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SYNTHESIS OF 3β-HYDROXY-5-CHOLENIC ACID FROM 3β-HYDROXY-5-PREGNEN-20-ONE AIMED AT THE PREPARATION OF LABELLED STEROID COMPOUNDS

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SUMMARY

3β-Hydroxy-5-cholenic acid was synthesized from 3β-hydroxy-5-pregnen-20-one in nine steps. The procedure would permit the labelling of C-22, C-23, and/or C-24 of the bile-acid side chain by choice of the appropriate labelled reagent.

INTRODUCTION

3β-Hydroxy-5-cholenic acid, a degradation product of cholesterol [1, 2], has been employed as the starting material for the synthesis of different steroid compounds [3–7]. However, in all published accounts to date, 3β-hydroxy-5-cholenic acid has been obtained either by oxidative degradation of 5, 6-dibromocholesteryl acetate [8], as a byproduct of the preparation of androstenolone, or as a transformation product from natural bile acids [9].

In this report we described a synthesis of the title compound from the inexpensive 3β-hydroxy-5-pregnen-20-one (pregnenolone) which is readily available from natural sources. The 3β-hydroxy-5-cholenic acid was required by us in connection with some of our biosynthetic studies [10], and the synthetic pathway described below would allow the preparation of bile acid-analogs labelled in the side chain.

EXPERIMENTAL

General. Optical rotations were measured with a Perkin–Elmer model 141 automatic polarimeter. Mass spectra were registered at 70 eV with a Varian-Mat CH-7 spectrometer provided with a direct-inlet system. Gas–liquid chromatography was conducted with a Hewlett–Packard dual chromatograph on glass columns filled with 2% OV-17 on silanized Chromosorb G. All other determinations of physical properties were performed as described previously [11].

3β-Acetoxy-24-norchol-5-en-23-al (VI). Compound V, prepared as previously reported [11], (3.1 g) in ethanol was hydrogenated at atmospheric pressure and room temperature over 10% palladium on charcoal (310 mg) for 2 h. The solid was filtered off and the filtrate was evaporated. The residue (3.1 g) was recrystallized from isopropanol yielding 2.3 g of compound VI, m.p. 123–125°, $[\alpha]_D^{25}$ −55.6° (*c* 0.6, $CHCl_3$); I.R. data: 2720, 1735, 1725, 1250, 800 cm^{-1}; p.m.r. data: 0.75 (s, 3H, Me-18), 0.91 (d, J = 6 Hz, Me-21 of 20R-isomer), 1.03 (s, 3H, Me-19), 2.03 (s, 3H, CH_3—COO), 4.58 (b.s., 1H, H-3), 5.42 (m, 1H, H-6), 9.77 (q, 1H, J = 1.5 Hz, H-23); M.S. data: (*m/e*) 386 (M^+), 326 (M-60) base peak. Compound VI is very unstable at room temperature in the presence of air.

3β-Acetoxy-24-norchol-5-en-23-ol (VII). Compound VI (2.2 g) was dissolved in ethanol (150 ml), treated with $NaBH_4$ (300 mg) and the solution was stirred at room temperature for 50 min. The excess of reagent was destroyed by addition of HCl:EtOH (1:2 V/V), the mixture diluted with water (700 ml) and extracted with ether. The ethereal extract was washed with water, saturated $NaCO_3H$ solution, and water, and dried over $MgSO_4$. Evaporation of the solvent afforded a residue which was chromatographed on a dry silica gel column. Elution with benzene, benzene:CH_2Cl_2, and CH_2Cl_2:MeOH produced 2.1 g of compound VII which after recrystallization from MeOH had m.p. 144–145°, $[\alpha]_D^{25}$ −56.3° (*c* 1.0, $CHCl_3$): I.R. data: 3350, 1730, 1250, 800 cm^{-1}; p.m.r. data: 0.72 (s, 3H, Me-18), 0.93 (d, J = 6 Hz, Me-21 of 20R-isomer), 1.03 (s, 3H, Me-19), 2.03 (s, 3H, $C\underline{H}_3$—COO), 3.75 (m, 2H, H-23), 4.58 (b.s., 1H, H-3), 5.42 (m, 1H, H-6), a hydroxyl signal at 1.55 disappears after treatment with D_2O; M.S. data: (*m/e*) 370 (M^+-18), 328 (M-60) base peak.

Anal. Calc. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.00; H, 10.40.

23-chloro-24-norchol-5-en-3β-ol-acetate (VIII). A mixture of compound VII (600 mg) and anhydrous triphenylphosphine (468 mg) in CCl_4 (3.3 ml) was

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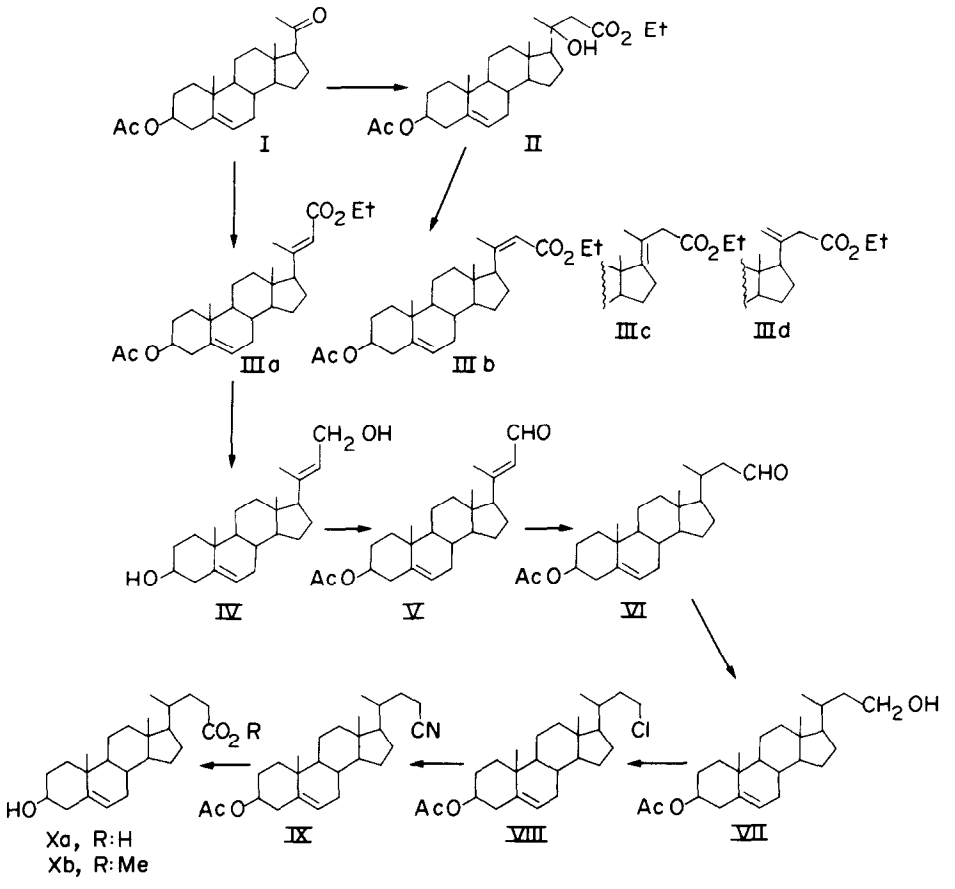

Fig. 1. Synthesis of 3β-hydroxy-5-cholenic acid (Xa) from pregnenolone acetate.

heated under reflux for 22 h. Light petroleum (3.3 ml) was added, the precipitate was filtered off, and the filtrate was evaporated. The residue was chromatographed on a dry column (silica gel, 45 g) eluting with mixtures of light petroleum: benzene of increasing polarity, affording 450 mg of pure compound VIII (t.l.c.). This was recrystallized from MeOH and had m.p. 128–129°, $[\alpha]_D^{25}$ −51.1° (*c* 1.0, $CHCl_3$); I.R. data: 1730, 1250 cm^{-1} (no hydroxyl band); p.m.r. data: 0.72 (s, 3H, Me-18), 0.95 (d, $J = 6$ Hz, Me-21 of 20R-isomer), 1.03 (s, 3H, Me-19), 2.03 (s, 3H, CH_3—COO), 3.55 (m, 2H, H-23), 4.58 (b.s., 1H, H-3), 5.41 (m, 1H, H-6); M.S. data: (*m/e*) 346:348 ($M^+ - 60$) base peak.

Anal. Calc. for $C_{25}H_{39}ClO_2$: C, 73.77; H, 9.66; Cl, 8.71. Found: C, 73.79; H, 9.90: Cl, 9.00.

3β-Acetoxychol-5-en-24-yl nitrile (IX). Compound VIII (204 mg) and dry KCN (110 mg) were treated with freshly distilled DMSO (3.8 ml) and the mixture was stirred and heated at 100° for 5 h. It was then poured into water and the solution was extracted with ether. The ethereal extract was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by preparative t.l.c. (silica gel) eluting with benzene: CH_2Cl_2 (1:1). Pure compound IX (179 mg) after recrystallization from MeOH had m.p. 141–142°, $[\alpha]_D^{25}$ −49.4° (*c* 1.0, $CHCl_3$); I.R. data: 2240, 1730, 1250 cm^{-1}; p.m.r. data: 0.72 (s, 3H, Me-18), 0.96 (d, $J = 6$ Hz, Me-21 of 20R-isomer), 1.03 (s, 3H, Me-19), 2.03 (s, 3H, CH_3—COO), 2.30 (m, 2H, H-23), 4.58 (b.s., 1H, H-3), 5.40 (m, 1H, H-6); M.S. data: (*m/e*) 397 (M^+), 337 (M-60) base peak.

Anal. Calc. for $C_{26}H_{39}NO_2$: C, 78.54; H, 9.89; N, 3.52. Found: C, 78.52; H, 9.79; N, 3.70.

3β-Hydroxychol-5-enic acid (Xa). A solution of compound IX (50 mg) in 90% EtOH (1.3 ml) containing NaOH (180 mg) was heated under reflux for 44 h. Water (50 ml) was then added and the solution was washed with ether. The alkaline aqueous solution was made acid with diluted HCl and extracted with ether. The ethereal extract was washed with water, dried over $MgSO_4$, and evaporated. The residue of 3β-hydroxy-5-cholenic acid (47 mg) was recrystallized from ethyl acetate. It had m.p. 216–217°, $[\alpha]_D^{24}$ −38.6° (*c* 0.5, pyridine), and its I.R. spectrum was identical with that of an authentic sample.

Methylation of compound Xa (22 mg) with diazomethane afforded the methyl ester Xb, p.m.r. data: 0.68 (s, 3H, Me-18), 0.92 (d, $J = 6$ Hz, Me-21 of 20R-isomer), 1.00 (s, 3H, Me-19), 1.22 (b.s., 1H, HO—) it disappears on treatment with D_2O, 3.58 (b.s., 1H, H-3), 3.65 (s, 3H, CH_3—COO), 5.33 (m, 1H, H-6).

RESULTS AND DISCUSSION

Pregnenolone acetate (I) reacted with ethyl bromoacetate in Refortmasky conditions producing, after acetylation of the reaction product, 3β-acetoxy-20-hydroxy-24-norchol-5-en-23-oic acid ethyl ester (II) [11]. The configuration at C-20 of compound II is thought to be mainly *S* following recent publications on the Grignard reactions of pregnenolone and other 20-keto-pregnane derivatives [12], although in the present experiment a small amount of the (20R) isomer was also formed. Dehydration of compound II afforded a mixture of four products (g.l.c.) two of which were isolated by column chromatography and fractioned crystallization; the compounds thus obtained, IIIa and IIIb, amounting 75% of the product mixture, were characterized as the isomeric (E) and (Z) 3β-acetoxy-24-norchola-5,20(22)-dien-23-oic acid ethyl ester respectively. The stereochemistry of IIIa and IIIb has been assigned on basis of the respective p.m.r. spectra [13]. Compounds IIIc and IIId, also formed in the reaction, were characterized as a mixture by p.m.r. spectroscopy.

The same compounds IIIa and IIIb were also prepared by the Wittig reaction between pregnenolone acetate and carboethoxymethylentriphenyl phosphorane. Recrystallization from ethanol gave pure IIIa whose physical properties were almost identical to those previously reported [11]. Compound IIIa was reduced by lithium aluminum hydride to the corresponding unsaturated alcohol IV. The physical properties of IV were in accord with those reported for the (E)20(22)-isomer [14]. In our previous synthesis [11] compound IV could have been contaminated with a small proportion of isomer (Z). Oxidation of compound IV with activated manganese dioxide produced, after acetylation, 3β-acetoxy-24-norchol-5, (E)20(22)-dien-23-al (V) previously prepared by ourselves [11], and then by Gut *et al.* [14] but following a different approach. Catalytic hydrogenation of compound V gave 3β-acetoxy-24-norchol-5-en-23-al (VI) of m.p. 123–125° and $[\alpha]_D$ −55.6°; in the literature there exist disagreement in the physical properties of the pure (20R)-isomer, for Sucrow [15] reported m.p. 140–142° and $[\alpha]_D$ −69.6° while Gut [16] gave m.p. 125–130° but no specific rotation value. From the p.m.r. spectrum of compound VI it could be inferred that our compound was mainly the (20R) isomer with a very small proportion of the (20S)-isomer. This assumption was confirmed after transformation of VI by reaction with sodium borohydride into 3β-acetoxy-24-norchol-5-en-23-ol (VII) whose p.m.r. spectrum presented a doublet at δ 0.93 which is typical of the 21-Me signal of the (20R)-epimer [17]. Treatment of compound VII with triphenylphosphine in carbon tetrachloride [18a] gave the chloro-derivative VIII in 70% yield. In turn, compound VIII on reaction with potassium cyanide in dimethylsulphoxide [18b] afforded 3β-acetoxychol-5-en-24-yl nitrile (IX). In both compounds VIII and IX the 21-Me signal, in the respective p.m.r. spectrum, was in a position which is indicative of the (20R)-epimer [19]. The signal from 21-Me of the (20S)-epimer ($\delta \simeq 0.85$) could not be discerned out of the methylenic absorption.

Alkaline hydrolysis of the nitrile IX gave the expected 3β-hydroxy-5-cholenic acid (Xa) which was purified by recrystallization leading to a product of m.p. 216–217° and $[\alpha]_D$ −38.6° (pyridine). A commercial sample of 3β-hydroxy-5-cholenic acid (supposedly pure 20R) had m.p. 230–232° and $[\alpha]_D$ −35.6° (pyridine). Analysis of Xa by t.l.c. presented two close spots one of which, the major and more polar, had the same R_F value as the standard; preparative t.l.c. allowed the separation and elution of the minor and less polar product which had m.p. 211–212° (the more polar product could not be eluted).

Infrared analysis of the eluted compound showed the carboxyl absorption at 1710 cm^{-1} while the commercial standard presented the carbonyl band at 1680 cm^{-1}. A raw product from the hydrolysis of IX showed both bands, therefore we could assume that the acid of m.p. 211–212° could be the (20S)-epimer while the one of m.p. 216–217° is mostly the (20R)-epimer, although it is believed that I.R. analysis is not a reliable procedure for the estimation of the amount of each isomer occurring in a (20R), (20S) epimeric mixture.

Methylation of Xa with diazomethane produced the methyl ester Xb which also confirmed the structure assigned to the final product. Hence, this work would allow the synthesis of cholenic acid labelled at C-22 and/or C-23—by the use of ethyl bromoacetate prepared from acetic acid labelled at different carbon-atoms—and at C-24 by the use of labelled sodium or potassium cyanide.

Moreover, the present work could be considered as a complement of our previous synthesis of [20-^{14}C]-pregnenolone [20] to which the present procedure could be applied.

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