (250 mg) in pyridine (5 ml) was added to a magnetically stirred mixture

of pyridine (20 ml), chromic oxide (1.0 g), and water (0.3 ml) at 0°. The mixture was stirred (4 hr) at 25°, then poured into water (150 ml) and the product recovered with ether. The organic phase was washed with cold dilute HCl, 5% NaOH, and water and dried (Na_2SO_4). Evaporation of the solvent gave a neutral material (110 mg), ν_{max} (KBr) 1700 and 1730 cm^{-1}, which was not further investigated. The alkaline extract was cooled in ice and acidified with dilute HCl. The product was extracted with ethyl acetate–ether (1:1). The extract was washed and dried (Na_2SO_4) and the solvent was removed. The obtained sold (141 mg) was crystallized from acetone to give (25*S*)-3β-tetrahydropyranyloxycholest-5-en-26-oic acid (**9a**): mp 158.5–160.5°; ν_{max} (KBr) 3500–3100 (COOH), 1725, 1700 cm^{-1} (C=O).



9

a, R_1 = THP; R_2 = H [derived from 25*S* (**6a**), ca. 23% optical purity]
b, $R_1 = R_2$ = H
c, $R_1 = R_2$ = H; 25ξ, recovered from crystalline quinine salt
d, R_1 = H; R_2 = H; 25ξ, recovered from the mother liquor of crystallization of the quinine salt
e, methyl ester of **9b**; R_1 = H; R_2 = CH_3

(25*S*)-Cholest-5-en-3β-ol-26-oic Acid (ca. 23% Optical Purity) (9b). A solution of the 25*S* 3-ether acid **9a** (350 mg) and *p*-toluenesulfonic acid (10 mg) in methanol (10 ml) was stored at 25° for 18 hr. The solvent was then removed in a stream of nitrogen. The residue was taken up in ether, washed with water, and dried and the solvent was removed. TLC and the ir spectrum indicated that the residue was a mixture of the hydroxy acid **9b** and the 26-methyl ester. The crude material was saponified with 5% methanolic KOH (reflux, 1 hr). The solution was acidified and the product recovered with ether. Two crystallizations from ethyl acetate furnished plates of (25*S*)-cholest-5-en-3β-ol-26-oic acid (**9b**): mp 170–172.5° (reported for 25*RS* acid 176–178° uncorrected[14] and 173–175°[15]); $[\alpha]^{22}D$ −23.9 ± 0.9° (*c* 2.21, CH_3OH) and −21.7 ± 2° (*c* 1.0, CH_3OH); ν_{max} (KBr) 3400 (OH), 1700 cm^{-1} (C=O).

The mother liquors of crystallizations were combined (98 mg) and recrystallized to give a second sample of the acid **9b**, mp 169–171°, $[\alpha]^{22}D$ −23.0 ± 1.4° (*c* 1.4, CH_3OH). Both specimens were homogeneous by TLC (ethyl acetate).

A portion of **9b** was treated with ethereal diazomethane and the product crystallized from methanol to give the methyl ester **9e:** mp 99–101° (reported for 25*RS* 26-Me ester 100–103° uncorrected[14] and 102–103°[15]); ν_{max} (KBr) 3430 (OH), 1730 cm^{-1} (ester).

Attempted Resolution of the Quinine Salt of (25*S*)-Cholest-5-en-3β-ol-26-oic Acid (ca 23% Optical Purity) (9b). Attempts to resolve the acids using the systems brucine–methanol, brucine–acetone, or strychnine–ethanol were not successful. However, it appears that at least a partial resolution was obtained using (−)-quinine–methanol.

A mixture of the 25*S* hydroxy acid **9b** (113.5 mg, 0.272 mmol) and (−)-quinine (88.0 mg, 0.272 mmol) was dissolved in hot methanol and stored at 0–5° overnight. The obtained salt was filtered (126 mg), dissolved in methanol (3.0 ml), and stored at 0–5° for 5 hr. The crystals were collected (89 mg) and the salt was decomposed by shaking with a mixture of ether (75 ml) and 1 *N* HCl (30 ml). The ether layer was washed, dried, and concentrated to yield the acid **9c** (47 mg), which was recrystallized from ethyl acetate–hexane as needles, $[\alpha]^{22}D$ −27.2 ± 1.1° (*c* 2.2, CH_3OH) and −25.3 ± 1.8° (*c* 0.9, CH_3OH).

The mother liquors of crystallization of the quinine salt were combined and the salt was decomposed as described above to give the acid **9d** (62 mg) which was crystallized from ethyl acetate–hexane. The product showed $[\alpha]^{22}D$ −19.1 ± 1.1° (*c* 1.9, CH_3OH).

A sample of the acid **9c** from a duplicate experiment showed $[\alpha]^{22}D$ −27.3 ± 1.4° (*c* 1.4, CH_3OH). Following preparative TLC and crystallization **9c** had $[\alpha]^{22}D$ −25.3 ± 1.1° (*c* 1.9, CH_3OH). The acid **9d** (from the duplicate experiment) showed $[\alpha]^{22}D$ −20.8 ± 1.4° (CH_3OH). After preparative TLC and crystallization this specimen of **9d** had $[\alpha]^{22}D$ −21.9 ± 1.1° (*c* 1.9, CH_3OH); NMR spectrum (in dimethylformamide-d_7) 0.71 (s, 3 H, 18-H), 1.00 (s, 3 H, 19-H), 1.11 (d, *J* = 7.0, 3 H, 27-H), ca. 3.38 (broad, 1 H, 3α-H), and 5.30 (1 H, 6-H).

(25ξ)-Cholest-5-ene-3β,26-diol (5d) from Acid 9c. A mixture of the acid **9c** (from the duplicate experiment), $LiAlH_4$, and dry tetrahydrofuran was refluxed in an atmosphere of dry nitrogen. The recovered product was purified by TLC [ethyl acetate–benzene (1:1)] and crystallized from ethyl acetate. The (25ξ)-cholest-5-ene-3β,26-diol (**5d**) showed $[\alpha]^{22}D$ −30.4 ± 3.0° (*c* 0.66, DMF), $[\alpha]^{21}D$ −27.1 ± 1.8° (*c* 1.18, dioxane), and $[\alpha]^{21}D$ −33.7 ± 1.8° (*c* 1.2, $CHCl_3$).

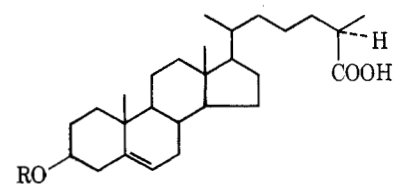
(25ξ)-Cholest-5-en-3β,26-diol (5e) from Acid 9d. The acid **9d** (from the duplicate experiment) was reduced with $LiAlH_4$ as described above. The obtained (25ξ)-cholest-5-ene-3β,26-diol (**5e**) showed $[\alpha]^{21}D$ −31.9 ± 2.2° (*c* 1.12, DMF), $[\alpha]^{21}D$ −33.3 ± 2.0° (*c* 1.0, dioxane), and $[\alpha]^{22}D$ −37.8° (*c* 1.1, $CHCl_3$).

(25*R*)-Cholest-5-en-3β-ol-26-oic Acid 3-Acetate (10a). A mixture of the 26-trityl ether **1c** (derived from kryptogenin) (1 g), pyridine (10 ml), and acetic anhydride (2 ml) was stored for 16 hr at ambient temperature. The product was recovered in the conventional manner to yield **1d,** ir no OH band, 1730 cm^{-1} (acetate), aromatic absorption bands.

A solution of (25*R*)-3β-acetoxycholest-5-ene 26-trityl ether (**1d,** 2.93 g) in 90% aqueous dioxane (60 ml) containing concentrated HCl (0.5 ml) was heated at 50–60° for 2 hr. The solution was diluted with water and extracted with ether. The organic phase was washed with dilute $NaHCO_3$ and water, and, after drying, the solvent was removed.

The resulting residue was adsorbed on a silica gel (100 g) column. Elution with benzene (2.7 l.) gave triphenylmethanol and some (25*R*)-cholest-5-ene-3β,26-diol diacetate (**1e**), mp 125.5–127.5° [reported[12] (for 25*R* diacetate) 128–129° (uncorrected)]. Subsequent elution with ethyl acetate–benzene (3:97, 1.8 l.) furnished (25*R*)-cholest-5-ene-3β,26-diol 3-acetate (**1f,** 952 mg). Crystallization from ethyl acetate gave plates: mp 130.5–131.5°; $[\alpha]^{24}D$ −35.6° (*c* 3.0, $CHCl_3$); ν_{max} (KBr) 3360 (OH) and 1735 cm^{-1} (acetate); NMR spectrum ($CDCl_3$) 0.68 (s, 3 H, 18-H), 0.91 (d, *J* = 6.5 Hz, 6 H, 21- and 27-H), 1.03 (s, 3 H, 19-H), 2.03 (s, 3 H, $-OOCCH_3$), 3.46 (d, *J* = 5 Hz, 2 H, 26-H), ca. 4.57 (broad, 1 H, 3α-H), and 5.37 (d, *J* = 5 Hz, 1 H, 6-H). Further elution of the column with ethyl acetate–benzene (3:7) gave (25*R*)-cholest-5-ene-3β,26-diol (**1a**) (410 mg), mp 167–168°.

The 3-acetoxy-26-alcohol **1f** (770 mg) in acetone (80 ml) was oxidized with Jones reagent at ambient temperature for 3 min. Conventional work-up gave a crystalline residue (728 mg), which was shown by TLC to contain mainly the required acetoxy acid (**10a**). Purification by preparative TLC [ethyl acetate–benzene (2:3)] and crystallization of the major product from ether–hexane gave (25*R*)-cholest-5-en-3β-ol-26-oic acid 3-acetate (**10a**): mp 142.5–149° (mostly 148–149°); ν_{max} (KBr) 3600–3000 (broad, –COOH) and 1725 cm^{-1} (C=O, acid and ester); $[\alpha]^{24}D$ −45.9 ± 1.3° (*c* 2.44, $CHCl_3$).



10

a, R = Ac [derived from 25*R* (**1f**) (100% optical purity)]
b, R = H
c, **b** recovered from crystalline quinine salt
d, **b** recovered from mother liquor of quinine salt

(25*R*)-Cholest-5-en-3β-ol-26-oic Acid (10b), 10c, and 10d. A. (25*R*)-Cholest-5-en-3β-ol-26-oic acid 3-acetate (**10a,** 650 mg) was dissolved in a mixture of ether (50 ml) and a saturated solution of Na_2CO_3 in 80% methanol (440 ml). After 24 hr at room temperature the solution was acidified with concentrated HCl (Congo Red) and the volume of solvent was reduced under vacuum at 35°. The residue was diluted with water, and the product was extracted with ether and worked up as usual to give a crystalline residue (620 mg). Two crystallizations from ethyl acetate furnished needles (400 mg) of (25*R*)-cholest-5-en-3β-ol-26-oic acid (**10b**), mp 168–170°, $[\alpha]^{24}D$ −34.1° (*c* 2.0, CH_3OH). From the mother liquor a second crop (125 mg) was obtained: $[\alpha]^{24}D$ −33.5° (*c* 2.0, CH_3OH) [reported for 25*RS* acid mp 176–178°[14] (uncorrected) and 173–175°;[15] $[\alpha]^{20}D$ −30.6° (no solvent or concentration reported)].

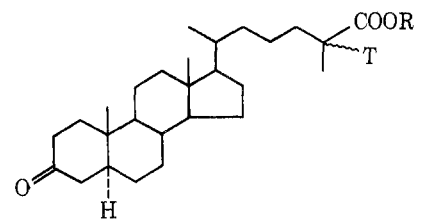
B. The above (25*R*)-cholest-5-en-3β-ol-26-oic acid (**10b,** 113.5

mg) (prepared in A) was subjected to resolution in the (−)-quinine–methanol system described previously. The twice-crystallized salt and the mother liquors were decomposed to give acids **10c** (71 mg) and **10d** (38 mg), respectively. Both samples had $[\alpha]^{24}D$ −33.6° (*c* 2.0, CH_3OH). Hence the acids **10** derived from kryptogenin are apparently optically pure 25*R* acids.

[25-^{3}H]-5α-Cholestane-3β,26-diol (12). A specimen of [25-^{3}H]cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether[6] (255 μg, 3.8 μCi) was mixed with nonradioactive material (500 mg) and converted to [25-^{3}H]cholest-5-ene-3β,26-diol (**5f**) as previously described.[6] The product was crystallized from ethyl acetate to constant specific activity.

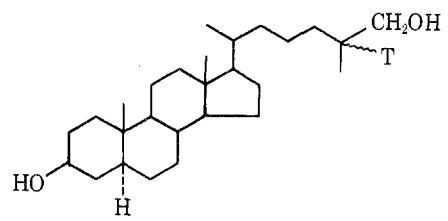
The [25-^{3}H]cholest-5-ene-3β,26-diol (**5f**, 400 mg) was hydrogenated in ethyl acetate–perchloric acid.[6] The partially acetylated reduced residue was treated with 1 *N* methanolic KOH (75 ml) at 25° for 18 hr. The product **12** was isolated and recrystallized from methanol. The specific activity was unchanged, mp 166.5–171°, $[\alpha]^{24}D$ +23.6 ± 1.2° (*c* 1.7, $CHCl_3$) [reported[12] for *R* diastereomer, 179–181° uncorrected, $[\alpha]^{20}D$ +28° (ca. 1%, $CHCl_3$)].

[25-^{3}H]-5α-Cholestane-3β,26-diol (**12**, 200 mg) in acetone (75 ml) was treated with Jones reagent (0.75 ml) dropwise at room temperature for 5 min. The usual work-up procedure gave a homogeneous product which was crystallized from ethyl acetate–hexane to give [25-^{3}H]-5α-cholestan-3-on-26-oic acid (**11a**). The specific activity of **11** remained unchanged.



11

a, R = H
b, R = CH_3



12

We repeated the oxidation of **12** with the pyridine (20 ml)–chromic acid (1 g)–water (0.3 ml) reagent. The resulting **11a** retained all the tritium.

The 3-keto 26-acid **11a** showed mp 145–147.5° [reported[12] for the 25*R* diastereomer 156–158° (uncorrected)]; ν_{max} (KBr) 3680–3640 and 1715 cm^{-1} (-COOH); $[\alpha]^{24}D$ +39.8 ± 1.3° (*c* 1.5, $CHCl_3$) [reported[12] for 25*R* (**11a**) $[\alpha]^{20}D$ +32° (*c* ~1, $CHCl_3$)].

Equilibration of [25-^{3}H]-5α-Cholestan-3-on-26-oic Acid (11a) and 26-Methyl Ester (11b) with Base. A. The keto acid **11a** (100 mg) was treated with a saturated solution of Na_2CO_3 in 80% methanol (25 ml) for 24 hr at room temperature. Isolation of the product and crystallization from ethyl acetate–hexane gave **11a** which showed an unchanged specific activity and $[\alpha]^{24}D$ +40.1° (*c* 1.6, $CHCl_3$).

B. A specimen of the acid **11a** (50 mg) was refluxed with 5% KOH in aqueous ethanol (1:9, 10 ml) for 20 hr. The product was isolated and crystallized from ethyl acetate–hexane. No loss of tritium occurred.

C. The material from experiment B was refluxed with KOH (600 mg) in propylene glycol (10 ml) and water (2 ml) for 20 hr. The product was isolated, purified by TLC [ethyl acetate–benzene (1:2)], and crystallized. The specific activity remained unchanged.

D. Another specimen of the keto acid **11a** (40 mg) was esterified with diazomethane. The resulting 26-methyl ester **11b** (38 mg) was added to a solution of sodium methoxide in dry methanol (860 mg of Na in 25 ml of MeOH) and the mixture was refluxed (6 hr) under N_2. Water (3 ml) was then added and the solution was refluxed for a further 2.5 hr. The product was isolated in the usual manner and after purification by TLC [ethyl acetate–benzene (1:3)] the keto acid **11a** was crystallized. The specific activity remained unchanged.

Preparation of Cholest-4-en-3-on-26-ol *p*-Bromobenzoates. The 26-*p*-bromobenzoates **3b** (derived from kyptogenin), **7b** (derived from hydroboration of Δ^{25} with (+)-diisopinocampheylborane), **8b** (derived from hydroboration of Δ^{25} with disiamylborane), and **8e** (derived from hydroboration of Δ^{25} with (−)-diisopinocampheylborane) were prepared as previously described.[4,16]

Preparation of Cholest-4-en-3β,26-diol 26-*p*-Bromobenzoates. (25*S*)-Cholest-4-en-3-on-26-ol *p*-bromobenzoate (**8g**, ca. 5 mg) [derived from microbially prepared 26-hydroxycholest-4-en-3-one] was dissolved in 0.5 ml of ether and treated with 1.5 mg of lithium aluminum tri-*tert*-butoxyhydride at 0°. The suspension was kept at 0° for 16 hr. The reaction mixture was poured into ice–water and extracted with ether. The crude product obtained after evaporation of the solvent was purified by preparative TLC [$CHCl_3$–MeOH (24:1)] and afforded ca. 1.8 mg of cholest-4-ene-3β,26-diol 26-*p*-bromobenzoate (**8h**): uv (MeOH) 245 nm (ε ca. 20000); NMR ($CDCl_3$) 0.70 (s, 3 H, 18-H), 0.94 (d, *J* = 6.5 Hz, 3 H, 21-H), 1.01 (d, *J* = 6.5 Hz, 3 H, 27-H), 1.04 (s, 3 H, 19-H), ca. 4.1 (broad, 1 H, 3α-H), 4.15 (2 H, 26-H), 5.27 (s, 1 H, 4-H), 7.59 and 7.90 ppm (4 H, benzoate protons) (the NMR spectrum was obtained on a Jeolco PS-100 instrument by the Fourier transform method).

The 26-*p*-bromobenzoate **8h** was further purified by liquid chromatography on a Waters-Alc instrument equipped with two linearly connected Corasil columns (3 ft × 0.375 in. each). The column was percolated with hexane–2-propanol (199:1) at a rate of 3 ml/min. A small amount of starting material (retention time 23 min) and a trace of an impurity were removed. The required 3β-ol-4-ene **8h** showed a retention time of 35 min.

The four remaining cholest-4-ene-3β,26-diol 26-*p*-bromobenzoates were prepared in a similar manner.

Results and Discussion

Our approach to the synthesis of 26-hydroxycholesterol analogs was the same as that previously used.[6] We planned to hydroborate[7-9] selectively the 25 double bond of the 5,25-diene 3-THP ether **4** and oxidize the resulting product with $NaOH–H_2O_2$ to yield the 26-hydroxycholesterol 3-tetrahydropyranyl ether (THP ether). The 26-hydroxy 3-THP ether could then be manipulated to give the required products.

It was considered likely that hydroboration of **4** with the achiral disiamylborane will give racemic (or nearly so) (25*RS*)-26-alcohol.[6a,b] On the other hand, hydroboration of **4** with (+)-diisopinocampheylborane (DIPCB) [derived from (−)-α-pinene] and (−)-DIPCB [derived from (+)-α-pinene] should result in 26-alcohols enriched with 25*R* and 25*S* isomers, respectively. These projections were based on certain generalizations on the possible orientation of the substrate (**4**) and the hydroborating reagents in the transition state.[7-9] The C-25(26) methylene moiety is located at the end of the aliphatic side chain and is removed from the chiral centers of **4**. We therefore considered it likely that the hydroboration of the C-25(26) double bond will be minimally influenced by the chiral centers of **4**. In essence, we thought that the reaction will proceed in a manner similar to that of a straight-chain aliphatic compound with a terminal methylene[7-9] moiety.

As reference we have synthesized (25*R*)-cholest-5-ene-3β,26-diol (**1a**) from kryptogenin diacetate[12] (**2**), which is known to have the 25*R* stereochemistry.[17] Authentic (25*S*)-cholest-4-en-3-on-26-ol was obtained from the incubation of cholesterol with *M. smegmatis*.[4,16,18] The 25*S* stereochemistry of the microbially obtained product was determined by X-ray crystallography[16a] of its 26-*p*-bromobenzoate (**8g**).

The determination of the C-25 stereochemistry (and eventually optical purity) of the 26-hydroxy specimens obtained via hydroboration of **4** was to be carried out by the circular dichroism (CD) method on the 26-*p*-bromobenzoates[19]. The CD spectra of these derivatives were to be

Table I
Circular Dichroism (CD) Data of Samples of Cholest-4-ene-3β,26-diol 26-*p*-Bromobenzoates[a]

Entry	Compd	Chirality	Estimated optical purity at C-25,[b] %	Origin of sample	CD Δε	CD λ, nm
1	**8h**	25*S*		Microbial transformation	+0.78	244
2	**3c**	25*R*		Kryptogenin	−0.80	244
3	**7c**	25*RS*	0	Hydroboration[c] with (+)-DIPCB		
4	**8f**	25*S*	83	Hydroboration[c] with (−)-DIPCB	+0.64	245
5	**8c**	25*S*	23	Hydroboration[c] with disiamylborane	+0.18	243

[a] The spectra were recorded in methanol–dioxane (9:1) solutions. [b] Calculated on the basis of Δε of **8h** and **3c**, which were assumed to be optically pure (100%). [c] The substrate for hydroboration was the 25(26)-olefin **4**.

compared with that of the (25*S*)-*p*-bromobenzoate **8g** whose 25*S* chirality was rigorously established. At this point we had two options, either to convert the microbially prepared (25*S*)-26-hydroxycholestenone to the (25*S*)-26-hydroxycholesterol analog, or convert the other chemically prepared compounds to the 26-hydroxycholestenones. Because of the scarcity of the microbially prepared material we chose to convert all the products to the 26-hydroxycholestenone analogs.

The transformation of the samples of 26-hydroxycholesterols to 26-hydroxycholestenones was carried out by two procedures. When the 26-hydroxycholesterol was the starting material (**1a, 5b,** and **6f**) the 26-hydroxyl was selectively protected by tritylation.[13] Treatment of **1a** with $(C_6H_5)_3CCl$ and pyridine gave the 26-trityl ether **1c,** which was separated from the accompanying 3,26-dityrityl ether **1b.** The formation of a dityrityl ether involving a primary and secondary hydroxyl was previously noted.[13] Oppenauer oxidation of **1c** and mild acid hydrolysis of the product provided the required **3a.** A similar sequence of transformations was used for the preparation of **7a** (from **5b** via **5c**) and of **8d** (from **6e** via **6f** and **6g**).

In the case of the 26-hydroxy 3-THP ether **6a** the 26-hydroxyl was acetylated and the resulting **6c** was treated with acid to yield the 3-hydroxy 26-acetate **6d.** The obtained **6d** was oxidized by the Oppenauer procedure to give 26-acetoxycholestenone, which was saponified to the required 26-hydroxycholestenone (**8a**).

The four samples of 26-hydroxycholestenone, 25*R* (**3a**), 25*RS* (**7a**), 25*S* (23% optical purity) (**8a**), and 25*S* (83% optical purity) (**8d**), were treated with *p*-bromobenzoyl chloride and pyridine to yield the 26-hydroxycholestenone 26-*p*-bromobenzoates **3b, 7b, 8b,** and **8e,** respectively. Attempts to determine the C-25 stereochemistry of these compounds by CD failed, since all gave essentially identical curves. The dominating effect of the 4-en-3-one chromophore obviously obscured the CD differences emanating from the stereochemical variations at C-25. To obviate this difficulty it was necessary to eliminate the 4-en-3-one chromophore. This was accomplished by reduction of the conjugated ketone to an allylic alcohol. Thus, treatment of the pertinent 26-hydroxycholestenone 26-*p*-bromobenzoates with $LiAlH(t\text{-}BuO)_3$ gave the required Δ^4-3β-ol-26-*p*-bromobenzoates **3c, 7c, 8c,** and **8f.** In a similar manner, reduction of the (25*S*)-26-hydroxycholestenone *p*-bromobenzoate **8g** (derived from the microbial preparation) gave **8h.**

The CD spectra of all samples were recorded in methanol–dioxane (9:1) solutions. Although the observed Cotton effects were not large, they sufficed for assigning the configurations[19] at C-25 (Table I). It is evident that the **8h** obtained microbiologically and **3c** obtained from kryptogenin gave CD Cotton effects of the same magnitude but of the opposite sign (entries 1 and 2). While the **8h** whose 25*S* configuration was determined by X-ray crystallography has a positive CD Cotton effect (Table I, entry 1), the 25*R* analog **3c** derived from kryptogenin has a negative CD Cotton effect (Table I, entry 2).[20] Rather unexpectedly, the derivative **7c** obtained by "asymmetric" hydroboration of **4** with (+)-DIPCB did not show a Cotton effect and proved to be racemic (25*RS*) (Table I, entry 3). In contrast, **8f,** which was derived from hydroboration of **4** with (−)-DIPCB, has a positive CD Cotton effect (Δε +0.64) for the 25*S* isomer. Assuming a 100% optical purity for **8h,** it follows that the optical purity of **8f** is ca. 83%. Finally, hydroboration of **4** with the achiral disiamylborane gave the 25*S* isomer **8c** which had an optical purity of ca. 23%.

The C-25 stereochemistry of the hydroboration products was unexpected and difficult to rationalize. However, the results become accountable if a "25*S*-inducing effect" is assigned to the steroidal moiety. The hydroboration of **4** with the achiral disiamylborane gave the 25*S* product (23% optical purity) due to the "25*S*" influence of the steroidal nucleus. The hydroboration of **4** with the (−)-DIPCB was expected to yield the 25*S* isomer and this was seemingly potentiated by the "inducing effect" of the steroidal molecule; hence **8f** shows a fairly high "25*S*" character (ca. 83% optical purity). In contrast, the (+)-DIPCB was expected to yield the 25*R* isomer, but this was counteracted by the influence of the steroidal moiety and resulted in the racemic (25*RS*) **7c.** Presumably the side chain of **4** is coiled during hydroboration so that the steroidal skeleton exerts a chiral (25*S*) influence.

In the initial stages of the study it was considered likely that **6a,** obtained via hydroboration of **4** with disiamylborane, is racemic.[6a,b] Attempts to resolve the 26-alcohol 3-THP ether **6a** with different reagents were not successful. Consequently we decided to convert the alcohol to a 25-carboxylic acid and resolve the acid.

We have previously proven[6a] that the transformation of [25-^{3}H]cholest-5-ene-3β,26-diol (**5f**) via [25-^{3}H]-5α-cholestane-3β,26-diol (**12**) to [25-^{3}H]-5α-cholestan-3-on-26-oic acid (**11a**) proceeds without epimerization at C-25 as evidenced by the retention of all the tritium in the acid (**11a**). We repeated the sequence of reactions except that the oxidation was carried out with the CrO_3–pyridine–water reagent.[21] Again no loss of tritium was observed. Since one of the steps in the projected preparation of the 26-acids involved saponification, we tested also the influence of common bases in protic solvents on the chirality at C-25. Under the conditions of equilibration tested (see Experimental Section) **11a** and **11b** retained all the tritium, indicating that epimerization at C-25 did not occur. Consequently, **6a** was oxidized under the same conditions (CrO_3–pyridine–water), to yield the acid **9a,** which was hydrolyzed to **9b.** The obtained acid **9b** was treated with (−)-quinine and the resulting salt was crystallized from methanol. The acid **9c** ($[\alpha]^{22}D$ −27.2°, −25.3°) was recovered from the crystalline quinine salt. The acid **9d** ($[\alpha]^{22}D$ −19.1°) was obtained from the mother liquor of crystallization of the quinine salt. It seems therefore that at least a partial resolution of

the acids was achieved by the procedure employed. The acids **9c** and **9d** were reduced ($LiAlH_4$) to the 26-alcohols **5d** and **5e**, respectively.

The authentic 25*R* acid **10b** was prepared from **1a** derived from kryptogenin. The diol **1a** was converted to the 26-trityl ether **1c** and acetylated to **1d**. Acid hydrolysis provided the 3-acetoxy-26-hydroxy **1f** which was oxidized to **10a** and saponified to **10b**. To test the optical purity of **10b** attempts were made to resolve it via the quinine salt as described above. However, both the acid **10c** recovered from the crystalline salt and the acid **10d** recovered from the mother liquor had the same $[\alpha]^{24}D$ −33.6°. No further attempts were made to determine the configurations of the acids **9c** and **9d** and the configurations of the derived alcohols **5d** and **5e**, respectively.

The identity of the $[\alpha]D$ of **10c** and **10d** tends to indicate their near 100% "optical purity" and consequently the near "optical purity" of **1a** and of the kryptogenin diacetate **2**.

Registry No.—**1a**, 20380-11-4; **1c**, 56792-57-5; **1f**, 56845-81-9; **2**, 56792-58-6; **3a**, 56792-59-7; **3c**, 56792-60-0; **4**, 24583-89-9; **5a**, 24583-90-2; **5b**, 13095-61-9; **6a**, 56845-82-0; **6b**, 56845-83-1; **6d**, 56906-69-5; **7a**, 19257-21-7; **7c**, 56845-84-2; **8a**, 41530-25-0; **8b**, 41530-31-8; **8c**, 56792-61-1; **9a**, 56792-62-2; **9b**, 56845-85-3; **9c**, 6561-58-6; **9e**, 56845-86-4; **10a**, 56792-63-3; **10b**, 56845-87-5; **11a**, 24583-91-3; **12**, 54575-47-8; triphenylchloromethane, 76-83-5; [25-^{3}H]cholest-5-ene-3β,26-diol 3-tetrahydropyranyl ether, 56792-64-4.

References and Notes

(1) (a) Worcester Foundation for Experimental Biology. (b) Postdoctoral Fellow, 1966–1969. (c) Postdoctoral Fellow, 1970–1972. (d) The work at the Worcester Foundation was supported by Grants AM 12156 and GM 19882 from the National Institutes of Health.

(2) (a) Columbia Univeristy. (b) The work at Columbia University was supported by Grant AI 10187 from the National Institutes of Health.

(3) J. B. Greig, K. R. Varma, and E. Caspi, *J. Am. Chem. Soc.*, **93**, 760 (1971).

(4) M. Galli Kienle, K. R. Varma, L. J. Mulheirn, B. Yagen, and E. Caspi, *J. Am. Chem. Soc.*, **95**, 1996 (1973).

(5) B. Yagen, J. S. O'Grodnick, E. Caspi, and C. Tamm, *J. Chem. Soc., Perkin Trans. 1*, 1994 (1974).

(6) (a) K. R. Varma, J. A. F. Wickramasinghe, and E. Caspi, *J. Biol. Chem.*, **244**, 3951 (1969). (b) The results reported in the present paper indicate that the 26-hydroxycholesterol described in the above reference is not 25*RS* as initially thought, but contains ca. 23% excess of the 25*S* isomer.

(7) E. Caspi and K. R. Varma, *J. Org. Chem.*, **33**, 2181 (1968).

(8) K. R. Varma and E. Caspi, *J. Org. Chem.*, **34**, 2489 (1969).

(9) K. R. Varma and E. Caspi, *Tetrahedron*, **24**, 6365 (1968).

(10) A. Sandoval, J. Romo, G. Rosenkranz, S. Kaufman, and C. Djerassi, *J. Am. Chem. Soc.*, **73**, 3820 (1951).

(11) C. Djerassi and R. Ehrlich, *J. Am. Chem. Soc.*, **78**, 440 (1956).

(12) I. Scheer, M. J. Thompson, and E. Mosettig, *J. Am. Chem. Soc.*, **78**, 4733 (1956).

(13) J. Wicha and E. Caspi, *Can. J. Chem.*, **45**, 707 (1967).

(14) P. D. G. Dean and M. W. Whitehouse, *Biochem. J.*, **98**, 412 (1966).

(15) J. Kucera and F. Sorm, *Collect. Czech. Chem. Commun.*, **23**, 116 (1958).

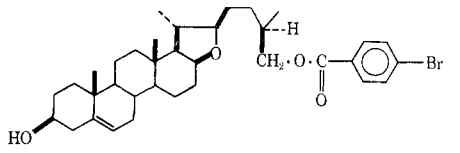
(16) D. J. Duchamp, C. G. Chidester, J. A. F. Wickramasinghe, E. Caspi, and B. Yagen, *J. Am. Chem. Soc.*, **93**, 6283 (1971).

(17) For pertinent references see L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, N.Y., 1959, pp 837–840.

(18) K. Schubert, G. Kaufman, and C. Hörhold, *Biochem. Biophys. Acta*, **176**, 163, 170 (1969).

(19) N. Harada and K. Nakanishi, *Acc. Chem. Res.*, **5**, 257 (1972); S. L. Chen, N. Harada, and K. Nakanishi, *J. Am. Chem. Soc.*, **96**, 7352 (1974), and references cited therein.

(20) A word of caution is in order. The presence of other chiral centers in the side chain will influence significantly the CD spectra of 26-*p*-bromobenzoates as exemplified by the CD of the dihydrodiosgenin derivative which showed $\Delta\epsilon_{245}$ −1.74 (MeOH).



(21) R. H. Cornforth, J. W. Cornforth, and G. Popjak, *Tetrahedron*, **18**, 1351 (1962).

Diterpenoid Total Synthesis, an A → B → C Approach. VII. Total Synthesis of DL-Sugiol, DL-Ferruginol, and DL-Nimbiol[1]

Walter L. Meyer,* George B. Clemans, and Richard A. Manning

Department of Chemistry, University of Arkansas, Fayetteville, Arkansas 72701

Received June 6, 1975

Cyanoethylation of 2,2,6-trimethylcyclohexanone followed by saponification affords 3-(1′,3′,3′-trimethyl-2′-ketocyclohexyl)propionic acid (**5**), the carboxyl of which is converted to acetyl (**9**) either by treatment of **5** with methyllithium or by sequential exposure to oxalyl chloride, diazomethane, hydrogen chloride, and zinc–acetic acid. Reaction of **5** with thionyl chloride affords chloro lactone **10** rather than the normal acid chloride. Pyrrolidine-catalyzed cyclodehydration of diketone **9** produces 4,4,10-trimethyl-Δ^5-7-octalone, which undergoes hydrogenation to a 4:1 mixture of the corresponding trans and cis decalones (**12** and **13**). Decalone **12** is also available from 10-cyano-4,4-dimethyl-*trans*-7-decalone by the sequence ketalization, lithium aluminum hydride reduction of cyano to imino, Huang-Minlon reduction of imino to methyl, and ketal hydrolysis, or from 10-carbethoxy-4,4-dimethyl-*trans*-7-decalone by the sequence ketalization, lithium aluminum hydride reduction, Sarett oxidation to the angular aldehyde, Huang-Minlon reduction, and ketal hydrolysis. 4,4,10-Trimethyl-*trans*-decalin was prepared by reduction of **12**. Condensation of **12** with ethyl formate affords exclusively the 8-hydroxymethylene derivative, which is dehydrogenated by 2,3-dichloro-5,6-dicyanoquinone to form the $\Delta^{8,9}$-unsaturated keto aldehyde **23**. Michael addition of the sodium enolate of *tert*-butyl isovalerylacetate or *tert*-butyl propionylacetate produces adducts **24**, which in the presence of *p*-toluenesulfonic acid in acetic acid undergo *tert*-butyl ester cleavage, decarboxylation, and cyclodehydration, thereby forming the tricyclic enediones **25a** and **25b**, respectively. On the basis of ^1H NMR data these are tentatively assigned the trans-syn-cis configuration, and the adducts **24** are formulated with a 9α side chain. Exposure of the enediones to pyridine hydrobromide perbromide in acetic acid brings about aromatization of ring C to form DL-sugiol and DL-nimbiol, respectively. Hydrogenolysis of the former affords DL-ferruginol.

Earlier we described a general scheme of synthesis which was planned to allow stereoselective construction of a variety of polycyclic members of the diterpenoid family of natural products.[1b] In essence this involves preparation of a 4,4,10-trisubstituted *trans*-7-decalone (**1**)[2] which is to become the A/B ring system of the terpenoid, followed by attachment of a C ring which carries the appropriate carbon substituents and functional groups. In this paper we de-