SYNTHESIS OF TRITIUM LABELLED 7-DEHYDROCHOLESTEROL 5β,6β-OXIDE

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SUMMARY

We report the synthesis of tritiated 7-dehydrocholesterol 5β,6β-oxide in high specific activity. Oxidation of 7α-bromocholesterol 5β,6β-oxide to the 3-keto epoxide followed by borotritide reduction, in a special buffer-organic solvent system to minimize undesired rearrangement, regenerated the 3β-hydroxyl group. Base-assisted elimination produced the title compound.

Key words: 7-dehydrocholesterol 5β,6β-oxide, cholesterol oxide hydrolase, inhibition, borohydride reduction, tritium.

INTRODUCTION

A great deal of interest has been generated recently by the demonstration that cholesterol 5,6-oxide is metabolized to the corresponding 5α,6β-glycol by liver microsomes.[1] Watabe et. al.[2] have shown that liver microsomes metabolize other steroidal 5,6-oxides to the corresponding 5α,6β-glycols as well. Levin et. al.[3] have shown that cholesterol oxide hydrolase in rat liver microsomes is antigenically and catalytically distinct from the well characterized xenobiotic epoxide hydrolase (EC 3.3.2.3). α-Iminocholestanol, the iminoanalog of cholesterol 5,6α-oxide, is a potent inhibitor for cholesterol oxide hydrolase.[4,5] This inhibition suggests that α-iminocholestanol is a transition state analog and that cholesterol oxide hydrolase catalyzes the hydration of epoxides by an acid-catalyzed mechanism which proceeds through a positively charged transition state[5] in contrast to general base-catalyzed mechanism for xenobiotic microsomal epoxide hydrolase.[6] Thus, in our investigation[5] of the properties of this newly described enzyme, there was a need for a mechanistic

0362-4803/86/040371-06$05.00
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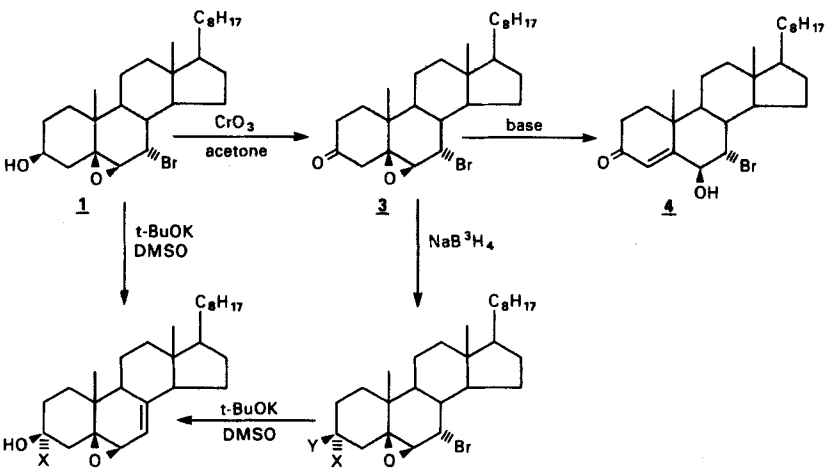
Revised August 26, 1985

probe for the action of cholesterol oxide hydrolase. The isomeric 7-dehydrocholesterol 5,6-oxides were envisioned as potential candidates, and synthetic procedures[7] for them were developed. Since the β-oxide 2, was shown to cause time and concentration dependent loss of catalytic activity for cholesterol oxide hydrolase,[7,8] a procedure was sought for radiolabelling the β-oxide so that studies of covalent binding to the protein could be undertaken.

RESULTS AND DISCUSSION

Since a hydroxyl group is present in all of the intermediates in the synthesis[7] of the β-oxide 2, we considered oxidation of an intermediate compound to a ketone and subsequent reduction of the ketone with ^{3}H-sodium borohydride to be the most effective and rapid way of achieving a radiolabelled substrate. Oxidation of 7-bromocholesterol 5,6-oxide 1, the immediate precursor to the β-oxide 2, with Jones reagent[9] a afforded the desired ketone 3 in very good yield despite the presence of an acid-sensitive epoxide group. Subsequent reduction of the ketone with sodium borohydride in several different solvents and at varied reaction times, however, gave erratic results. The reason for the poor reproducibility in this reduction is that the ketone 3 is extraordinarily sensitive to base. For example, at pH 10.3 (25% THF-water, μ 1.0, 25°C) the half-life of the ketone is 80 sec, as measured by the increase in absorption at 243 nm. Examination of the reaction product by NMR reveals that ketone 3 undergoes base-catalyzed deprotonation at C-4 followed by epoxide ring opening via β-elimination at C-5 to produce 7-bromo-6-hydroxycholest-4-en-3-one 4. This reaction in base is typical of β,γ-epoxy steroidal ketones[10] and has been used to advantage in the synthesis of γ-hydroxy steroidal enones.[10,11] Conditions were therefore required in which the rate of ketone reduction would be significantly greater than either its rearrangement to enone at higher pH or the decomposition of borohydride at lower pH. In their investigation of the kinetics kinetics of the reduction of cholestanone by borohydride, Wheeler and Mateos reported[12] that dioxane-water mixtures were unsuitable for kinetic determinations because the observed rates were too rapid. For this very reason their



solvent system appeared to be an appropriate choice for our needs. The stability of the ketone was determined in 33% aqueous dioxane at various pH's, and the ketone was found to be sufficiently stable in aqueous buffer at pH ~ 9 (before organic solvent addition) to attempt the reduction. Addition of $NaBH_4$ to the solvent mixture followed immediately by the ketone resulted in the production of the desired 3β-ol as the major component (56%) along with smaller amounts of the 3α-ol (13%) and the enone (12%). The reduction procedure was repeated using ^3H-sodium borohydride. Chromatographic analysis of the reaction mixture indicated formation of ketone 3 (15%), 3α-ol 5, (12%), 3β-ol 6, (54%), enone 4, (17%), and an unidentified product (2%). The 3β-ol fractions were collected, pooled, and the solvent removed. Treatment of the 3β-ol with potassium t-butoxide in DMSO at 55°C yielded the desired β-ene-oxide 7 which was purified by HPLC. The oxide 7 was 99.5% radiochemically pure by TLC with a specific activity of 60 mCi/mmol.

EXPERIMENTAL

All chemicals were used as obtained from the manufacturer except where noted otherwise. Melting points were obtained on a Thomas Hoover melting point

apparatus and are uncorrected. NMR spectra were recorded in deuteriochloroform using a Varian 220 MHz spectrometer with tetramethylsilane as the internal reference. Mass spectra and accurate mass determinations were obtained using a VG Micromass 7070F spectrometer. UV spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. HPLC analyses were performed using a Waters Associates Model 6000A pump equipped with a Model R401 differential refractometer. Liquid scintillation spectrophotometry was performed using an Intertechnique SL 4000 liquid scintillation counter with Hydrofluor scintillation cocktail.

7-Bromo-5β,6β-epoxycholestan-3-one 3

A solution of 1 (100 mg) in 5 ml of acetone was treated with 0.12 ml of Jones reagent.[9] After 3 min with occasional agitation, the mixture was poured into dilute aqueous bicarbonate solution and extracted with ethyl acetate. The organic layer was washed with water and dried over sodium sulfate. The crude material was recrystallized from ethanol to afford 71 mg (71% yield) of product as colorless plates; mp 159 - 161°C. NMR: δ 0.70 (s, 3H, C-18), 1.10 (s, 3H, C-19), 2.98 (d, 1H, J = 17 Hz, H-4), 3.30 (d, 1H, J = 4 Hz, H-6), 4.48 (t, 1H, J = 4 Hz, H-7). HRMS $C_{27}H_{43}{}^{79}BrO_2$ requires: 478.2446, found: 478.2482. MS 480(5), 478(5), 399(100), 381(59).

Reduction of 3 with sodium borohydride

Ketone 3 (18 mg) was dissolved in 2 ml of freshly distilled dioxane. To this solution was added at once a mixture of dioxane (2 ml), phosphate buffer (2 ml, 0.033 M, pH 9.1), isopropanol (1 ml) and sodium borohydride (3 mg). After 45 min at room temperature the mixture was extracted with ethyl acetate, and the organic layer was washed with water. The mixture contained, as determined by NMR, the 3β-ol (56%), 3α-ol (13%), starting ketone (19%), and enone 4 (12%). NMR of 3α-ol: 0.70 (s, 3H, C-18), 1.02 (s, 3H, C-19), 3.41 (d, 1H, J = 3.2 Hz, C-6), 4.17 (m, 1H, W1/2 = 12 Hz, C-3), 4.52 (t, 1H, J = 3 Hz, C-7). NMR of enone 4: 0.77 (s, 3H, C-18), 1.36 (s, 3H, C-19), 4.30 (t, 1H, J = 2.6 Hz, C-7), 4.50 (d, 1H, J = 2.6 Hz, C-6), 5.86 (s, 1H, C-4).

Reduction of 3 with ^{3}H-sodium borohydride

The ketone (25.5 mg) was dissolved in 5 ml of freshly distilled dioxane and ^{3}H-sodium borohydride (25.0 mCi, sp. act. 532.0 mCi/mmol, New England Nuclear) was dissolved in isopropanol (10 ml) and added to a mixture of dioxane (5 ml) and phosphate buffer (5 ml, 0.33 M, pH 9). This mixture was then added to the ketone solution. After one hour at room temperature the mixture was extracted with ethyl acetate. The ethyl acetate was washed with water and dried over sodium sulfate. The residue (20 mg) was dissolved in chloroform (200 µl) and chromatographed on a Waters Z-module using 10% dioxane in hexane as eluant at a flow rate of 3 ml/min to yield the desired product (6, retention time = 8.6 min) in 54% yield.

7-Dehydro[3α-^{3}H]cholesterol, 5β,6β-oxide 7

The labelled alcohol 6 (10 mg) was dissolved in freshly distilled DMSO (1 ml). Potassium tert-butoxide (10 mg) in DMSO (1 ml) was added, and the mixture was heated at 55°C for 1 hr. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with dilute bicarbonate and dried over anhydrous sodium carbonate. The residue was chromatographed on a Waters Z-module using 10% dioxane in hexane as eluant at a flow rate of 3 ml/min. The desired product 7 retention time = 12.9 min) was obtained in 33% yield as determined spectrophotometrically in acetonitrile (221 nm, ε 8300). The specific activity obtained was 60 mCi/mmol with 99.5% radiochemical purity as measured by TLC on Silica Gel GHLF (Analtech Uniplates) plates eluted with 20% dioxane/cyclohexane (R_f = 0.35).

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