Partial Synthesis of 25D- and 25L-Cholestanoic Acids from Some Common Bile Acids[1]

THOMAS BRIGGS

Department of Biochemistry and Molecular Biology, The University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104

Received September 17, 1969

Cholestanoic acids isomeric at C-25 have been partially synthesized by an improved application of the Kolbe electrolytic cross-coupling reaction. Electrolysis was carried out with optically pure methyl 2-methyl-3-carboxypropanoate, *i.e.*, one of the half-esters of methylsuccinic acid, and a C_{24} bile acid. A single step produced the extended-chain 25D- or 25L-bile acid methyl ester, after which the free acid was easily obtained. Cholestanoic acids corresponding to cholic, deoxycholic, chenodeoxycholic, and lithocholic acids were made, in yields of 3–5% (pure crystalline products). The contribution of side-chain extension to molecular rotation is *ca.* −24 in 25D-cholestanoic acids and +47 in the 25L series, when compared with the parent C_{24} cholanoic acid.

Bile acids with 27 carbon atoms are interesting from the standpoint of both comparative biochemistry and metabolism. They occur in the bile of some primitive existing vertebrates, and are also a stage in the formation of cholic acid[2] from cholesterol in more highly evolved forms. The pathway of bile acid biosynthesis in a mammal such as the rat apparently recapitulates the evolutionary history of bile acids in vertebrates.[3]

In partial synthesis of 27-carbon bile acids, introduction of an asymmetric center at C-25 presents a major difficulty. Using the Kolbe electrolytic cross-coupling reaction, Bridgwater[4] converted cholic acid into optically pure 3α,7α,12α-trihydroxy-5β-cholestanoic acids by a procedure which has remained the only unambiguous synthesis of these substances. Others[5] have reported syntheses, but without attempting to produce asymmetry at C-25.

This paper describes the partial synthesis of two known and six new C_{27} bile acids corresponding to some of the common C_{24} acids. Asymmetry has been introduced at C-25 by an electrolytic method based on that of Bridgwater, but with modifications which have simplified the synthesis and improved the yields.

Discussion

The synthesis described here differs from the method of Bridgwater[4] in the asymmetric half-ester used during electrolysis. Owing to the lack of a satisfactory method for preparing optically pure half-esters of methylsuccinic acid, Bridgwater used half-esters of D- or L-β-methylglutaric acid. Electrolysis produced methyl esters of homocholestanoic acids which had to be converted into C_{27} acids by a laborious Barbier–Wieland degradation.

Bridgwater attempted to employ half-esters of methylsuccinic acid, but the method of preparation (from D- or L-methylsuccinic anhydride) actually gave a mixture of $HOOCCH_2CH(CH_3)COOMe$ (A), and $HOOCCH(CH_3)CH_2COOMe$ (B) (both D or both L). Although B theoretically should not have undergone anodic coupling, the mixture gave anomalous results, and the products of electrolysis were not considered reliable with respect to configuration at C-25.

Methylsuccinic acid half-methyl ester prepared by the method of Ställberg[6] exists entirely as A above. Electrolysis conducted with the optically pure form should give unequivocal results. This was verified by preliminary syntheses with cholic acid, which yielded the expected products (**1** and **2**) whose configuration was established by Bridgwater.

Preparing optically pure half-ester is tedious. However, for making 25D- and 25L-cholestanoic acids, the present synthesis has the advantage that electrolysis with C_{24} bile acid yields the product (methyl ester) in one step. Once the half-ester is available, it can easily be used to make a variety of C_{27} acids, since purification in each case is not difficult.

Equimolar amounts of bile acid and half-ester were an arbitrary choice for electrolysis. Excess half-ester would have improved the yield (based on C_{24} bile acid) but wasted precious half-ester. Excess bile acid might have conserved half-ester, but produced larger amounts of steroid impurities which could have hindered recovery of C_{27} acid. With a 1:1 ratio of reactants, the extent of cross coupling which would form the desired compound could not be expected to result in a yield of more than 25%. Yields of pure crystalline products were usually 3–5%.

Rotational Relationships.—Side-chain extension with introduction of a new center of asymmetry changes the optical rotation of bile acids in a manner which causes systematic differences in molecular rotation (MD). These MD increments (ΔMD) should be the same for a series of cholestanoic acids having the same asymmetry at C-25, assuming no vicinal effects since the side-chain terminus is well separated from the rest of the molecule.

With two exceptions, pure synthetic cholestanoic acids have consistent ΔMD values. Compared with the parent C_{24} bile acids, three C_{27} acids, made from

(1) Supported in part by a general research support grant (NIH) from the University of Oklahoma School of Medicine. The work was begun while the author was in the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn., where it was supported by USPHS Grants FR 00356-01 and HE 03558-10. An abstract has appeared: T. Briggs, *Federation Proc.*, **26**, 3333 (1967).

(2) Systematic names: cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid. In the nomenclature of cholestanoic acids according to the sequence rule, 25D corresponds to (25*R*) and 25L to (25*S*).

(3) Recent reviews: (a) comparative biochemistry and evolution, G. A. D. Haslewood, *J. Lipid Res.*, **8**, 535 (1967); (b) metabolism, H. Danielsson and T. T. Tchen in "Metabolic Pathways," Vol. 2, D. M. Greenberg, Ed., Academic Press Inc., New York, N. Y., 1968; (c) extended-chain bile acids and alcohols, T. Hoshita and T. Kazuno, *Advan. Lipid Res.*, **6**, 207 (1968).

(4) R. J. Bridgwater, *Biochem. J.*, **64**, 593 (1956).

(5) (a) P. D. G. Dean and R. T. Aplin, *Steroids*, **8**, 565 (1966); (b) N. Hoshita and K. Okuda, *J. Biochem.* (Tokyo), **62**, 655 (1967); (c) K. Morimoto, Y. Kurata, N. Hoshita, and K. Okuda, *Hiroshima J. Med. Sci.*, **17**, 1 (1968).

(6) G. Ställberg, *Arkiv Kemi*, **12**, 79 (1957).

TABLE I

PHYSICAL CONSTANTS OF C_{27} BILE ACIDS

Compd	Parent acid	Isomerism at C-25	Mp, °C	Methyl ester Mp, °C	α_D, deg	M_D, deg	M_D[a] of parent acid, deg	ΔM_D of chain extension
1	Cholic	L	199–201	160–161	+44	198	151	+47
2		D	183–184	153–154	+28	126		−25
3	Chenodeoxycholic	L			+18[b]	79[b]	45	+34[b]
4		D	175–176		+5	21		−24
5	Deoxycholic	L	177–179		+63	274	208	+66
6		D	130, 174–175	92–94	+47	204		−4
7	Lithocholic	L	169–170	112–113	+43	180	132	+48
8		D	155–157	108–109	+26	109		−23

[a] Based on α_D values (EtOH): Cholic +37°; deoxycholic, +53°; chenodeoxycholic, +11.5°; lithocholic, +35°. [b] Noncrystalline product.

cholic, chenodeoxycholic, and lithocholic acids, exhibit ΔM_D values close to −24 when configuration at C-25 is D (Table I). Two 25L products, made from cholic and lithocholic acids, show ΔM_D about +47. The 25L product made from chenodeoxycholic acid could not be purified by crystallization and has M_D lower than the expected value. The presence of 12% impurity (optically inactive) could, however, account for the discrepancy.

Molecular rotations for 25D- and 25L-cholestanoic acids made from deoxycholic acid show increments (−4 and +66) which deviate from the other values (−24 and +47). However, the difference between the 25D and 25L Δ values is nearly the same in all cases (*ca.* 70). Since Δ values are derived in part from α_D of the parent C_{24} acid, a simple explanation for the anomalous figures could be that the accepted α_D value for deoxycholic acid (+53°) is low by about 10%; that is, it should be about +58°. In fact, calculation of the specific rotation of deoxycholic acid from α_D values of other bile acids and M_D contributions of various hydroxyl groups gives a figure of +58°. Further, Wieland and Sorge[7] found that deoxycholic acid holds solvents with exceptional tenacity. After careful purification and exhaustive drying, their sample had α_D +57°.

On balance, we may assign, for the M_D contribution of side-chain extension in various bile acids, tentative values of −24 for 25D-cholestanoic acids and +47 for the 25L series, when compared with parent C_{24} cholanoic acids.

Biological.—Several 5β-cholestanoic acids occur naturally,[3c,5a,8] but a number remain to be found, which the compounds described in this report will be useful in identifying. A series of 5α-cholestanoic acids, corresponding to the 5α (allo) cholanoic acids, may also occur. One, a C_{27} homolog of allocholic acid, has already been isolated from the bile of the lizard *Iguana iguana*.[9] From M_D considerations, the 25D isomer should have α_D *ca.* +16°, and the 25L isomer *ca.* +31° (taking α_D +23° for allocholic acid[10]).

Optically pure cholestanoic acids will also be valuable in metabolic investigations. Mendelsohn and Mendelsohn[11] have shown that rat liver enzymes of cholesterol catabolism exhibit a stereochemical preference in sterol side-chain oxidation. A search for cholestanoic acid as an intermediate *in vitro* revealed mainly the 25D form. Partially synthetic C_{27} acids can be used both as substrates and as reference substances in the analysis of unknown metabolites. Work along these lines is in progress.

Experimental Section

Half-Methyl Esters of Optically Active Methylsuccinic Acid.—Methylallylacetic acid was synthesized, resolved with quinine,[12] and converted into (−)-methyl 2L-methyl-3-carboxypropanoate,[6] α_D (pure substance) −8.52° (lit.[6] α_D −8.71°). The opposite enantiomer of methylallylacetic acid[13] was prepared *via* the (+)-phenylethylamine[14] salt, and converted into (+)-methyl 2D-methyl-3-carboxypropanoate.[6] Since optical purity of the half-ester was unsatisfactory, resolution was completed by means of the cinchonidine salt,[6] giving $\alpha^{20}D$ +9.55° (lit.[6] $\alpha^{20}D$ +9.44°).

Electrolysis.[15]—The two electrodes were of platinum mesh, 3 cm × 8 cm, rolled into cylinders and mounted coaxially 2.5 mm apart on two Teflon rings. A typical electrolysis was conducted in a 150-ml beaker, in which the electrodes were suspended with platinum wire. Sodium (1.7 mg-atoms) was dissolved in redistilled methanol (100 ml, dried by reaction with magnesium). Bile acid (17 mmol) was added; the mixture was stirred and warmed if necessary to promote solution. Optically active half-ester of methylsuccinic acid (17 mmol) was added and the assembly was immersed in an ice bath. Electrolysis was carried out with constant stirring (magnetic bar) at 15–25° using direct current from a 20-V power source. Current polarity was reversed every 15 min. Initially *ca.* 0.9 A, the current decreased after 3–4 hr to *ca.* 0.7 A. The reaction caused a change in pH from *ca.* 4.5 to 6.5.

Purification.—The methanolic solution, which contained products of electrolysis, was poured into water (700 ml), acidified (HCl) to about pH 2, and extracted twice with ethyl acetate (150 ml and 100 ml) and twice with ether (100 ml each). The combined extracts were washed with 5% aqueous $NaHCO_3$ (100 ml), water (50 ml), and saturated NaCl solution (50 ml). The combined washings were extracted with ether (50 ml), which was added to the main ethyl acetate–ether solution and filtered through Na_2SO_4. Evaporation left neutral material, usually about 6.5 g. Acidic material (usually 1 g or less) could be recovered from the aqueous washings.

The neutral fraction, which consisted of a complex mixture including the desired C_{27} bile acid methyl ester, was partially purified by adsorption chromatography on alumina (acid; 80–200 mesh, Fisher Scientific Co., Houston, Texas). Fractions were concentrated and analyzed by thin layer chromatography (tlc). Those with product were combined and saponified by refluxing 1.5 hr with 2 *N* ethanolic KOH (25 ml). After dilution with water (250 ml), ether extraction removed nonsaponifiable material. The aqueous solution was acidified (HCl) and extracted with ethyl acetate (50 ml) and twice with ether (50 ml each); the combined extracts were washed with water (25 ml) and saturated NaCl solution (25 ml). The combined washings

(7) H. Wieland and H. Sorge, *Z. Physiol. Chem.*, **97**, 1 (1916).
(8) T. Briggs, M. W. Whitehouse, and E. Staple, *Arch. Biochem. Biophys.*, **85**, 275 (1959).
(9) K. Okuda, M. G. Horning, and E. C. Horning, *Proc. Int. Congr. Biochem., 7th, Tokyo, IV*, 721 (1967) (Abstracts).
(10) I. G. Anderson and G. A. D. Haslewood, *Biochem. J.*, **85**, 236 (1962).
(11) D. Mendelsohn and L. Mendelsohn, *ibid.*, **114**, 1 (1969).
(12) S. Ställberg-Stenhagen, *Arkiv Kemi, Mineral., Geol.*, **23A**, 1 (1946).
(13) G. Ställberg, *Acta Chem. Scand.*, **11**, 1430 (1957).
(14) A. W. Ingersoll in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley & Sons, Inc., New York, N. Y., 1943.
(15) The method in general is that of Bridgwater,[4] with modifications. I thank Dr. B. Preiss for help in designing electrodes.

were extracted with ether (15 ml), which was added to the main ethyl acetate–ether solution and filtered through Na_2SO_4. Evaporation left crude C_{27} acid, usually 0.5–0.8 g, which often crystallized readily. Yields, calculated from the total amount of starting material (17 mmol of C_{24} bile acid), are given for pure recrystallized products.

Characterization of Products.—Melting points were determined on a block-type apparatus under a magnifying lens and are uncorrected. Galbraith Laboratories, Inc., Knoxville, Tenn., performed the microanalyses. Optical rotation measurements were made with a Franz Schmidt and Hoensch polarimeter. Unless stated otherwise, all determinations were done at or near 25°, in 2% ethanolic solutions with a 2-dm light path. Methyl esters were made with CH_2N_2. Thin layer chromatography was carried out with silica gel H (E. Merck, A. G., Darmstadt, Germany) in 250-μ layers. Solvent systems were modified from Usui.[16] Spots were made visible with an anisaldehyde spray reagent.[17] Since R_f values varied, analyses always included standards. Cholestanoic acids and their methyl esters migrated farther than the corresponding C_{24} compounds containing the same number of hydroxyl groups (Table II).

TABLE II

THIN LAYER CHROMATOGRAPHY OF BILE ACIDS

	R_f of free acid in system 1[a]	R_f of Me ester in system 2[b]
Cholic	0.04	0.02
1 and **2**	0.09	0.04
Chenodeoxycholic	0.25	0.18
3 and **4**	0.35	0.27
Deoxycholic	0.28	0.18
5 and **6**	0.41	0.31
Lithocholic	0.74	0.72
7 and **8**	0.78	0.79

[a] Benzene–acetic acid (80:20). [b] Benzene–ethyl acetate (50:50).

3α,7α,12α-Trihydroxy-5β-25L-cholestanoic Acid (1).—Electrolysis of cholic acid (7.0 g, Mann Research Laboratories, New York) with L half-ester (2.5 g) and Na (40 mg) for 4 hr gave neutrals (5.7 g) and acids (1.8 g). The neutrals were dissolved in ether and applied to a column of alumina (150 g, activity grade I) prepared in ether. Eluting solvents (collected in 250-ml portions) and residues after evaporation follow: ether (0.5 l.) and ether–acetone (1:1) (0.5 l.), 1.44 g; acetone (1 l.), 2.15 g; 2% methanol in acetone (0.5 l.), 0.78 g; 10% methanol in acetone (0.5 l.), 1.33 g; and methanol (0.5 l.), 0.92 g. Substances with the mobility (tlc) expected of methyl trihydroxycholestanoate emerged in fractions eluted by acetone and by 2% methanol in acetone. Saponification yielded neutrals (1.8 g), and an acid (0.72 g), which crystallized on standing with ethyl acetate. Recrystallization from ethyl acetate and from ethanol–water gave **1** (393 mg, 5%), mp 199–201°, α_D +44° (lit.[4] mp 195–196°, α_D +43°).

The methyl ester, crystallized from ether–petroleum ether (bp 30–60°) melted at 160–161°. Komatsubara[18] reported mp 156° for the methyl ester of the acid (mp 195–196°) from the bile of *Rana nigromaculata nigromaculata*, and from partial synthesis.

3α,7α,12α-Trihydroxy-5β-25D-cholestanoic Acid (2).—Electrolysis of cholic acid (7.0 g) with D half-ester (1.5 g) and Na (40 mg) for 2 hr, followed by addition of half-ester (1.0 g) and further electrolysis (total, 4.5 hr), gave neutral material (6.0 g) and acids (1.0 g). Neutrals were separated on alumina as before. Appropriate fractions were combined (2.2 g) and saponified yielding neutrals (1.4 g, discarded) and an acid (0.9 g), which crystallized on standing with ether. Recrystallization from ethyl acetate, acetone (twice), ethanol–water, and acetone gave **2** (357 mg, 4.6%), mp 183–184°, $\alpha^{20}D$ +28° (MeOH) (lit.[4] mp 180–182°, α_D +27°).

The methyl ester, crystallized from ether, melted at 153–154°. Haslewood[19] reported mp 153–155° for the methyl ester of the acid (mp 171–173°) from alligator bile.

(16) T. Usui, *J. Biochem.* (Tokyo), **54**, 283 (1963).

(17) D. Kritchevsky, D. S. Martak, and G. H. Rothblat, *Anal. Biochem.*, **5**, 388 (1963).

(18) T. Komatsubara, *Proc. Jap. Acad.*, **30**, 618 (1954); *Chem. Abstr.*, **50**, 387 (1956).

3α,7α-Dihydroxy-5β-25L-cholestanoic Acid (3).—Electrolysis of chenodeoxycholic acid (6.7 g, "A" grade, CalBiochem, Los Angeles, Calif.) with L half-ester (1.5 g) and Na (40 mg) for 1.5 hr, followed by addition of half-ester (1.0 g) and further electrolysis (total, 4.25 hr), gave neutrals (6.25 g) and acids (0.5 g). The neutrals were dissolved in ether and applied to a column of alumina (180 g, activity grade I) prepared in ether. Eluting solvents and residues follow: ether (0.5 l.) 0.2 g; ether–acetone (1:1) (0.5 l.), 3.0 g; acetone (1.5 l.), 4.8 g; 2% methanol in acetone (1.0 l.), 0.5 g; and 10% methanol in acetone (0.5 l.), 0.7 g. Substances with the mobility (tlc) expected of methyl dihydroxycholestanoate emerged with the first 0.75 l. of acetone.

The combined fractions (4.5 g), which contained much pungent oil,[20] were dissolved in ether and rechromatographed on a column of alumina (135 g) prepared in ether. Eluting solvents and residues follow: ether (0.25 l.), 0.05 g; 50% acetone in ether (1.0 l.), 7.9 g; 60% acetone in ether (1.0 l.), 3.5 g; 75% acetone in ether (1.0 l.), 0.7 g; and acetone (1.0 l.), 0.7 g. The product was eluted by the last 250 ml of 50% acetone and the first 500 ml of 60% acetone. The combined residues (2.4 g) after saponification yielded neutrals (1.6 g, partially crystalline) and acids (0.75 g), which did not crystallize even after purification by reversed phase column partition chromatography.[21] The amorphous material had α_D +18°.

Attempts to crystallize the methyl ester, the diacetate, and the diacetate methyl ester also failed.

3α,7α-Dihydroxy-5β-25D-cholestanoic Acid (4).—Electrolysis of chenodeoxycholic acid (6.7 g) with D half-ester (2.5 g) and Na (40 mg) for 4 hr yielded a small amount of acid material and neutrals (6.6 g), which were dissolved in benzene and applied to a column of alumina (150 g, deactivated with 6% water), prepared in benzene. Eluting solvents (1.0 l. each, collected in 200-ml portions) and residues follow: benzene, 0.95 g; 20% ether in benzene, 1.9 g; and 50% ether in benzene, 1.1 g. Fractions eluted by the last 600 ml of 20% ether appeared (tlc) to contain most of the product and were combined (0.87 g). Saponification gave neutrals (413 mg) and acids (415 mg). The acid fraction was purified by reversed phase column partition chromatography: stationary phase, chloroform–heptane (9:1, 25 ml) on Reversil 4 (100 g, Applied Science Laboratories, State College, Pa.); moving phase, 58% methanol in water.[21] Fractions (5 ml) were titrated with 0.02 *N* NaOH. The peak emerging at 170–615 ml contained material which was recovered by concentration, acidification, and extraction with ethyl acetate–ether. Evaporation left crystals (198 mg), mp 173–175°. Two recrystallizations from methanol–water yielded **4** (161 mg, 2.2%), mp 175–176°, α_D +5°.

Anal. Calcd for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.7; H, 10.8.

The methyl ester did not crystallize from any solvent tried.

3α,12α-Dihydroxy-5β-25L-cholestanoic Acid (5).—Electrolysis of deoxycholic acid (6.7 g, recrystallized "C" grade, CalBiochem) with L half-ester (2.5 g) and Na (44 mg) gave neutrals (6.8 g), which were dissolved in benzene and applied to a column of alumina (150 g, deactivated with 6% water) prepared in benzene. Eluting solvents (0.8 l. each) and residues follow: benzene, 0.75 g; 20% ether in benzene, 3.5 g; and 50% ether in benzene, 1.2 g. Substances with appropriate mobility on tlc emerged in 50% ether and in the last 600 ml of 20% ether. Saponification yielded neutrals (1.5 g) and an acid (0.5 g), which crystallized on standing with ethyl acetate. Recrystallization from methanol–water (three times) gave **5** (229 mg, 3.1%), mp 177–179°, α_D +63°.

Anal. Calcd for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.1; H, 10.8.

The methyl ester failed to crystallize.

3α,12α-Dihydroxy-5β-25D-cholestanoic Acid (6).—Electrolysis was carried out with D half-ester and deoxycholic acid exactly as it was with the L form. Neutrals (6.75 g) were dissolved in benzene and applied to a column of alumina (165 g, deactivated with 3% water) prepared in benzene. Eluting solvents (1.0 l. each) and residues follow: benzene, 1.0 g; 20% ether in benzene,

(19) G. A. D. Haslewood, *Biochem. J.*, **52**, 583 (1952).

(20) Activated alumina apparently causes condensation or polymerization of acetone; evaporation leaves an oil which interferes with product purification. A better procedure is to deactivate the alumina with 6% water; bile acid methyl esters will then be eluted with benzene or a mixture of ether and benzene.

(21) S. Bergström and J. Sjövall, *Acta Chem. Scand.*, **5**, 1267 (1951); A. Norman, *ibid.*, **7**, 1413 (1953).

1.0 g; 50% ether in benzene, 1.7 g; and 75% ether in benzene, 0.1 g. Substances (2.0 g) with appropriate mobility on tlc were eluted by the last 400 ml of 20% ether, by 50% ether, and by 75% ether. Saponification yielded neutrals (1.55 g) and a crystalline acid (526 mg). Recrystallization from ethyl acetate, methanol–water (containing a little ethyl acetate), and ethyl acetate gave **6** (245 mg, 3.3%), mp 130° with subsequent recrystallization and remelting at 174–175°, α_D +47°.

Anal. Calcd for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.6; H, 11.0.

The methyl ester, crystallized from methanol, melted at 92–94°.

3α-Hydroxy-5β-25L-cholestanoic Acid (7).—Electrolysis of lithocholic acid (6.5 g, Nutritional Biochemicals Corp., Cleveland, Ohio) with L half-ester (2.5 g) and Na (40 mg) for 4 hr yielded neutrals (7.1 g), which were dissolved in benzene (10 ml), diluted with petroleum ether (20 ml), and applied to a column of alumina (150 g, deactivated with 6% water) prepared in petroleum ether. Eluting solvents (1.0 l. each) and residues follow: 33% benzene in petroleum ether, 1.9 g; 50% benzene in petroleum ether, 0.8 g; and benzene, 1.2 g. Solvents and residues of further elution follow: 5% ether in benzene (0.4 l.), 0.4 g; 20% ether in benzene (0.6 l.), 0.8 g; and ether (0.4 l.), 0.4 g. Substances (750 mg) with the mobility (tlc) expected of methyl monohydroxycholestanoate were eluted by the first 400 ml of benzene. Saponification yielded no neutrals but an acid (723 mg) which crystallized from ether. Several recrystallizations from ether gave **7** (278 mg, 3.9%), mp 169–170°, α_D +43°.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.7; H, 11.8.

The methyl ester, crystallized from ether–petroleum ether, melted at 112–113°.

3α-Hydroxy-5β-25D-cholestanoic Acid (8).—A similar electrolysis with lithocholic acid (recrystallized), Na, and D half-ester for 3.75 hr yielded neutrals (6.8 g) and traces of acid. After chromatography the product emerged with the first 800 ml of benzene. Saponification yielded crystalline neutrals (556 mg) and an acid (940 mg) which crystallized on standing with ether–petroleum ether. Recrystallization from methanol–water, ether, and methanol–water gave **8** (392 mg, 5.4%), mp 155–157°, α_D +26°.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.7; H, 11.6.

The methyl ester, crystallized from ether–petroleum ether, melted at 108–109°.

Registry No.—**1**, 23047-29-2; **1** methyl ester, 23740-21-8; **2**, 23740-14-9; **2** methyl ester, 23740-22-9; **3**, 23740-15-0; **4**, 23740-16-1; **5**, 23740-17-2; **6**, 23740-18-3; **6** methyl ester, 23740-23-0; **7**, 23740-19-4; **7** methyl ester, 23740-24-1; **8**, 23740-20-7; **8** methyl ester, 23829-36-9.

Acknowledgments.—I thank Mrs. Louis J. Bussjaeger for expert technical help during part of this work. Numerous colleagues have been most generous in lending equipment.

The Reaction of 5-Bromouracil Derivatives with Sulfur Nucleophiles, and a Novel Synthetic Route to 5-Sulfur-Substituted Uracils and Nucleotides[1a,b]

L. SZABO, T. I. KALMAN, AND T. J. BARDOS[1c]

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214

Received October 3, 1969

1-Methyl-5-bromouracil (**1**) reacts with sodium hydrosulfide in dimehtyl sulfoxide at room temperature to give 1-methyl-5-mercaptouracil (**2**, isolated as the disulfide **3**) and 1-methyluracil (**4**). Deuterium-exchange studies are consistent with 1,4 addition of the reagent to **1**, followed by tautomerization, to give two stereoisomeric adducts which, after nucleophilic displacement of the bromine by another anion of the reagent, undergo *trans* elimination reactions leading to **2** and **4**, respectively. Based on this mechanism, a useful, novel synthetic route was developed for the introduction of a 5-sulfur substituent into the pyrimidine ring of uracil derivatives *via* addition of methyl hypobromite to the 5,6 double bond, followed by reaction of the adduct with sodium disulfide. By use of this method, **3** was synthesized in 74% yield, and the disulfide of the nucleotide 5-mercapto-2′-deoxyuridine 5′-phosphate (**15**) was synthesized in an overall yield of 68%.

The synthesis of 5-mercapto-2′-deoxyuridine, a structural analog of thymidine, was recently reported.[2] This compound was found to be an effective antimetabolite in various test systems[3] in which it was apparently converted into its 5′-phosphate (**17**). It appeared of interest to synthesize the nucleotide **17** and some of its derivatives; this prompted the investigation of the feasibility of introducing a thiol group at the 5 position of 1-substituted uracil derivatives, to provide a relatively simple method applicable for the preparation of various 5-sulfur-substituted pyrimidine nucleotides. Although 5-halogenopyrimidines are characterized by low reactivity of the halogen atom,[4] Roth and Hitchings reported[5] that thiophenol salts react readily with with 5-bromouracil in ethylene glycol at 150° to give 40–50% yield of 5-arylthiouracils, in addition to uracil and diaryl disulfides obtained as by-products. The observed side reaction was attributed to electron transfer, resulting in the reductive removal of the halogen.[5]

1-Methyl-5-bromouracil (**1**) was selected as a model compound for the determination of the optimal conditions for the substitution reaction. Preliminary experiments indicated that **1** reacted with excess sodium hydrosulfide only at high temperature when ethylene glycol[5] was used as the solvent, but the reaction proceeded readily at room temperature in dimethylacetamide (DMAA) or dimethyl sulfoxide (DMSO). Although in the latter case the reaction appeared to be essentially complete in 1 hr, the presence of both 1-methyl-5-mercaptouracil (**2**) and its disulfide (**3**) in the reaction

(1) (a) This work was supported by U. S. Public Health Service Research Grant R01-CA06695 from the National Cancer Institute, National Institutes of Health, Bethesda, Md. (b) A preliminary report of this work was presented at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969. (c) To whom inquiries should be directed.

(2) (a) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966); (b) M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, in press.

(3) (a) K. Baranski, T. J. Bardos, A. Bloch, and T. I. Kalman, *Biochem. Pharmacol.*, **18**, 347 (1969); (b) T. I. Kalman and T. J. Bardos, *Fed. Proc.*, **27**, 650 (1968).

(4) G. W. Kenner and A. Todd, "Heterocyclic Compounds," Vol. 6, R. C. Elderfield, Ed., J. Wiley & Sons, Inc., New York, N. Y., 1957, p 301.

(5) B. Roth and G. H. Hitchings, *J. Org. Chem.*, **26**, 2770 (1961).