1545 (broad NO_2, asymmetrical stretching), 1340–1310 (broad NO_2, symmetrical stretching), 1080, 1060 sh (C–O, C–O–C).

Anal. Calcd for $C_{16}H_{14}N_8O_{10}$: C, 38.63; H, 3.03; N, 24.03. Found: C, 38.42; H, 3.21; N, 23.84.

9-(2,3-*O*-Isopropylidene-L-erythrofuranosyl)adenine Picrate (7).—To 2.74 g of **2** in 10 ml of hot ethanol was added 20 ml of 10% ethanolic picric acid, the solution was refluxed for 5 min and chilled.[15] A yellow precipitate (2.28 g) was obtained and recrystallized from boiling methanol to yield 1.26 g of product, mp 230–232° dec, with sublimation beginning near 180°. The infrared spectrum had the following peaks: ν_{max}^{KBr} (cm^{-1}) 3400 (OH, NH), 1690 ($NH_2C{=}N$), 1638, 1610 (phenyl and purine ring), 1570, 1545 (NO_2, asymmetrical stretching), 1362 (*gem*-dimethyl), 1325 (NO_2, symmetrical stretching), 1100–1080, 1055 (C–O, C–O–C).

Anal. Calcd for $C_{18}H_{18}N_8O_{10}$: C, 42.69; H, 3.58; N, 22.13. Found: C, 42.60; H, 3.57; N, 22.44.

9-(2,3-*O*-Isopropylidene-L-erythrofuranosyl)adenine (3).—All attempts to crystallize the regenerated[15] isopropylidene nucleoside from common solvents failed. The anomers were not separated by chromatography on neutral alumina or Dowex 1 (OH) resin, which gave only one peak. Ultraviolet and infrared spectra are as follows: λ_{max}^{EtOH} 258 mμ; ν_{max}^{film} (cm^{-1}) 3300, 3140 (doublet, NH), 1655 sh, 1640, 1600, 1580 ($NH_2C{=}N$, purine ring), 1372 (*gem*-dimethyl), 1110–1090, 1050 (C–O, C–O–C).

(15) J. R. Parikh, M. E. Wolff, and A. Burger, *J. Amer. Chem. Soc.*, **79**, 2778 (1957); M. L. Wolfrom, A. B. Foster, P. McWain, W. von Bebenburg, and A. Thompson, *J. Org. Chem.*, **26**, 3095 (1961).

9-β-D-Xylopyranosyladenine (10) was prepared by condensation of tetra-*O*-acetyl-D-xylopyranosyl bromide with 6-benzamidochloromercuripurine by the method of Davoll and Lowy:[16] mp 302–305° dec; $[\alpha]^{23}D$ −22.5° (*c* 0.62, 1 *N* HCl); lit.[9a] 290° dec; $[\alpha]^{14}D$ −24 ± 4° (*c* 0.17, H_2O). A melting point of 298° has also been reported.[9b]

Polarimetric Studies.—To 14.6 mg of **10** in a 2-ml volumetric flask was added 1.5 ml of 0.08 *M* sodium periodate. The sample was dissolved by heating for several minutes in a steam bath; the flask was stored in the dark for 24 hr. The volume was adjusted to 2 ml with water and the optical rotation obtained—$[\alpha]^{23}D$ +34° based upon the concentration of dialdehyde **11**.

Similarly, 13.9 mg of **5** was treated with periodate and after 24 hr 0.12 ml of 0.503 *M* formic acid was added to adjust for the presence of liberated formic acid in the above experiment. The volume was adjusted to 2 ml and the optical rotation obtained—$[\alpha]^{23}D$ −33° based upon **9**.

Registry No.—3β, 18031-26-0; 3α, 18031-43-1; 4β picrate, 18031-27-1; 4α picrate, 18031-22-6; **5**, 17019-48-6; **6**, 14266-04-7; 7β picrate, 18031-21-5; 7α picrate, 18031-25-9.

Acknowledgment.—The author is deeply grateful to Dr. Paul Kohn for his interest and encouragement with regard to this work.

(16) J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, **73**, 1650 (1951).

Reassignment of Configuration to the 22-Hydroxycholesterols. Synthesis of (22*S*)- and (22*R*)-^{3}H-Cholesterols[1]

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Stereospecifically labeled (22*S*)- and (22*R*)-^{3}H-cholesterols were synthesized *via* (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl 3-benzoates, respectively. The (22*S*)- and (22*R*)-hydroxycholesterols, the (22*S*)- and (22*R*)-hydroxycholesteryl 3-benzoates and the (22*S*)- and (22*R*)-hydroxycholesteryl 3-methyl ethers were interrelated. Assignments of configuration at C-22 were made on the basis of the Horeau and Prelog procedures; the two methods lead to identical assignments. Our results demonstrate that the configurations assigned previously to the 22-hydroxycholesterols and their derivatives are incorrect and should be reversed.

For our continuing studies of the mechanisms of biosynthesis of sterols in plants and animals, samples of cholesterols labeled stereospecifically with a tritium atom at C-22 were required. The synthesis and configurational assignments of the (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl 3-benzoates and of the (22*S*)- and (22*R*)-^{3}H-cholesterols derived therefrom is the subject of this paper.

The requisite starting material, 22-ketocholesteryl acetate (**1**, Scheme I), was prepared from 3β-acetoxy-23,24-bisnor-5-cholenic acid according to the published procedure.[2] Because the 3-acetoxy group is known not to survive borohydride treatment,[3] it was necessary to replace it by a benzoate prior to introduction of a tritium atom at C-22 by reduction with sodium borotritide. Hydrolysis of the acetate with sodium carbonate was reported to give 22-ketocholesterol without epimerization[2] at C-20, and we have confirmed this by comparison of the nmr spectrum of this product with that of material obtained by potassium hydroxide treatment (see Experimental Section).

In a cold run, 22-ketocholesteryl benzoate on treatment with a large excess (greater than tenfold) of sodium borohydride gave a high yield of (22*R*)- and (22*S*)-hydroxycholesteryl benzoates (**2a** and **2b**) in a ratio of 1:3. The alcohols were separated by column chromatography followed by repeated thin layer chromatography (tlc) on alumina. The minor alcohol (**2a**, eluted first from the column) was assigned the (*R*) configuration and the major alcohol (**2b**, eluted second) the (*S*) configuration by the Horeau method[4] (Table I).

TABLE I

ASSIGNMENT OF CONFIGURATION TO 22-HYDROXYCHOLESTERYL 3-BENZOATES AND 3-METHYL ETHERS BY THE α-PHENYLBUTYRIC ACID METHOD

Alcohol	Mp, °C	Wt of alcohol, mg	α-Phenylbutyric acid $[\alpha]_{D_{obsd}}$, deg	α-Phenylbutyric acid $[\alpha]_D$, deg	Optical yield,[a] %	Configuration of alcohol
2a	190	202.2	+0.87	+3.55	15	(22*R*)
2b	162	203.0	−3.28	−13.2	55	(22*S*)
5a	161.5–163.5	164.7	+0.71	+2.81	59	(22*R*)
5a	90–92	167.9	−3.49	−14.2	12	(22*S*)

[a] Calculated as in W. Herz and H. B. Kagan, *J. Org. Chem.*, **32**, 216 (1967).

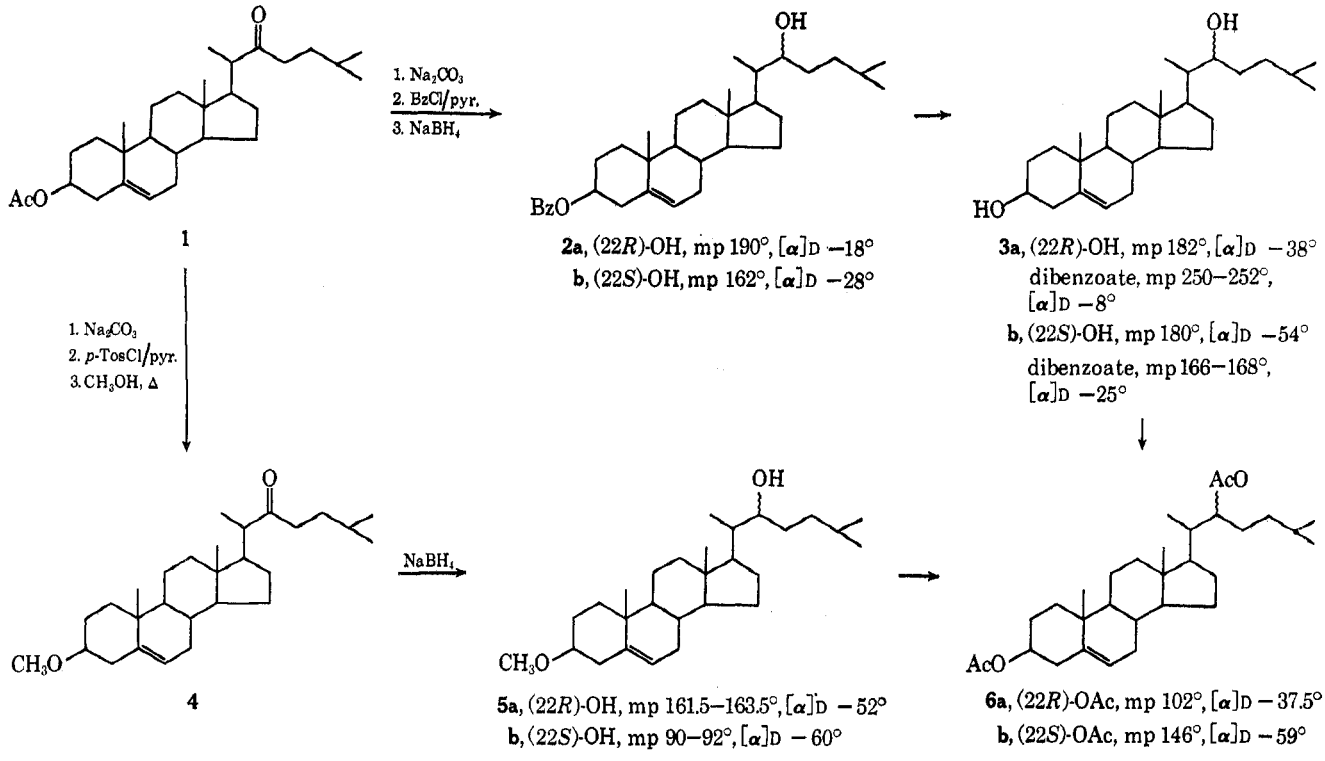
(1) The work was supported by Grants GB 5832 from the National Science Foundation, American Cancer Society P-500H and K3 16614 from the National Cancer Institute.

(2) W. Cole and P. L. Julian, *J. Amer. Chem. Soc.*, **67**, 1369 (1945).

(3) K. Tsuda and R. Hayatsu, *ibid.*, **81**, 5987 (1959).

(4) A. Horeau, *Tetrahedron Lett.*, 506 (1961); 965 (1962); A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).

SCHEME I



To substantiate further the configurational assignments the hydroxy benzoates were each reduced to the respective diols (**3a**, mp 182°, and **3b**, mp 180°) which were characterized by diacetates **6a** and **6b** and dibenzoates as described previouslyl.[3] Surprisingly we found that the major hydroxy benzoate (**2b**) to which we had assigned the (22*S*) configuration gave the diacetate with mp 146° and the dibenzoate with mp 169°. The earlier workers (Scheme II) claimed that the diacetate with mp 146° was obtained from a 22-hydroxycholesteryl 3-methyl ether assigned the (22*R*) configuration[5] by the Prelog method.[6] In addition the minor hydroxy benzoate [**2a**, (22*R*) configuration by the Horeau procedure] gave the diacetate with mp 102° and the dibenzoate with mp 254°. Tsuda and Hayatsu[3] stated that the diacetate with mp 102° was obtained from a (22*S*)-hydroxycholesteryl 3-methyl ether. In view of these discrepancies it became of importance to determine whether the Horeau and Prelog methods do lead to opposite configurational assignments, and to establish unambiguously the correct configurations.

We undertook the preparation and separation of the 22-hydroxycholesteryl methyl ethers described previously,[3] and determined their configurations by the Prelog and Horeau procedures. Subsequently we related these compounds to the diacetates obtained from the hydroxy benzoates. The results are summarized in Scheme I. Separation of the hydroxy methyl ethers (obtained in a nearly 1:1 mixture from sodium borohydride reduction) proved far more difficult than that of the hydroxy benzoates. By a lengthy process of column chromatography on silica gel followed by multiple successive tlc plate separations and finally several recrystallizations, pure specimens (each consisting of approximately 10% of the initial mixture) were obtained. The alcohol eluted first from the column (**5b**, mp 90–92°) was subjected to the Horeau procedure (Table I) as well as to the Prelog procedure (Table II) and in both cases the (*S*) configuration was indicated. This alcohol (**5b**) on treatment with acetic anhydride–*p*-toluenesulfonic acid gave diacetate **6b** (mp 146°), previously obtained from the (22*S*)-hydroxy benzoate **2b**. An analogous series of reactions with the alcohol eluted second (**5a**, mp 161.5–163.5°) gave similarly self-consistent results establishing the (*R*) configuration. Thus it is evident that the two methods do lead to identical assignments and that the previous assignment of configuration to the 22-hydroxycholesterols is incorrect and must be reversed. A comparison of the physical constants of the synthetic 3,22-diols and their derivatives with those reported for the naturally occurring product[7] conclusively establishes the (22*R*) configuration for the latter. A comparison of melting points of the 22-hydroxycholesteryl methyl ethers and their phenyl glyoxylates (Table II) reported previously with those determined by us suggests that the earlier investigators had not obtained pure specimens. This fact could reasonably account for the erroneous configurational assignments.

Reduction of 22-ketocholesteryl benzoate with a slight excess of sodium borotritide gave the 22-hydroxy-22-^{3}H-cholesteryl benzoates in a radioactive yield of *ca.* 5% (*ca.* 80% of the ketone was recovered unchanged). The ratio of (22*R*)-hydroxy-22-^{3}H to (22*S*)-hydroxy-22-^{3}H was 1:10, and the specific activity

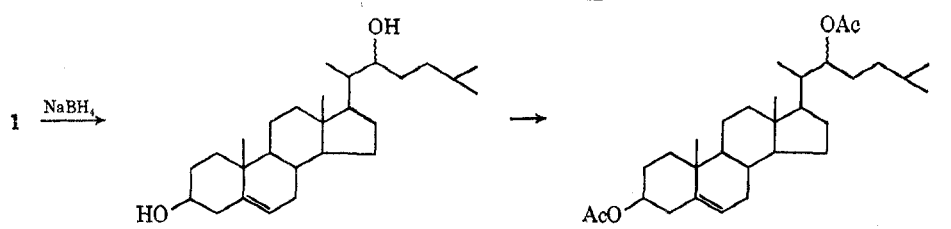
(5) The Japanese workers used Fieser's convention for definition of stereochemistry in the side chain: (a) L. F. Fieser and M. Fieser, *Tetrahedron*, **8**, 360 (1960); (b) "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp 337–340. A clearer presentation of the convention is given in ref 5a; see particularly the drawing of the models at the right bottom side on p 361. For simplicity we shall use the (*R*),(*S*) system in our discussion throughout. The Fieser designation 22α (OH) is equivalent to 22(*S*), and 22β (OH) is equivalent to 22(*R*).

(6) V. Prelog, *Helv. Chim. Acta*, **36**, 308 (1953).

(7) A. Stabursvik, *Acta Chem. Scand.*, **7**, 1220 (1953); ref 5, pp 343–344.

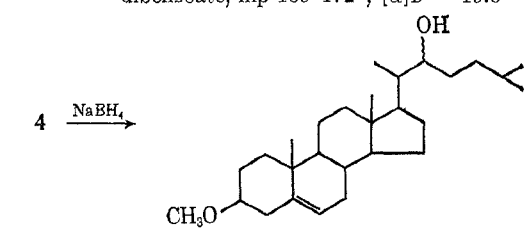
SCHEME II

PHYSICAL DATA AND PREVIOUS ASSIGNMENT[8] OF CONFIGURATION AT C-22



7a, (22*S*)-OH, mp 184–185°, $[\alpha]_D$ − 39.0°
dibenzoate, mp 254–256°, $[\alpha]_D$ − 9.6°
b, (22*R*)-OH, mp 181–182°, $[\alpha]_D$ − 52.0°
dibenzoate, mp 169–172°, $[\alpha]_D$ − 19.6°

8a, (22*S*)-OAc, mp 102°, $[\alpha]_D$ − 37.1°
b, (22*R*)-OAc, mp 146°, $[\alpha]_D$ − 51.5°



9a, (22*S*)-OH, mp 109–111°, $[\alpha]_D$ − 45.3° ⟶ **8a**, (22*S*)-OAc, mp 102°
b, (22*R*)-OH, mp 78–80°, $[\alpha]_D$ −60.1° ⟶ **8b**, (22*R*)-OAc, mp 146°

of the major isomer was more than twice that of the minor isomer. In terms of radioactivity actually isolated after repetitive separations on alumina, 200 mCi of sodium borotritide yielded 6.2 mCi of pure (22*S*)-hydroxy-22-^{3}H-cholesteryl benzoate and 0.25 mCi of pure (22*R*)-hydroxy-22-^{3}H-cholesteryl benzoate. Treatment of the methanesulfonates of (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl benzoates with lithium aluminum hydride yielded, respectively, (22*S*)-^{3}H-cholesterol and (22*R*)-^{3}H-cholesterol. Assignment of configuration to the two specimens of cholesterol is based on the fact that hydrogenolysis of sulfonate esters of secondary alcohols with lithium aluminum hydride is known to proceed largely with inversion.[8] In each case the tritiated cholesterol was separated by tlc from the olefin and diol formed by competing elimination and oxygen–sulfur cleavage reactions. The purity of the resulting (22*S*)-^{3}H- and (22*R*)-^{3}H-cholesterols (1.9 mCi and 94 μCi, respectively) was demonstrated by tlc on silver nitrate–silica gel and by co-crystallization.

Jones oxidations of (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl benzoates followed by crystallization of the ketones showed that *ca.* 99.6% of the tritium in each case was at C-22.

Experimental Section[9]

22-Ketocholesterol.—22-Ketocholesteryl acetate (1) was synthesized according to the published procedure[2] except that steam distillation was not found necessary. Hydrolysis was carried out essentially as described previously. A mixture of 3.5 g of sodium carbonate, 150 ml of methanol and 5 ml of water was used for 5.3 g of the acetate. The 22-ketocholesterol so obtained had mp 139–140.5° (lit.[2] mp 142–143°) and its nmr spectrum (deuteriochloroform) showed methyl signals at τ 9.29 (s, C-18), 9.10 (d, J = 4 cps, C-26 and C-27), 8.98 (s, C-19) and 8.90 (d, J = 7 cps, C-21).[10] The mixture of C-20 epimers obtained by potassium hydroxide treatment[2] of 22-ketocholesteryl acetate showed additional methyl signals at τ 9.33, 9.02, and 8.93.

22-Ketocholesteryl Benzoate.—A mixture of 22-ketocholesterol (2.00 g), benzoyl chloride (1.2 ml) and pyridine (1 ml) was kept at 65° for 1.5 hr and then allowed to stand at room temperature overnight. The crystalline mass was suspended in 30 ml of water, filtered, resuspended in 20 ml of warm water and filtered again. The benzoate so obtained was homogenous to tlc (silica gel). Its mass spectrum showed m/e 382 ($M^+ - C_6H_5CO_2H$) as the most prominent peak; no molecular ion was found. The analytical sample, mp 179–180°, $[\alpha]_D$ −35°, was recrystallized from ethyl acetate.

Anal. Calcd for $C_{34}H_{48}O_3$: C, 80.90; H, 9.59. Found: C, 81.40; H, 9.24.

(22*R*)- and (22*S*)-Hydroxycholesteryl Benzoates (2a and 2b).—To a stirred solution of the ketobenzoate (1.70 g) in a mixture of tetrahydrofuran (25 ml) and methanol (12.5 ml) was added in portions 0.50 g of sodium borohydride. The mixture was stirred 3 hr at room temperature and allowed to stand 24 hr longer. It was then evaporated to near dryness and shaken with water (50 ml) and ether (100 ml). The layers were separated and the water layer was extracted with ether (25 ml). The combined ether solutions were washed twice with sodium chloride solution, dried and evaporated. From the residue (1.71 g, >95% hydroxy benzoates in a ratio 1:3 as determined by tlc) were obtained, by column chromatography on alumina (benzene, then benzene–ether mixtures) followed by repeated separations on alumina plates (benzene), **2a** (238 mg, mp 186–187°, $[\alpha]_D$ −18°, eluted first) and **2b** (529 mg, $[\alpha]_D$ −28°, mp 162°). Mass spectra of the two isomers did not differ significantly and each displayed m/e 384 ($M^+ - C_6H_5CO_2H$) as the most prominent peak. No molecular ion peak was found in either case. Analytical samples, mp 190 and 162°, were recrystallized from ethyl acetate.

Anal. Calcd for $C_{34}H_{50}O_3$: C, 80.58; H, 9.95. Found (**2a**): C, 80.01; H, 9.58. Found (**2b**): C, 80.35; H, 10.03.

22-Ketocholesteryl Methyl Ether (4).—A solution of 22-ketocholesterol (7.1 g) and *p*-toluenesulfonyl chloride (3.7 g) in pyridine (70 ml) was stirred at room temperature for 3 days. Most of the pyridine was evaporated and the residue was stirred with water for 1 hr. The crude tosylate was filtered, dried *in vacuo* overnight, and then refluxed for 20 hr in dry methanol (120 ml). The crude methyl ether (7.1 g) showed only trace amounts of 22-ketocholesterol by tlc (silica gel) and was used without purification for the borohydride reduction. A sample collected by tlc and recrystallized from methanol had mp 82–84° (lit.[11] mp 84–85°).

(8) G. K. Helkamp and B. F. Rickborn, *J. Org. Chem.*, **22**, 479 (1957).

(9) Melting points were taken on a hot stage and are corrected. Optical rotations were determined as 1–2% solutions in chloroform unless otherwise stated. Silica gel and alumina used for tlc were both Merck HF_{254}. Silica gel plates were developed in 9:1 benzene–ethyl acetate unless noted otherwise. Microanalyses were performed by Ilse Beetz, Kronach, West Germany. Tritiated samples were counted on a Packard Tri-Carb, Model 314-DC, liquid scintillation spectrometer as solutions in toluene containing 0.4% 2,5-diphenyloxazole and 0.01% *p*-bis-2(5-phenyloxazolyl)benzene. Mass spectra were determined using a Varian Associates M-66 instrument.

(10) For the basis of these assignments see A. Mijares, D. I. Cargill, J. A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).

(11) A. Romeo and R. Villotti, *Ann. Chim.* (Rome), **47**, 618 (1957).

TABLE II

ASSIGNMENT OF CONFIGURATION TO 22-HYDROXYCHOLESTERYL 3-METHYL ETHERS BY THE ATROLACTIC ACID METHOD

Compd (amt)	Mp, °C	Phenyl glyoxylate[a] Amt	Phenyl glyoxylate[a] Mp, °C	Atrolactic acid $[\alpha]_{D obsd}$, deg	Atrolactic acid $[\alpha]_D$, deg	Atrolactic acid Amt	Optical yield,[b] %	Configuration of alcohol
5a (552 mg)	161.5–163.5	576 mg (79%)	141–144	−0.20	−1.67	150 mg (86%)	4.4	(22*R*)
5b (747 mg)	90–92	856 mg (87%)	161–166	+2.35	+12.2	192 mg (76%)	32	(22*S*)

[a] The two phenyl glyoxylates were previously reported as "mp 68–70°" and "oil," respectively.[3] [b] Based on specific rotation of 37.7° or optically pure atrolactic acid in ethanol.

(22*R*)- and (22*S*)-Hydroxycholesteryl Methyl Ethers (5a and 5b).—To a stirred suspension of **4** (6.8 g) in methanol (75 ml) was added in portions 2.5 g of sodium borohydride. The methyl ether gradually dissolved and the product later precipitated. The mixture was allowed to stand 2.5 days, then water was added and the products (6.75 g, *ca.* 90% alcohols and 5–10% unchanged ketone as estimated by tlc) were extracted with ether as described for the previous borohydride reduction. Partial separation of the alcohols was achieved on a silicic acid column (benzene, then 0.25% chloroform in benzene) and enriched mixtures were further purified by preparative tlc.[12] Mixtures enriched to 90–95% of either could be freed from the other by several recrystallizations from methanol. In this way pure **5b** (800 mg, mp 90–92°, $[\alpha]_D$ −60°, eluted first from the column) and **5a** (552 mg, $[\alpha]_D$ −52°, mp 161.5–163.5°) were obtained. In contrast to the hydroxy benzoates, the two most prominent peaks in the mass spectrum of each methyl ether were *m/e* 416 (M^+) and 384 ($M^+ - CH_3OH$).

Atrolactic Acid from 5a and 5b.—The Prelog procedure[6] was carried out essentially as outlined previously[3] except that progress at each stage was monitored by tlc. The phenyl glyoxylates were crystalline single-spot materials as were the sterols recovered from alkaline hydrolysis of the Grignard products. The sterols were recrystallized to constant melting point before use in subsequent experiments. The results are summarized in Table II. Optical rotations were measured in ethanol.

α-Phenylbutyric Acid from 2a, 2b, 5a and 5b.—The modified procedure of Herz and Kagan[13] was followed using 0.4 mmol of each steroidal alcohol and 2.5 mmol of α-phenylbutyric anhydride in each case. Results are summarized in Table I. Optical rotations were measured in benzene.

(22*R*)- and (22*S*)-Hydroxycholesterols (3a and 3b).—α-Phenyl butyrates of **2a** and **2b**, homogenous to tlc, were recovered from the Horeau experiments and were reduced with excess lithium aluminum hydride in ether. The resulting sterols were recrystallized from ether and from methanol. Diol **3a** had mp 182°, $[\alpha]_D$ −38°; **3b** had mp 180°, $[\alpha]_D$ −54°. Portions of the infrared spectra of **3a** and **3b** which show characteristic differences are reproduced in Figures 1 and 2, respectively.

22-Hydroxycholesteryl Diacetates (6a and 6b) from 2a and 2b.—Benzoates **2a** and **2b** (20 and 50 mg, respectively) were each reduced with lithium aluminum hydride in ether, and pyridine solutions of the resulting diols were treated overnight with acetic anhydride. The diacetates, recrystallized from methanol, had mp 101.5–103°, $[\alpha]_D$ −37.5°, and 145–146°, $[\alpha]_D$ −59°, respectively.

22-Hydroxycholesteryl Diacetates (6a and 6b) from 5a and 5b.—Methyl ethers **5a** and **5b** (10 and 50 mg, respectively) were each heated at 100° with acetic anhydride and *p*-toluenesulfonic acid according to the published procedure.[3] The resulting diacetates, recrystallized from methanol, had mp 98–100 and 146–146.5°, respectively, and mixture melting points with the diacetates from the previous experiment were not depressed.

(22*S*)-Hydroxy-22-^{3}H- and (22*R*)-Hydroxy-22-^{3}H-cholesteryl Benzoates.—To a stirred solution of 22-ketocholesteryl benzoate (1.53 g) in tetrahydrofuran (20 ml) was added a solution of sodium borotritide[14] (40.0 mg, specific activity 190 mCi/mmol) in methanol (8 ml). The mixture was allowed to stand 90 hr at room temperature. Acetone (8 drops) was then added and the mixture was stirred 0.5 hr. The solvents were removed under a stream of nitrogen and the residue was stirred with water (30 ml) and ether (100 ml) until all solids had dissolved. A work-up procedure and chromatographic separations similar to those described in the cold run followed. Column chromatography

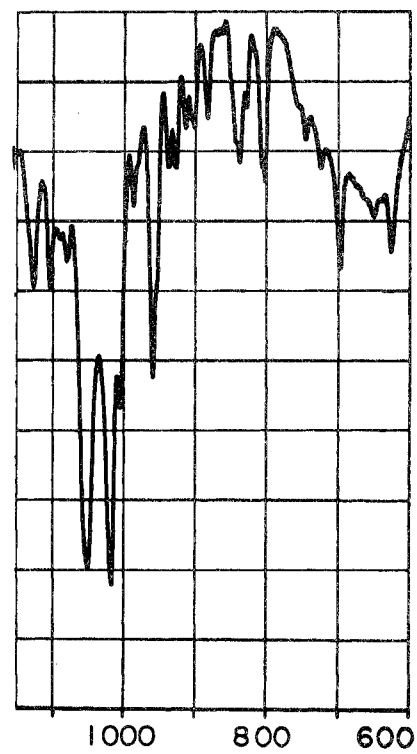


Figure 1.—Infrared spectrum of **3a**.

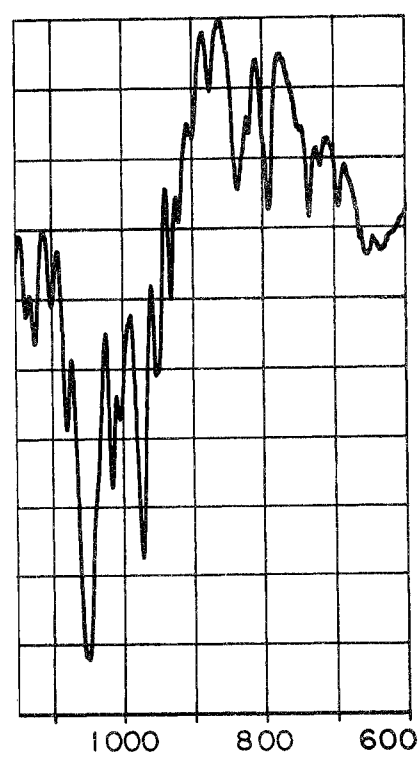


Figure 2.—Infrared spectrum of **3b**.

(12) Extent of purity was readily demonstrable by tlc but for preparative purposes the plates were easily overloaded.

(13) See Table I, footnote *a*.

(14) New England Nuclear Corp., Boston, Mass.

served to separate the mixture of alcohols (9.2 mCi) from unchanged ketone (1.17 g, 77%). Actually isolated after repetitive tlc were (22*R*)-hydroxy-22-^{3}H-cholesteryl benzoate (11.3 mg, 0.24 mCi) and (22*S*)-hydroxy-22-^{3}H-cholesteryl benzoate (110 mg, 6.2 mCi), each shown to be radiochemically pure and free from the other.

Jones Oxidation of (22*S*)-Hydroxy-22-^{3}H-cholesteryl Benzoate.—An aliquot was cocrystallized with 45 mg of cold material (specific activity of first crop, 4.03×10^8 dpm/mmol; first crop recrystallized, 4.06×10^8 dpm/mmol). The alcohol (40 mg) was dissolved in acetone (5 ml) and was stirred at 15–20° for 25 min with 3 drops of Jones reagent. The resulting ketone was crystallized from ethyl acetate to constant specific activity (first crop, 1.74×10^6 dpm/mmol; first crop recrystallized, 1.16×10^6 dpm/mmol; first crop recrystallized twice, 1.20×10^6 dpm/mmol). Thus >99.6% of the tritium was at C-22 in the alcohol.

Jones Oxidation of (22*R*)-Hydroxy-22-^{3}H-cholesteryl Benzoate.—An aliquot was cocrystallized with 23 mg of cold material (specific activity of first crop, 5.33×10^6 dpm/mmol; first crop recrystallized, 5.36×10^6 dpm/mmol). Jones oxidation (20 mg of alcohol, 4 ml of acetone, 1 drop of Jones reagent, 20 min) gave 22-ketocholesteryl benzoate (first crop, 1.5×10^4 dpm/mmol; first crop recrystallized, 1.4×10^4 dpm/mmol). Thus >99.6% of the tritium was at C-22.

(22*S*)-^{3}H-Cholesterol.—To a cooled, stirred solution of (22*S*)-hydroxy-22-^{3}H-cholesteryl benzoate (66 mg, 3.7 mCi) in pyridine (1 ml) was added an excess (5 drops) of methanesulfonyl chloride. The mixture stood 16 hr at room temperature and then was cooled and stirred while water and ether were added. The aqueous layer was extracted with ether and the combined ether extracts were washed successively with 0.5 *N* hydrochloric acid, sodium bicarbonate solution and water, and dried. The methanesulfonate so obtained was stirred with excess lithium aluminum hydride in ether overnight. The mixture was cooled while water was added dropwise followed by sufficient 2 *N* sulfuric acid to dissolve the hydroxides. The ether layer was washed with dilute sodium carbonate solution and twice with saturated sodium chloride. (22*S*)-^{3}H-Cholesterol (1.3 mCi) was separated from the mixture of products by preparative tlc on silica gel. Its purity was demonstrated by tlc on silica gel–silver nitrate (19:1 benzene–methanol, single radioactive peak), and by cocrystallization with cold material which had been purified *via* the dibromide (first crop, 6.68×10^6 dpm/mmol; first crop recrystallized, 6.71×10^6 dpm/mmol).

(22*R*)-^{3}H-Cholesterol.—To a cooled stirred solution of (22*R*)-hydroxy-22-^{3}H-cholesteryl benzoate (11.3 mg, 0.24 mCi) in pyridine (0.5 ml) was added 3 drops of methanesulfonyl chloride. After standing 16 hr at room temperature the mixture was worked up as described above and the lithium aluminum hydride reduction and separation of the products were carried out in a similar manner. (22*R*)-^{3}H-Cholesterol (94 μCi) showed a single radioactive peak on tlc (silica gel–silver nitrate) and its purity was also demonstrated by cocrystallization with cold material (first crop, 4.21×10^6 dpm/mmol; first crop recrystallized, 4.23 dpm/mmol).[15]

Registry No.—**2a**, 17954-94-8; **2b**, 17954-95-9; **2a** (22-^{3}H), 17954-96-0; (**2b** (22-^{3}H), 17954-97-1; **3a**, 17954-98-2; **3b**, 17954-99-3; **3a** dibenzoate, 17955-00-9; **3b** dibenzoate, 17955-01-0; **5a**, 17955-02-1; **5b**, 17955-03-2; **6a**, 17955-04-3; **6b**, 17955-05-4; 22-ketocholesteryl benzoate, 17976-38-4; (22*S*)-^{3}H-cholesterol, 17955-06-5; (22*R*)-^{3}H cholesterol, 17955-07-6.

(15) NOTE ADDED IN PROOF.—While this paper was at the printers, Mori *et al.* [*Chem. Pharm. Bull. Jap.*, **16**, 1407 (1968)], had reversed their previous configurational assignment[3] from (22*S*)- to (22*R*)-hydroxycholesterol for the product isolated by Stabursvik.[7] This is in agreement with our conclusions, and provides added support for our results.

The Total Synthesis of Some (±)-18-Methyl-9β,10α-androstanes and (±)-18-Methyl-9β,10α-D-homoandrostanes

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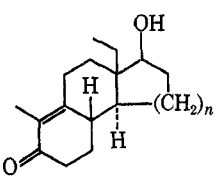
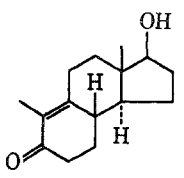
Synthetic methods developed for the preparation of retrotestosterone have been extended to the synthesis of its analog containing an 18-methyl group and to the 18-methyl-D-homo structure. The synthesis of the corresponding 17α-ethinyl and 17α-ethyl compounds is also described.

In 1966, the nonphotolytic partial synthesis of retrosteroids (*i.e.*, 9β,10α-steroids) of the pregnane series was reported[1] by workers in these laboratories. The synthesis used as the key intermediate a BCD tricyclic compound. More recently the total synthesis, in both racemic[2] and optically active modifications,[3] of such a tricyclic compound in the androstane series was described. As it is known that 18-methyl steroids of the normal series have interesting biological activities, it was decided to extend the synthetic methods that had been developed to the preparation of the title compounds. The initial targets in the present work were thus the tricyclic compounds **1** and **2** which correspond to the intermediate **3** used in the synthesis of retrotestosterone.



1, $n=1$
2, $n=2$



3

The method used to prepare compound **1** (Scheme I) closely paralleled the procedures of the previous workers.[2,4] The known 2-ethylcyclopentane-1,3-dione[5] **4** was converted into the indanone **5** by Michael addition of methyl vinyl ketone and cyclization of the resultant adduct with *p*-toluenesulfonic acid. The bicyclo[3.2.1]octane derivative **6** was isolated as a very minor by-product of this reaction and was characterized by analytical and spectral data. It was not possible to ascertain if **6** was a single stereoisomer because, while it appeared to be homogeneous on thin

(1) M. Uskokovic, J. Iacobelli, R. Philion, and T. Williams, *J. Amer. Chem. Soc.*, **88**, 4538 (1966).

(2) Z. G. Hajos, R. A. Micheli, D. R. Parrish, and E. P. Oliveto, *J. Org. Chem.*, **32**, 3008 (1967).

(3) Z. G. Hajos, D. R. Parrish, and E. P. Oliveto, *Tetrahedron*, **24**, 2039 (1968).

(4) C. B. C. Boyce and J. S. Whitehurst, *J. Chem. Soc.*, 2022 (1959).

(5) H. Smith, *et al.*, *ibid.*, 4472 (1964).