Sterol synthesis: chemical synthesis of 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile

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Summary 3β-Hydroxy-5α-cholest-7-ene-9α-carbonitrile was obtained via hydrocyanation of 3β-acetoxy-5α-cholest-8-en-7-one by diethylaluminum cyanide. The resulting 9α-cyano-7-ketone was reduced with sodium borohydride to 7α-hydroxy-compound. These compounds were characterized by infrared, nuclear magnetic resonance, and mass spectral measurements.—**Anastasia, M., and P. Allevi.** Sterol synthesis: chemical synthesis of 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile. *J. Lipid Res.* 1981. **22:** 370–372.

Supplementary key words sterols · 9α-cyano sterols

In connection with our studies on the possible inhibitory action of modified cholesterol biosynthetic precursors (1–4) having an "unnatural" substituent in the nucleus, we planned the preparation of the previously undescribed 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile. The cyano group present in 9α-position precludes the equilibrium of the Δ^7 double bond to Δ^8 position and thus different biological behavior can be expected for this sterol compared to alkyl substituted and oxygenated steroids (5, 6).

In this study we describe a simple synthesis of 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile from the known compound 3β-acetoxy-5α-cholest-8(9)-en-7-one (**Fig. 1**).

EXPERIMENTAL PROCEDURES AND RESULTS

General procedures

IR spectra were taken for solutions in $CHCl_3$. NMR spectra were determined on $CHCl_3$ solutions of the sterols at 100 MHz on a Varian XL-100 spectrometer using tetramethylsilane as internal standard. Mass spectra were recorded on a Varian 112-S by direct inlet. TLC was performed on precoated silica gel G plates (E. Merck, HF_{254}), visualized by spraying with 70% sulfuric acid followed by heating. GLC analyses were made using a Carlo Erba Fractovap 2400 T unit using either 3% OV-17 or 1% SE-30 on Gas Chrom Q (100–120 mesh) columns operating at 220–240°C. All compounds gave satisfactory (±0.2%) elemental analysis.

Materials

3β-Acetoxy-5α-cholest-8(9)-en-7-one (I; mp 155–156°C; purity in excess of 98% on the basis of GLC analyses on a 1% SE-30 column) was prepared as described by Fieser (7). Diethylaluminum cyanide was purchased from Alfa Division of Ventron Corporation, Danvers, MA.

3β-Acetoxy-7-oxo-5α-cholestane-9α-carbonitrile (II)

The enone I (900 mg; 2.0 mmol) in anhydrous benzene (4 ml) was treated with diethylaluminum cyanide (10 ml of 0.7 M solution in benzene) at 0°C for 1 hr. The mixture was poured dropwise into a solution of sodium potassium tartrate (500 ml, 20%) and extracted with dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The resulting crude residue was crystallized from diethyl ether to give 3β-acetoxy-7-oxo-5α-cholestane-9α-carbonitrile (II) (770 mg) melting at 175–176°C; $[\alpha]_D^{23}$-30° (c.,1); IR, 2240, 1735, and 1720 cm^{-1}; NMR δ 1.24 (3 H, s, 19-CH_3), 0.66 (3 H, s, 18-CH_3; calcd (8, 9) 0.658); MS, 469 (100%, M), 451 (35%, M-H_2O), 391 (52%, M-H_2 O-AcOH), 315 (46%), 273 (60%). The compound was pure as judged by TLC (20% benzene in hexane).

3β-Acetoxy-7α-hydroxy-5α-cholestane-9α-carbonitrile (III)

The ketone II (900 mg; 1.9 mml) in diethyl ether (15 ml) and methanol (50 ml) was treated with sodium borohydride (600 mg; 15.8 mmol) at 20°C for 30 min. Water was added and the resulting mixture was extracted with benzene. The extracts were washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated to dryness under reduced presssure. The resulting crystalline residue was crystallized from diethyl ether to give compound III (720 mg) melting at 162–163°C; $[\alpha]_D^{23}$-15°; IR, 3600, 2220, and 1730 cm^{-1}; NMR, δ 3.9 (1 H, m, 7β-H), 0.98 (3 H, s, 19-CH_3; calcd (8) 0.957, 9α-CN = + 0.140 ppm), 0.68 (3 H, s, 18-CH_3; calcd (8, 9) 0.658; MS, 471 (1%, M), 453 (3%), 393 (100%), 378 (13%), 280 (22%), 253 (15%); it was pure as judged by TLC (20% ethyl acetate in benzene).

3β-Acetoxy-5α-cholest-7-ene-9α-carbonitrile (Va)

A solution of the 7α-ol (III) (500 mg; 1.1 mmol) in dry pyridine (20 ml) was cooled to 0°C and added

Abbreviations: IR, infrared; NMR, nuclear magnetic resonance; MS, mass spectra; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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with methanesulphonyl chloride (1 ml; 12.8 mmol). The solution was allowed to warm to 4°C and after 2 hr was poured into ice-cold water. Compound IV separated as a filterable solid that was filtered off, washed, and crystallized from methanol to yield a solid (450 mg), melting at 170–171°C (with decomposition; variable with rate of heating). A solution of the methanesulphonate (IV) (450 mg; 0.82 mmol) in dry collidine (15 ml) was refluxed under nitrogen for 12 hr. After cooling to room temperature, water (100 ml) was added and the resulting mixture was extracted three times with diethyl ether (20-ml portions). The combined extracts were washed with dilute HCl, aqueous sodium bicarbonate, water, and saline, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The resulting crystalline residue was subjected to chromatography on a silica gel G–celite–$AgNO_3$ (50:50:30; 25 g) column (100 cm × 2 cm) using 10% benzene in hexane as eluting solvent. Fractions of 30 ml were collected. The contents of fractions 30 through 38 were pooled and, after evaporation of the solvent under reduced pressure, crystallized from methanol to give compound Va (270 mg) melting at 139–140°C; $[\alpha]_D^{20}$-10 (c. 1); IR, 2220, 1730 cm^{-1}; NMR δ 5.4 (1 H, m, 7-H), 0.94 (3 H, s, 19-CH_3; calcd (8) 0.940, 9α-CN = 0.140 ppm); MS, 453 (3%, M), 393 (100%), 378 (13%), 280 (22%), 253 (14%), 238 (13%); the compound was pure by TLC (90:10 silica gel G–$AgNO_3$) (solvent 10% diethyl ether in hexane).

3β-Hydroxy-cholest-7-ene-9α-carbonitrile (Vb)

A solution of the acetate Va (70 mg) in methanol (10 ml) was treated at room temperature with 2N methanolic KOH for 24 hr. Water was added (15 ml) and the resulting mixture was extracted three times with diethyl ether (10-ml portions). The combined extracts were washed twice with a saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The resulting crystalline residue was crystallized from methanol to give Vb (45 mg) melting at 149–150°C; $[\alpha]_D^{21}$-12 (c. 1); IR 3480, 3225, 2220 cm^{-1}; NMR, δ 5.3 (1 H, m, 7-H), 0.95 (3 H, s, 19-CH_3), 0.55 (3 H, s, 18-CH_3); MS, 411 (19%; M) 393 (100%) 378 (17%), 298 (25%), 280 (25%), 253 (20%), 238 (13%); it was pure by TLC (20% ethyl acetate in benzene).

DISCUSSION

Described herein are chemical procedures for the synthesis of 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile (Vb) having a 9α-cyano group in place of the 9α-hydrogen present in 3β-hydroxy-5α-cholest-7-ene, a well known biological precursor of cholesterol. The starting material for the synthesis was 3β-acetoxy-5α-cholest-8-en-7-one (I) (7). Conjugated addition of cyanide to I by diethylaluminum cyanide (10) affords in good yield 3β-acetoxy-7-oxo-5α-cholestane-9α-carbonitrile (II) as the only product. The "natural" 8β,9α-configuration for II was demonstrated as follows. Treatment with methanolic sodium hydroxide and reacetylation had no effect on the properties of this "ketone"; therefore C-8 is in the thermodynamically most stable configuration. Molecular models indicate that, in 14α series, the 8α, 9α and 8α,9β configurations are considerably less stable than the 8β,9α and 8β,9β configurations and, even if formed initially, the 8α-structure would be expected to rearrange in the presence of base to the more stable epimer of 8β configuration. Therefore, if II possesses a 9α-cyano group, reduction of the 7-ketone with sodium borohydride would be expected to proceed predominantly by β-attack and so produce relatively more of the 7α-alcohol than does 3β-hydroxy-5α-cholestan-7-one (73% α, 27% β) (11). In facts only one alcohol (as shown by GLC, TLC, and NMR) was obtained in crystalline form. The axial configuration of the hydroxy group in the product 3β-acetoxy-7α-hydroxy-5α-cholestane-9α-carbonitrile (III) was evident from the relative difficulty of acetylation and from the NMR spectrum. A peak at δ 3.9, identical in shape with the peak at δ 3.95 in the spectrum of 3β-acetoxy-

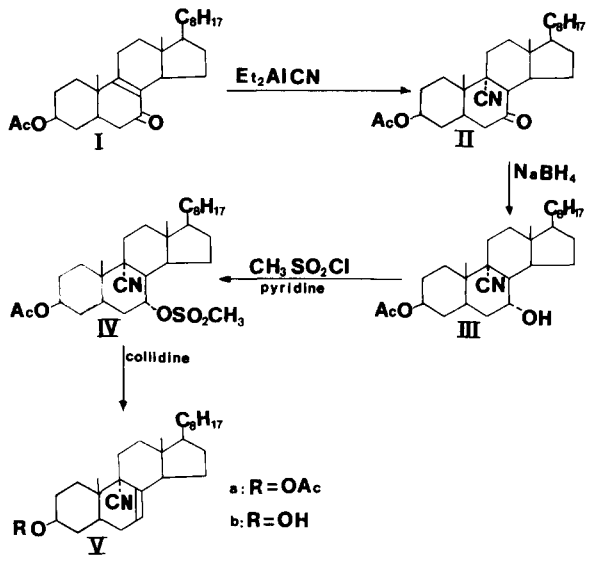

Fig. 1. Synthetic scheme for the synthesis of 9α-cyano sterols. I, 3β-acetoxy-5α-cholest-8(9)-en-7-one; II, 3β-acetoxy-7-oxo-5α-cholestane-9α-carbonitrile; III,3β-acetoxy-7α-hydroxy-5α-cholestane-9α-carbonitrile; IV, 3β-acetoxy-7α-methylsulphonyloxy-5α-cholestane-9α-carbonitrile; Va, 3β-acetoxy-5α-cholest-7-ene-9α-carbonitrile; Vb, 3β-hydroxy-cholest-7-ene-9α-carbonitrile.

7α-hydroxy-14α-methyl-5α-cholestane (12), was assigned to the 7β-proton. A 7α-proton would be strongly coupled and should give rise to a broad multiplet. The C-19 methyl resonance shows a downfield shift (0.140 ppm) with respect to the calculated value (8) for the same methyl group in the 9-unsubstituted compound. This appears in accord with the downfield shift observed for the same methyl group in 5α-cyano steroids (13). In addition, a very close agreement between the observed chemical shift of the angular C-18 methyl group of III and the value calculated according to the tables of Zürcher (8) was observed. The 9α-cyano group does not modify the chemical shift of the C-18 methyl group as observed in the case of 9α-cyano-19-nortestosterone (9). Attempted dehydration of 3β-acetoxy-7α-hydroxy-5α-cholestane-9α-carbonitrile (III) to the 3β-acetoxy-5α-cholest-7-ene-9α-carbonitrile (Va) with thionyl chloride or phosphoryl chloride in pyridine was unsuccessful. However, compound Va was obtained by treating compound III with methanesulphonyl chloride in pyridine to furnish 3β-acetoxy-7α-methylsulphonyloxy-5α-cholestane-9α-carbonitrile (IV), followed by heating in collidine for 12 hr. NMR and mass spectra confirmed the structure. The presence of an olefinic proton resonance at 5.50 ppm was compatible with the presence of a Δ^7 double bond. The C-19 proton signal occurred at 0.94 ppm, downfield with respect to that of 9α-unsubstituted compound, thus indicating the location of the cyano group in 9α-position. Saponification of 3β-acetoxy group affords 3β-hydroxy-5α-cholest-7-ene-9α-carbonitrile (Vb). The present synthesis reported herein is the first case of the introduction of a cyano group in 9α position of a steroidic compound starting from a Δ^8-7-ketone via hydrocyanation (10). Due to the fact that the cyano group is a versatile synthon, our results open the way to the C-9-substituted steroids. These substances possess a nonmodified stereochemistry with respect to natural steroids and may interfere with enzymes involved in the metabolism of steroids.

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