notes on methodology

A sterospecific synthesis of 7α-hydroxycholesterol

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Summary The five step synthesis of 7α-hydroxycholesterol utilizes the solvolysis of 7α-bromocholesterol benzoate with potassium acetate in acetic acid as the key step in controlling the stereospecificity of the reaction sequence. This reaction yields 7α-acetoxycholesterol benzoate with retention of configuration at position seven. The diester is readily reduced with lithium aluminum hydride to 7α-hydroxycholesterol.

Supplementary key words 7α-hydroxycholesterol • synthesis • 7α-bromocholesterol

In 1964, Danielsson and Einarsson (1) found that 7α-hydroxycholesterol was an important intermediate in the enzymatic conversion of cholesterol to bile acids. The need for gram quantities of 7α-hydroxycholesterol in our studies of the cholesterol 7α-hydroxylase enzyme system prompted us to consider a more convenient and direct method for the synthesis of this compound.

Reported syntheses (2–4) of 7α-hydroxycholesterol involve either the reduction of 7-ketocholesterol acetate or the allylic bromination of cholesterol benzoate. The reduction of 7-ketocholesterol acetate gives a mixture of 7α- and 7β-hydroxycholesterols where the 7β isomer predominates 4:1. The isomer separation by preparative thin-layer or column chromatography results in very low yields of 7α-hydroxycholesterol, which is usually contaminated with small amounts of the 7β epimer. The allylic bromination of cholesterol benzoate results in low yields of impure 7α-bromocholesterol benzoate (4). The sequences of synthetic reactions, **Fig. 1**, can yield gram amounts of 7α-hydroxycholesterol without 7β-hydroxycholesterol contamination.

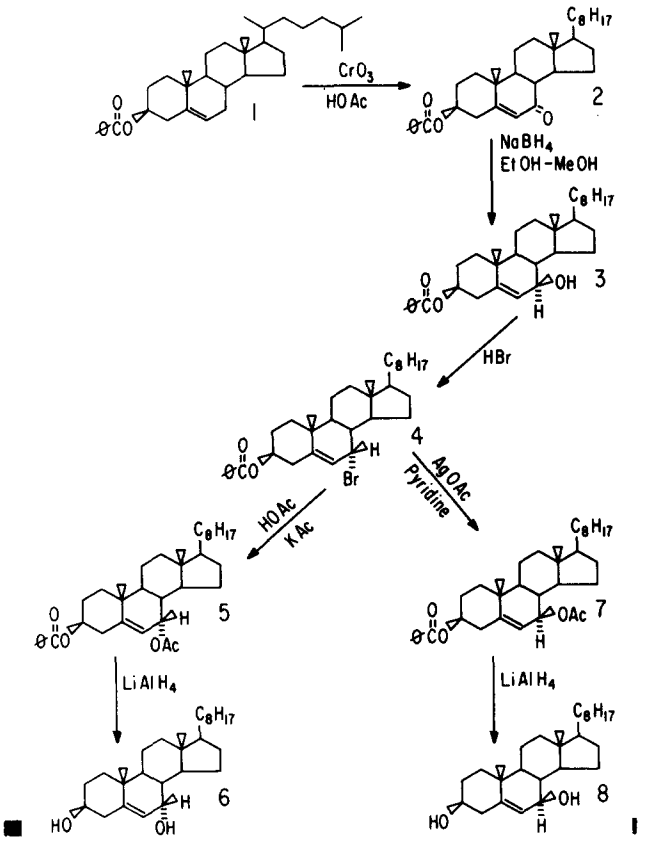

Fig. 1. Sequence of chemical reactions leading to the synthesis of 7α-hydroxycholesterol.

METHODS

The cholesterol benzoate was purchased from Sigma Biochemical Corporation, St. Louis, Mo. All other chemicals were reagent grade and purchased from Ventron (Beverly, Mass.), Eastman (Rochester, N.Y.) or Baker (Phillipsburg, N.J.) Chemicals Corporations. Solvents were used without further purification. Melting points were determined with a Fisher-Johns melting point apparatus and are corrected. The infrared spectroscopic data was obtained from a Perkin Elmer Model 150 infrared spectrophotometer (Perkin Elmer Corp., Norwalk, Conn.). Nuclear magnetic resonance spectra were recorded at 100 mHz on a JEOL MH-100 spectrometer (JEOL, Inc., Medford, Mass.), through the courtesy of Dr. Peter Jeffs and the Duke University Chemistry Department. The indexes of rotation were obtained with a Cenco polarimeter (Central Scientific Co., Chicago, Ill.).

7-Ketocholesterol benzoate (no. 2, Fig. 1)

A mixture of cholesterol benzoate (24.5 g, 50.0 mmoles) in acetic acid (300 ml) was heated to 52–58°C with rapid stirring before adding solid chromium trioxide (15 g) at the rate of 1.25 g every 10 min for 2 hr. After an additional 2 hr, thin-layer chromatography showed no starting material. The reaction was quenched with 95% ethanol (5 ml) and water (50 ml) and allowed to crystallize overnight at 5°C. The solid was filtered and washed with 80% acetic acid. Crystallization from 95% ethanol gave 7.75 g (30%) of colorless needles. mp 158.5–159.5°C; IR 1718 cm^{-1} (ArC = 0), 1667 and 1656 cm^{-1} (C = 0).

7β-Hydroxycholesterol benzoate (no. 3, Fig. 1)

The 7-ketocholesterol benzoate (7.00 g, 13.9 mmoles) was reduced with sodium borohydride by the procedure of Corey and Gregoriou (5). The product, 4.2 g (60%), was obtained as white flakes after crystallization from cyclohexane. mp

192–193.5°C (192°C reported, see ref. 6); IR 3280 cm^{-1} (OH), 1718 cm^{-1} (ArC = 0).

7α-Bromocholesterol benzoate (no. 4, Fig. 1)

The 7β-hydroxycholesterol benzoate (2.50 g, 4.94 mmoles) was added to a dry saturated ethereal hydrogen bromide solution according to the procedure of Corey and Gregoriou (5). The 7α-bromocholesterol benzoate, 2.00 g (70%), was isolated as colorless heavy crystals after two crystallizations from hexane. mp 140–141.5°C (139–140.5°C reported, see ref. 5).

7α-Acetoxycholesterol benzoate (no. 5, Fig. 1)

The 7α-bromocholesterol benzoate (585 mg, 1 mmole) was mixed with acetic acid (50 ml) containing potassuim acetate (1.25 g). The mixture was stirred until all of the bromo compound had dissolved, then it was left standing for 24 hr. The mixture was diluted with water, cooled, and the solid was filtered. After two crystallizations from methanol–acetone 3:1, 310 mg (55%) of colorless needles were isolated. mp 129.5–130.5°C (132°C reported, see ref. 7); IR 1718 and 1702 cm^{-1} (C = 0), nmr (100 mHz) δ 8.16–7.36 (5H, M, ArH), 5.68 (1H, d, J = 6 Hz, H_6), 5.00 (1H, M, H_7), 2.04 (S, 3H, CH_3).

7β-Acetoxycholesterol benzoate (no. 7, Fig. 1)

The 7α-bromocholesterol benzoate (500 mg, 0.855 mmole) was added to a solution containing silver acetate (1 g) in dry pyridine (10 ml) and the mixture was kept at room temperature for 24 hr. The mixture was diluted with water, extracted with ethyl ether and the ethereal solution was washed with cold 5% HCl to remove the pyridine. The colorless oil gave 350 mg (73%) of long white needles from methanol–benzene 3:1. mp 154–155°C (153°C reported, see ref. 7), nmr (100 mHz) δ 8.1–7.1 (ArH), 5.08 (S, 1H, H_6), 5.00 (d, 1H, J = 9Hz, H_7), 2.00 (S, 3H, CH_3). The spectroscopic and chromatographic properties of this compound were identical to a standard synthesized from 7β-hydroxycholesterol benzoate and acetic anhydride.

7α-Hydroxycholesterol (no. 6, Fig. 1)

The 7α-acetoxycholesterol benzoate (2.00 g, 3.65 mmole) in dry ethyl ether (40 ml) was added dropwise to a suspension of lithium aluminum hydride in dry ethyl ether at room temperature.

After stirring overnight, the reaction was quenched with 10% ammonium chloride and filtered. The aluminum salts were washed with hot ethyl acetate and the combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed in vacuo. The white solid was crystallized twice from methanol to yield 1.00 g (68%) of colorless needles. mp 186–188°C $[\alpha]_D^{24°}$ − 94° (C 0.9) (reported values mp 188–188.5°C, $[\alpha]_D$ − 94° see ref. 8, 9); IR 3600 cm^{-1} (OH), 1470 cm^{-1}, 1390 cm^{-1}, 1112 cm^{-1}, 1060 cm^{-1}, 1017 cm^{-1}, nmr (100 mHz) δ 5.60 (d, 1H, J = 6 Hz, H_6), 3.96–3.84 (m, 1H, H_7), 3.44 (S, 1H, D_2O replaceable).

7β-Hydroxycholesterol (no. 8, Fig. 1)

The 7β-acetoxycholesterol benzoate (200 mg, 0.36 mmole) was reduced with lithium aluminum hydride in the same manner as the 7α-acetoxycholesterol benzoate. Two crystallizations from methanol–water gave 100 mg (68%) of a gelatinous precipitate. mp 178–180°C, $[\alpha]_D^{24°}$ + 6° (C 1.0) (reported values mp 177–178.5°, $[\alpha]_D$ + 7° see ref. 8); IR 3600 cm^{-1} (OH), 1470 cm^{-1}, 1383 cm^{-1}, 1136 cm^{-1}, 1047 cm^{-1}, 1010 cm^{-1}, 974 cm^{-1}, nmr δ 5.28 (s, 1H, H_6), 3.88–3.76 (m, 1H, H_7).

DISCUSSION

The five step synthesis of 7α-hydroxycholesterol involves the oxidation of cholesterol benzoate (1, Fig. 1) to 7-ketocholesterol benzoate (2, Fig. 1) with chromium trioxide in acetic acid (9). The 7-ketocholesterol benzoate was reduced to 7β-hydroxycholesterol benzoate (3, Fig. 1) with sodium borohydride in a methanol–95% ethanol mixture (5). When treated with dry hydrobromide gas at −78°C, the 7β-hydroxycholesterol benzoate was smoothly converted to 7α-bromocholesterol benzoate (4, Fig. 1) (5). This method circumvents the allylic bromination of cholesterol benzoate which is difficult to reproduce, and often gives low yields of impure material (10).

The key step in the synthesis is the solvolysis of the bromide (4, Fig. 2) with potassium acetate in acetic acid (7). This reaction probably involves the participation of the double bond, helping to displace the bromide ion to yield a highly reactive cyclopropyl carbonium ion (11). This intermediate allows for attack of the acetate ion from the less sterically hindered α face to yield the 7α-acetoxycholesterol benzoate (5, Fig. 1).

The 7α-acetoxycholesterol benzoate was readily reduced to 7α-hydroxycholesterol (6, Fig. 1) with lithium aluminum hydride at room temperature. After two crystallizations from methanol, the 7α-hydroxycholesterol was isolated as colorless needles. The thin-layer chromatogram showed only one spot, corresponding to the same R_f as the known material. Also, there was no detectable 7β-hydroxycholesterol when the plate was sprayed with 1 N H_2SO_4 and heated and then exposed to ultraviolet light. Comparison of the 100 mHz nuclear magnetic resonance spectrum of 7α-hydroxycholesterol with that of 7β-hydroxycholesterol gave definitive evidence of the stereochemistry of the two compounds at position seven.

The 7β-hydroxycholesterol (8, Fig. 1) was prepared by treating 7α-bromocholesterol benzoate with silver acetate in pyridine (7). The 7β-acetoxycholesterol benzoate (7, Fig. 1), was reduced to 7β-hydroxycholesterol with lithium aluminum hydride and crystallized twice from methanol. A thin-layer chromatogram of the 7β-hydroxycholesterol showed only trace amounts of 7α-hydroxycholesterol.

The nuclear magnetic resonance spectrum of 7α-hydroxycholesterol was characterized by a doublet at 5.60 ppm with a coupling of J = 6 Hz, corresponding to the proton at C-6. This should be contrasted with the spectrum of 7β-hydroxycholesterol which has a sharp singlet at 5.28 ppm, also cor-

responding to the proton at C-6. The singlet is indicative of a vicinal angle of 90° between the vinyl proton at C-6 and the proton at C-7. This confirms the axial orientation of the proton at C-7 and results in having the hydroxyl group in the equatorial position at C-7 of 7β-hydroxycholesterol. In the 7α-hydroxycholesterol spectrum, the coupling of 6 Hz between the C-6 vinyl proton and the proton at C-7 is in good agreement with the expected coupling for protons with vicinal angle of 0°. This confirms the equatorial orientation of the proton at C-7 and therefore the hydroxyl group is in the axial configuration of 7α-hydroxycholesterol.

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