A FACILE SYNTHESIS OF 5β-CHOLESTANE-3α,7α,12α,25-TETROL*

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ABSTRACT

A convenient procedure for the synthesis of 5β-cholestane-3α,7α,12α, 25-tetrol via a modified homologation sequence of the intermediate 3α, 7α,12α-triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane involving a homogeneous medium is described. This involves treating the intermediate α-diazoketone in methanol with a solution of silver benzoate in triethylamine. Grignard reaction of the resulting triformyloxy methyl homocholate yielded 5β-cholestane-3α,7α,12α,25-tetrol. Large amounts of this bile alcohol were needed to further investigate the defect of cholic acid biosynthesis in patients with cerebrotendinous xanthomatosis (CTX).

INTRODUCTION

The cerebrotendinous xanthomatosis (CTX) patients described by Setoguchi, et al. (1) were first suggested to lack a normal 26-hydroxylase activity which would explain that the major part of cholic acid in these patients was formed through an alternate pathway involving 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24,25-pentol as intermediates (2,3). Recent investigations by Salen, et al.(4) suggest that the accumulation of various 25-hydroxylated cholestanols in the CTX patients could be due to a low 5β-cholestane-3α,7α,12α,25-tetrol-24β-hydroxylase activity as compared with normal individuals (4). Furthermore, 5β-cholestane-3α,7α,12α,25-tetrol has been demonstrated to be a key intermediate in the alternate pathway of cholic acid biosynthesis in CTX and normolipidemic subjects (5,6).

In order to further investigate this defect of cholic acid biosynthesis in CTX patients, we required larger amounts of the 25-hydroxylated tetrol. A facile synthesis of this bile alcohol (fig. 1 comp. V) via a

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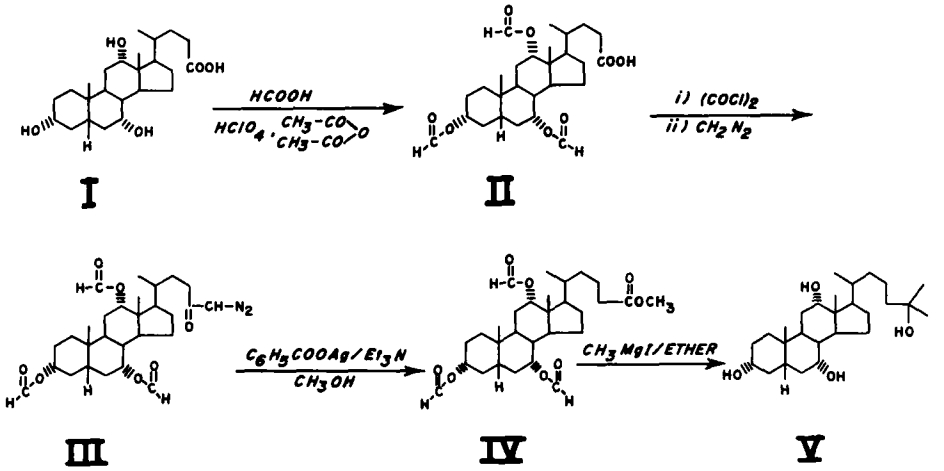

Fig. 1

modified homologation sequence of the intermediate 3α,7α,12α-triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane (figure 1, compound III) is described.

METHODS

Physical measurements: Melting points were determined on a Thermolyne apparatus, model MP-12600, and are uncorrected.

Optical rotations were determined at 25°C in methanol on a Carey model 60 spectropolarimeter.

GLC: 5β-cholestane-3α,7α,12α,25-tetrol, as a TMSi-derivative, was analysed on a 180cm x 4mm column packed with 3% QF-1, 230°C (Hewlett-Packard model 7610 gas chromatograph).

Mass spectra were obtained with a Varian MAT-111 gas chromatograph-mass spectrometer (Varian Associates, Palo Alto, Ca.). High resolution mass spectra were recorded on a model CEC-110 (Consolidated Electrodynamics Corp., Monrovia, Ca.).

TLC: Performed on silica gel G plates (Brinkmann, 0.25 mm thickness) The spots were detected with phosphomolybdic acid (3.5% in isopropanol), sulphuric acid (10%) and heating for one minute at 110°C. Bands on preparative TLC were made visible with iodine or water.

EXPERIMENTAL

(A) Preparation of silver benzoate reagent.

Silver benzoate was prepared by mixing equivalent solutions of silver nitrate and sodium benzoate. The precipitate thus formed was filtered under reduced pressure, washed with copious amounts of distilled water and dried in a vacuum dessicator at room temperature. In order to obtain maximum yields of the desired homologation product, it is advisable to prepare the silver benzoate reagent fresh each time this modified Wolff rearrangement is run (7).

(B) Preparation of 3α,7α,12α-triformyloxy-5β-cholan-24-oic acid (triformyloxy-cholic acid) (fig. 1, compound II).

Triformyloxy-cholic acid (m.p. 205-207°C) was synthesized from cholic acid and purified as described by Tserng and Klein (8).

(C) Preparation of 3α,7α,12α-triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane (fig. 1, compound III).

Compound III, m.p. 124-126°C (reported m.p. 128-129°C (9) was synthesized and identified by mass spectrometry as described previously (10,11) except that for the preparation of the starting compound, 3α,7α, 12α-triformyloxy-5β-cholan-24-yl chloride, oxalyl chloride ($COCl)_2$ was substituted for thionyl chloride ($SOCl_2$). For each gram of dried triformylated bile acid placed in a 100 ml flame dried flask with magnetic stirrer 11.1 ml of dry benzene and 2.2 ml of oxalyl chloride are added. The reaction mixture is allowed to stir for 2 hrs. at room temperature with subsequent workup as previously described (12). The triformyloxy-acid chloride was then treated with diazomethane (10), yielding 3α,7α, 12α-triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane (fig. 1, compound III) which was used without further purification. The mass spectrum of the diazoketone exhibited a major fragment of m/e 432 ($M^+ - C_3H_4ON_2$) due to McLafferty style cleavage of the side chain between carbon-22 and carbon-23 suggesting the presence of a ketonic group at carbon-24 (10).

(D) Silver benzoate-triethylamine catalysed rearrangement of 3α,7α,12α, triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane: Preparation of the methyl ester of 25-homo-triformyloxy-cholic acid (fig. 1, comp. IV (7).

A solution of 1.1 g (.002 mol.) of III, in 25 ml of absolute methanol was placed in a 100 ml three necked flask fitted with dropping funnel, magnetic stirrer, reflux condenser and connection to an azotometer. Twenty-five drops of catalyst prepared from 0.5 g (2.2 mmol.) of silver benzoate and 5 ml of triethylamine (distilled from barium oxide) was added to the diazoketone solution at room temperature. The addition of the catalyst promoted a rapid reaction in which the reaction mixture turned black and the evolution of nitrogen commenced. As soon as the evolution of nitrogen slackened, a new addition of silver benzoate solution was made. A total of 1.0 g of silver benzoate solution was added over a period of one hour with the subsequent generation of 96% of the theoretical amount of nitrogen obtained and the cessation of

further reaction. The mixture was then heated to reflux for one hour after the addition of charcoal and filtered. The solvents were removed from the filtrate under reduced pressure. The residue was dissolved in ethyl acetate and washed with 250 ml of 10% $NaHCO_3$ solution and twice with 250 ml of distilled water. The solvent layer was subsequently drie over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product (790 mg) showed four spots on TLC (solvent system: chloroform, acetone, methanol: 70:20:2.5 v/v/v), which coalesced to one after reaction with Grignard reagent (See experimental part E).**

(E) Preparation of 5β-cholestane-3α,7α,12α,25-tetrol (fig. 1, comp. V).

A mixture of mono, di and triformylated methyl homocholates** (790 mg) obtained from the preceding Wolff rearrangement was used for a Grignard reaction without further purification as described by Dayal, et al. (10). The residue was dissolved in 30 ml of anhydrous ether and ben zene (20:10 v/v) and added to 15 ml of methyl magnesium iodide and worke up as previously reported (9,10). The reaction yielded 630 mg of crude product (fig. 1, compound V) tentatively confirmed on TLC, solvent syste chloroform, acetone, methanol: 70:50:7.5 (v/v/v), R_f = 0.30. This compound was further purified on a column of neutral alumina grade V, using an effluent of ethyl acetate with increasing amounts of methanol (up to 5-7% methanol v/v). Column chromatography yielded pure 5β-cholestane-3α,7α,12α,25-tetrol (480 mg), m.p. 188-190°C (reported m.p. 189-190°C (10), TLC, GLC, $[\alpha]_D^{25}$, NMR and mass spectral characteristics matched those in the literature (1,3,5,9).

DISCUSSION

Cholic acid biosynthesis is defective in individuals with cerebrotendinous xanthomatosis (CTX) and is associated with the excretion of 5β-cholestane-3α,7α,12α,25-tetrol, an intermediate in the 25-hydroxylati pathway of cholic acid in CTX (5). Furthermore, the results of the in vivo as well as the in vitro experiments suggested that the site of the enzymatic defect in CTX is at the 24(S)-hydroxylation of 5β-cholestane-3α,7α,12α,25-tetrol (4). The need for appreciable quantities of 25-hydroxy-tetrol in our studies of the 24β-hydroxylase system prompted us to consider a more convenient and direct method for the synthesis of this compound.

Tserng and Klein have recently developed a simple effective synthetic route for the preparation of bile acid formates (8). These bile acic formates are usually crystalline and are easily purified, making this the

procedure of choice for the preparation of 3α,7α,12α-triformyloxy-24-oxo-25-diazo-25-homo-5β-cholane (fig. 1, comp. III) from the easily prepared triformate of cholic acid (fig. 1, comp. II).

In earlier literature reports, the homologation of α-diazoketones (10,11) has been achieved by a high temperature (180-200°C) Arndt-Eistert reaction on the appropriately substituted α-diazoketone. In contrast to the rather severe conditions of this method, the approach described herein, which is essentially a new modification of the Wolff rearrangement as described by Newman and Beal (7), proceeds under mild conditions (See experimental), thus providing a facile route for the synthesis of a variety of 25-hydroxylated bile alcohols. The yields of this modified rearrangement are usually 75-80%. In addition to its facility, one avoids the use of highly toxic, carcinogenic materials like 2,4,6-trimethylpyridine and benzyl alcohol necessary to the high temperature Arndt-Eistert reaction previously described (10,11). However, experiments performed by Newman and Beal on various substituted α-diazoketones show that the α-hydrogen is necessary for the reaction to take place. This is a limitation of this procedure.

After this homologation step, which directly gives the methyl ester of 25-homocholic acid in the form of mono, di and triformates, it is subjected to a Grignard reaction with methyl magnesium iodide (in slight excess) which not only reacts with the methyl ester functionality to generate the 25-hydroxy group but also assists in the removal of the remaining formyl groups present in the nucleus of the molecule, thus regenerating the hydroxyl groups at positions 3,7 and 12.

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** Personal communication with Professor M.S. Newman, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. Initial trial of this reaction by us first yielded results that were ambiguous and difficult to interpret, i.e. the formation of four different products as demonstrated by TLC. Professor Newman suggested to us that these may be trans-esterification products of the original triformate esters and/or products of partial deformylation; hence their lability in the presence of Grignard reagent.

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