IMPROVED SYNTHESIS OF 3α ,7α ,12α ,24ξ -TETRAHYDROXY-5β -CHOLESTAN-26-OIC ACID

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ABSTRACT

This paper describes three simple and short methods for the conversion of cholic acid into cholylaldehyde with protected hydroxyl groups. The first method involves lithium aluminum hydride reduction of the tetrahydropyranyl ether of methyl cholate and oxidation of the resulting primary alcohol with pyridinium chlorochromate. The second method employs diborane for the reduction of the -COOH group to the $-CH_2OH$ group, while the third method involves the reduction of 3α ,7α ,12α -triformyloxy-5β -cholan-24-oic acid (as the acid chloride) directly into 3α ,7α ,12α -triformyloxy-5β -cholan-24-al with TMA-ferride (tetramethylammonium hydridoirontetracarbonyl). The aldehyde obtained by any of the above methods underwent smooth Reformatsky reaction with ethyl α -bromopropionate to yield 3α ,7α ,12α ,24ξ -tetrahydroxy-5β -cholestan-26-oic acid.

INTRODUCTION

The biosynthesis of cholic acid from cholesterol is thought to proceed via the 26-hydroxylation of the obligatory intermediate, 5β -cholestane-3α ,7α ,12α -triol (1). The 26-hydroxylated bile alcohol is transformed into 3α ,7α ,12α -trihydroxy-5β -cholestan-26-oic acid (2) which is considered to yield the C_{24} bile acid via the 24-hydroxylated C_{27} bile acid (3,4). Recent studies in our laboratory have

demonstrated a new pathway for cholic acid biosynthesis which involves 25-hydroxylation of 5β -cholestane-3α ,7α ,12α -triol (5,6). The 25-hydroxylated bile alcohol is then transformed into cholic acid via the intermediary formation of 5β -cholestane-3α ,7α ,12α ,24S,25-pentol (7). In order to further study the relative roles of the 25- and 26-hydroxylation pathways in mammals (8), we needed a quantity of 3α , 7α ,12α ,24ξ -tetrahydroxy-5β -cholestan-26-oic acid. This acid has been synthesized by the Raformatsky reaction of cholyl aldehyde and ethyl α -bromopropionate (9), but the reported synthesis of cholyl aldehyde involves a number of steps (10,11). In the present paper, we report three short, simple methods for the synthesis of cholyl aldehyde with protected hydroxyl groups in good yield. The aldehyde thus obtained underwent smooth Reformatsky reaction to yield 3α ,7α , 12α ,24ξ -tetrahydroxy-5β -cholestan-26-oic acid.

MATERIALS AND METHODS

Materials

Tetramethylammonium hydridoirontetracarbonyl (TMA-ferride) was purchased from Aldrich Chemical Co., Milwaukee, Wis.

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

Infrared spectra were recorded in chloroform solution on a Perkin-Elmer model 421 grating spectrophotometer.

GLC. The bile acid as its methyl ester trimethylsilyl (TMSi) derivative was analyzed on a 180cm x 4mm column packed with 1% HiEFF-8BP on 80/100 mesh Gas Chrom Q; column temp. 240°C (Hewlett-Packard model 7610 gas chromatograph).

Mass spectra was obtained with a Varian MAT - 111 gas chromatograph-mass spectrometer (Varian Associates, Palo Alto, CA.).

TLC was done on silica gel G plates (Brinkmann, 0.25mm thickness). The spot were detected by spraying the plate with phosphomolybdic acid

(3.5% in isopropanol) and sulfuric acid (20%) and heating for 2 minutes at 110°C.

Preparation of 3 α,7 α,12 α-tritetrahydropyranyl-5 β-cholan-24-al (III, Fig. 1).

To 1.5 gm of cholic acid in freshly distilled dihydropyran (10ml), p-toluene-sulfonic acid (100mg) was added and the reaction mixture was stirred at room temperature for 2 hours. Ether (100ml) was added and the solution was washed with water (2x10ml). The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness. The oily product (Ia) (1.65gm) was divided into two equal portions.

One portion of Ia was taken in ether (20ml) and poured over an ethereal solution of CH_2N_2 (prepared from 1.5gm of N-nitroso-N-methylurea) at -10°C. After standing overnight at 0°C, the ether was evaporated. The pale yellow oily product thus obtained was dissolved in dry tetrahydrofuran (10ml) and $LiAlH_4$ (1gm) was added to the solution. The mixture was refluxed under anhydrous conditions for 3 hours and then cooled. Excess $LiAlH_4$ was destroyed by the addition of moist ethyl acetate (1ml) and then the careful addition of a few drops of water. After the addition of 10ml of 1N HCl, the contents were extracted with ether (2x50ml). The ether layer was washed with water (3x10ml) and dried over anhydrous sodium sulfate. Evaporation of ether yielded 0.45gm of a colourless glassy oil (II). The compound failed to crystallize, but it showed a single spot on TLC (solvent system, chloroform:methanol, 98:2), R_f 0.28, IR (chloroform solution), 3450cm^{-1} (-OH). It showed the following important peaks in the mass spectrum: m/z 561 (0.2%, M^+-85); 461 [0.7%, M^+-(2x85+CH_3)]; 377 [6%, M^+-(2x85+84+CH_3)]; 376 [2%, M^+-(3x85+CH_3)]; 359 [15%, M^+-(2x85+84+CH_3+H_2O)]; 358 [4%, M^+-(3x85+CH_3+H_2O)]; 341 [19%, M^+-(2x102+101)]; 340 [4%, M^+-(3x102)]; 253 [3%, M^+-(3x102+side chain)]; 213 [1%, M^+-(3x102+side chain + ring D)] and 85 (100%).

The second portion of Ia was dissolved in dry tetrahydrofuran (5ml) and the solution was cooled to -5°C. To this solution, a 2M solution of diborane in tetrahydrofuran (1.5ml) was added under strictly anhydrous conditions, and the reaction mixture was stirred for 5 minutes. Excess diborane was destroyed by dropwise addition of water (0.2ml) and the reaction mixture was stirred with 2N H_2SO_4 (2ml) for 2 hours. The contents were extracted with ether (50ml), the ether extract was washed with water (5ml), 5% NaOH (5ml) and water (3x5ml), respectively and dried over anhydrous sodium sulfate. Evaporation of ether yielded 0.42gm of a colourless glassy oil (II) which could not be crystallized, but showed a single spot on TLC, identical with that obtained for II prepared above and the IR spectra of the two compounds were identical.

Compound II (0.4gm) obtained by either method was dissolved in dry methylene chloride (2ml) and pyridinium chlorochromate (0.4gm) was added. The reaction mixture was shaken at room temperature for

2.5 hours and then diluted with ether (25ml). The almost black solid was filtered off and the pale yellow filtrate thus obtained was washed with 10% Na_2CO_3 (2x5ml) and then with water (3x5ml) and dried over anhydrous sodium sulfate. Evaporation of ether in a current of N_2 and slightly reduced pressure yielded 0.37gm of a pale yellow semi-solid (III). On TLC (solvent system, chloroform: methanol 98.2), it showed a single spot, R_f 0.7. In the IR spectrum, the peak at 3450cm^{-1} was absent and a peak due to C=O group was observed at 1710cm^{-1}. It showed the following important fragments in the mass spectrum: m/z 644 (0.01%, M^+); 559 (0.06%, M^+-85); 474 (0.15%, M^+-2x85); 389 (0.5%, M^+-3x85); 338 (2%, M^+-3x102); 253 [2%, M^+-(3x102+side chain)]; 213 [1%, M^+-(3x102+side chain + ring D)]; 85 (100%).

This product (III) (0.36gm) was kept overnight at room temperature. TLC now revealed the presence of an additional compound with the same R_f value as the tritetrahydropyranyl ether of cholic acid (Ia). The product was dissolved in ether (50ml) and washed with 10% Na_2CO_3. The aqueous layer was acidified with dilute HCl at 0°C and extracted with ether to yield 0.27gm of the acid Ia. Afterwards, compound III was used for further reaction immediately after isolation.

Preparation of 3 α,7 α,12 α-triformyloxy-5 β-cholan-24-al (IV).

Triformyloxy cholic acid (Ib, 500mg) (12) was dissolved in dry benzene (5ml) and solution was cooled in an ice bath. Freshly distilled thionyl chloride (1.5ml) was added to this solution and the contents were stirred at room temperature for 2 hours. Solvents were distilled off under reduced pressure and the last traces of thionyl chloride were removed by distilling off twice under reduced pressure with 5ml portions of dry benzene. The pale yellow semi-solid acid chloride thus obtained was dissolved in dry methylene chloride (4ml) and to the solution 0.5gm of tetramethylammonium hydridoirontetracarbonyl (TMA-ferride) was added. The contents were stirred at room temperature for 4 hours under a current of dry N_2. Methylene chloride was evaporated under reduced pressure and at low temperature (0° to -5°C) and the residue was extracted with ether (2x50ml). The combined ether extract was washed with 10% Na_2CO_3 (2x5ml) and then with water (3x10ml) and dried over anhydrous sodium sulfate. Evaporation of ether under a current of N_2 and slightly reduced pressure yielded 0.35gm of pale yellow semi-solid (IV). TLC of this product showed a single spot R_f 0.6 (solvent system, chloroform:methanol, 98.2). On keeping , the compound was slowly converted into the starting compound (Ib); it was quickly dissolved in dry toluene and used for the next reaction.

Preparation of 3 α,7 α,12 α,24 ξ-tetrahydroxy-5 β-cholestan-26-oic acid (V).

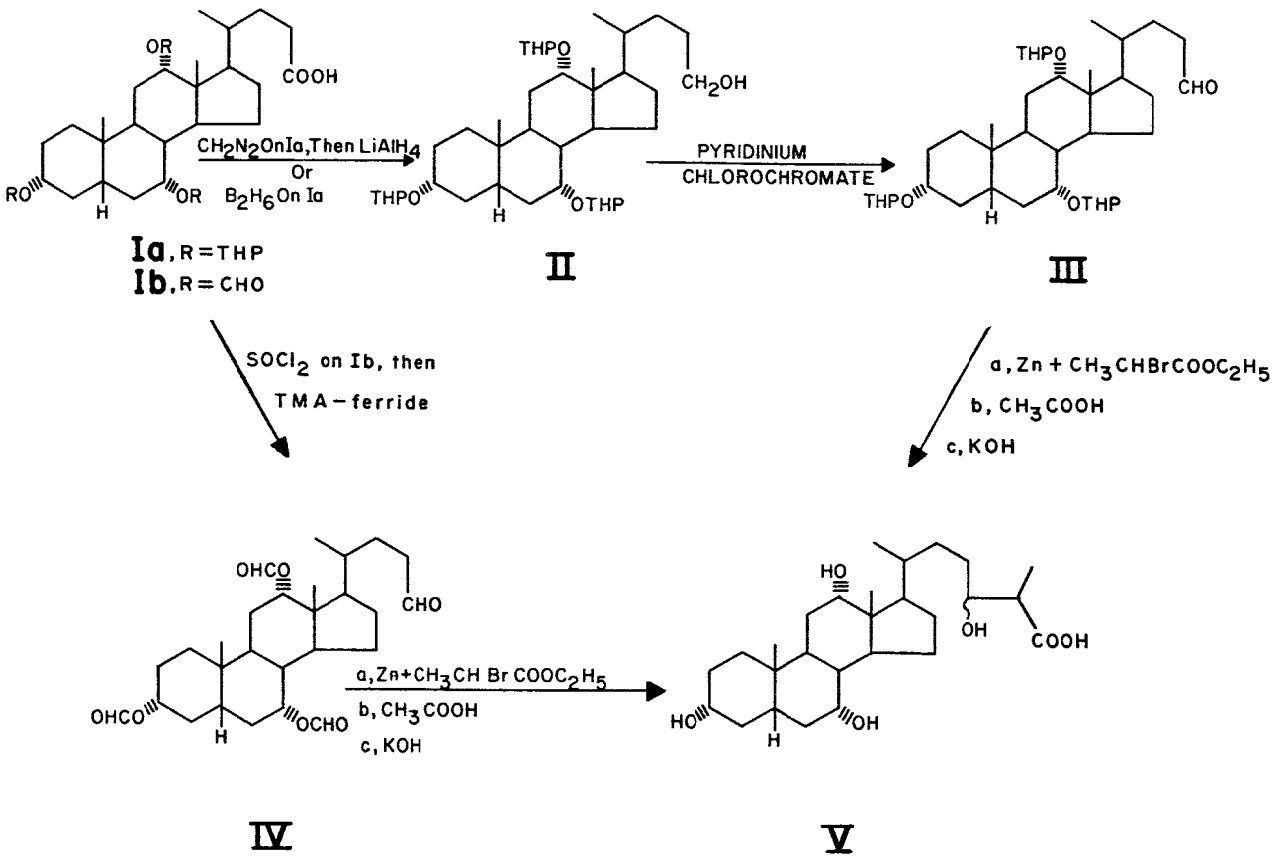
To a solution of III or IV (0.9gm) in dry toluene (20ml) were added ethyl α-bromopropionate (1ml), granulated zinc (500mg); a few crystals of iodine and powdered copper (5mg). The mixture was refluxed in an oil bath when a vigorous reaction set in and the solution

became cloudy. After 1.5 hours, the reaction mixture was cooled and poured over 10% sulfuric acid (10ml) at 0°C. After adding ethyl acetate (20ml), the organic layer was separated, washed with water (4x10ml), dried over anhydrous sodium sulfate and the solvents were evaporated (9). The residue was refluxed with 70% acetic acid (10ml) for 1 hour and the solvent was evaporated. (This step was not required when (IV) was used as the starting material). The residue was hydrolyzed by refluxing with 10% ethanolic potassium hydroxide (20ml) for 3 hours. Water (30ml) was added and most of the ethanol was removed under reduced pressure. The resulting solution was cooled and acidified with dilute HCl and extracted with ethyl acetate (2x25ml). The ethyl acetate extract was washed with water (4x10ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 0.5gm of the acid V as pale yellow semi-solid. Attempts to crystallize it failed. TLC in benezene:isopropanol:acetic acid (30:10:1) showed the presence of one major spot R_f 0.24 (9).

The semi-solid V was dissolved in methanol (2ml) and esterified with an ethereal solution of diazomethane (prepared from 2gm of N-nitroso-N-methylurea). The product obtained was dissolved in ethyl acetate (2ml) and poured over a column containing neutral alumina (10gm) suspended in ethyl acetate. The column was eluted with 100ml of ethyl acetate:methanol (95:5). Evaporation of solvents from the eluate yielded 0.48gm of a pale yellow semi-solid which was crystallized from ethyl acetate as colorless needles, m.p. 164-65°C [Lit. m.p. 164°C (9)], R_f 0.6 [solvent system, benzene:isopropanol:acetic acid (30:10:1)], Its trimethylsilyl ether had a GLC retention time of 4.12 relative to 5 α-cholestane (retention time of 5 α-cholestane, 5.28 minutes) and showed the following major peaks in the mass spectrum: m/z 753 (3%, M^+-15); 678 (0.8%, M^+-90); 588 (8%, M^+-2x90); 501 [1%, M^+-(2x90+87)]; 498 (22%, M^+-3x90); 411 [4%, M^+-(3x90+87)]; 408 (7%, M^+-4x90); 321 [11%, M^+-(4x90+87)]; 343 [27%, M^+-(2x90+side chain)]; 281 (22%, M^+-(3x90+C-22 to C-27)]; 253 [72%, M^+-(3x90+side chain)]; 211 [10%, M^+-(3x90+ ring D)]; 189 (9%, cleavage a); 73 [100%, $(CH_3)_3Si^+$].

RESULTS AND DISCUSSION

Inai et al have described the synthesis of 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestan-26-oic acid by the Reformatsky reaction of the tetrahydropyranyl ether of cholyl aldehyde with ethyl α-bromopropionate (9). However, their method is laborious and involves six steps. Furthermore, we have observed that this cholyl aldehyde readily undergoes oxidation to the tetrahydropyranyl ether of cholic acid on exposure to air. Since Inai et al converted cholyl aldehyde

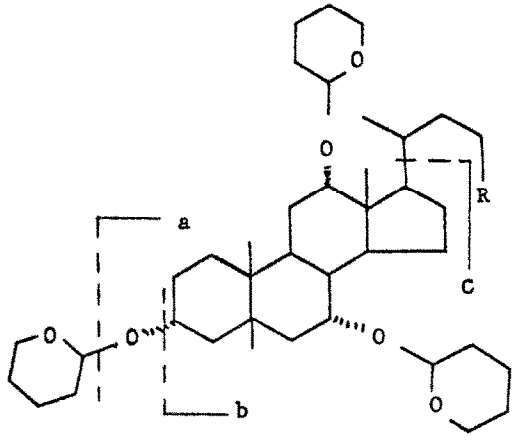

OR
COOH
RO
OR
H
CH2N2 On Ia, Then LiAlH4
Or
B2H6 On Ia
THPO
CH2OH
OTHP
PYRIDINIUM
CHLOROCHROMATE
CHO
Ia, R = THP
Ib, R = CHO
II
III
SOCl2 on Ib, then
TMA-ferride
a, Zn + CH3CHBrCOOC2H5
b, CH3COOH
c, KOH
OHCO
OCHO
a, Zn+CH3CH Br COOC2H5
b, CH3COOH
c, KOH
HO
OH
IV
V

into its tetrahydropyranyl ether before performing Reformatsky reaction, it is probable that there was appreciable loss of the starting material. The present paper describes three methods for the synthesis of the tetrahydropyranyl or the formyloxy derivative of cholyl aldehyde by the direct conversion of the -COOH group to the -CHO group. The substituted aldehyde thus obtained was used for Reformatsky reaction without isolation.

The first method involves lithium aluminium hydride reduction of the tetrahydropyranyl ether of methyl cholate to the corresponding alcohol II in almost quantitative yield. This alcohol on oxidation with Corey's reagent (13) was completely converted into the desired aldehyde (III). Yoon et al (14) have reported a convenient and extremely fast reaction of diborane in tetrahydrofuran with carboxylic acids. This reaction was successfully employed to convert Ia into II. In order to get optimum yields, it was found necessary to avoid the use of large excess of diborane, to perform the reaction at low temperatures and to limit the reaction time to 5-10 minutes. Also, Yoon et al (14) have reported that under these very mild conditions, the -COOH group is reduced in preference to the carbonyl and ester groups. This permitted the reduction of triformyloxycholic acid to the corresponding alcohol. However, the reaction was found to require very controlled conditions and the addition of a small excess of diborane resulted in partial deformylation. Therefore, the tetrahydropyranyl ether (Ia) only was used for this reaction.

The structures of II and III were confirmed by their mass spectra. The alcohol II did not show a molecular ion peak but fragments due to the successive losses of one, two and three tetrahydropyran

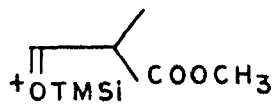
groups (m/z 85) were observed at m/z 561, 461 [M^+-(2x85+CH_3)] and 376 (cleavage type a). Cleavage type b gave fragments due to successive losses of 102 mass units at m/z 544, 442 and 340. Fragment at m/z 253 formed by loss of the side chain from the fragment at m/z 340 (cleavage type c) confirmed the molecular weight of the side chain. Fragments at m/z 377, 359 and 341 probably resulted from a hydrogen transfer to the fragments at m/z 376, 358 and 340 respectively. The base ion peak was observed at m/z 85 due to the tetrahydropyranyl moiety. The aldehyde III showed a very weak molecular ion peak at m/z 644. The base ion peak was again observed at m/z 85 and the other fragments were generally of very poor intensity. The fragmentation pattern of this compound was very similar to that of II except that the fragments were 2 mass units less. The fragment at m/z 253 [M^+-(3x102+side chain)] revealed the molecular weight of the side chain as 85 mass units corresponding to $CH_3-\underset{|}{CH}-CH_2-CH_2-CHO$.



II. R= CH_2OH
III. R = CHO

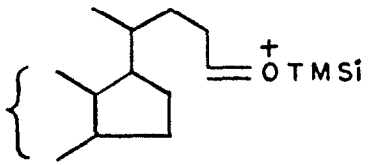
Recently, Cole and Pettit employed TMA-ferride for the reduction of acid chlorides to aldehydes in excellent yields (15). This led to the direct conversion of triformyloxycholic acid into the corresponding aldehyde IV via the preparation *in situ* of the acid chloride. The aldehyde was obtained smoothly in approximately 70% yield; the small amounts of unreacted acid chloride were removed as the free acid during washing with sodium carbonate.

The aldehyde obtained by any of the above methods was quite stable in toluene but underwent aerial oxidation to the starting acid when stored free of solvent. It was found that as much as 70% of the aldehyde was oxidized on keeping overnight at room temperature. It was, therefore, considered best to use the compound for Reformatsky reaction soon after it was prepared. The Reformatsky reaction was performed according to Inai *et al* (9). 3α,7α,12α,24ξ-Tetrahydroxy-5β-cholestan-26-oic acid thus obtained could not be crystallized but showed the same mobility on TLC as described previously (9). Its methyl ester could, however, be crystallized as colorless needles from ethyl acetate, m.p. 164-65°C, which agreed with the reported m.p. of 164°C. The structure of the methyl ester was confirmed from a study of the mass spectrum of its trimethylsilyl ether derivative.



m/z 189

(a)



m/z 501

(b)

The molecular ion peak was not observed but, the M^+-15 peak was observed at m/z 753 (3%). Four consequitive losses of 90 mass units due to $(CH_3)_3SiOH$ confirmed the presence of four -OH groups. The base ion peak was observed at m/z 73 [$(CH_3)_3Si^+$]. Peaks at m/z 189 (a) and at m/z 501 (b) and corresponding peaks at m/z 411 and 321 by subsequent losses of one and two molecules of $(CH_3)_3SiOH$ from (b) confirmed the presence of a trimethylsilyloxy group at C-24 and -COOH group at C-26 (16).

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