Synthesis of New Nitro and Amino Sterols; Potential Inhibitors of 4-Methyl Sterol Oxidase

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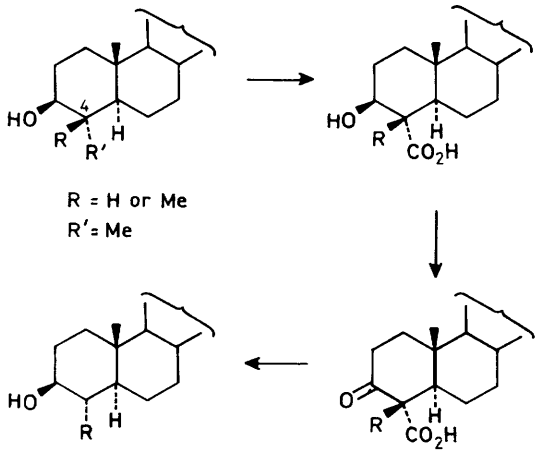
New 4-nitro and 4-amino 5α-steroids were made regioselectively from cholesterol *via* nitration of 3-acetoxy-5α-cholesta-1,3-diene (**2**). Nitration of enol acetate (**2**) gave 4α-nitro-5α-cholest-1-en-3-one (**3**), which was catalytically reduced to 4α-nitro-5α-cholestan-3-one (**4**). Sodium borohydride reduction of (**4**) gave 4α-nitro-5α-cholestan-3β-ol (**5**). Reduction of 4α-nitro-5α-cholestan-3β-ol with lithium aluminium hydride furnished 4-amino-3,4-seco-5α-cholestan-3-ol (**6**), rather than the desired 4α-amino-5α-cholestan-3β-ol (**8**). The synthesis of compound (**8**) required prior derivatization of the 4α-nitro sterol as the 3-(tetrahydropyran-2-yl) ether. The related 4α-aminomethyl-5α-cholestan-3β-ol (**12**) has also been synthesized, *via* a 4α-formyl intermediate.

It has been shown by Gaylor and co-workers [1] that a sterol 4α-carboxylic acid is an intermediate in the oxidative 4-demethylation of lanosterol by enzymes of the microsomal fraction of rat liver homogenate. This 4α-carboxylate is considered to be the result of successive oxidations of the 4α-methyl group leading to 4α-hydroxymethyl and 4α-formyl intermediates. This proposed sequence is supported by the experimentally demonstrated metabolism of postulated intermediates and of synthetic steroids. The 4-demethylation process is completed by the action of an NAD^+-linked decarboxylase which oxidizes the 3β-hydroxy group to a 3-ketone prior to decarboxylation of the resulting 4α-carboxy-3-ketone. The process ends with enzymic reduction of the 3-ketone to a 3β-hydroxy group [2] (Scheme 1).[2]

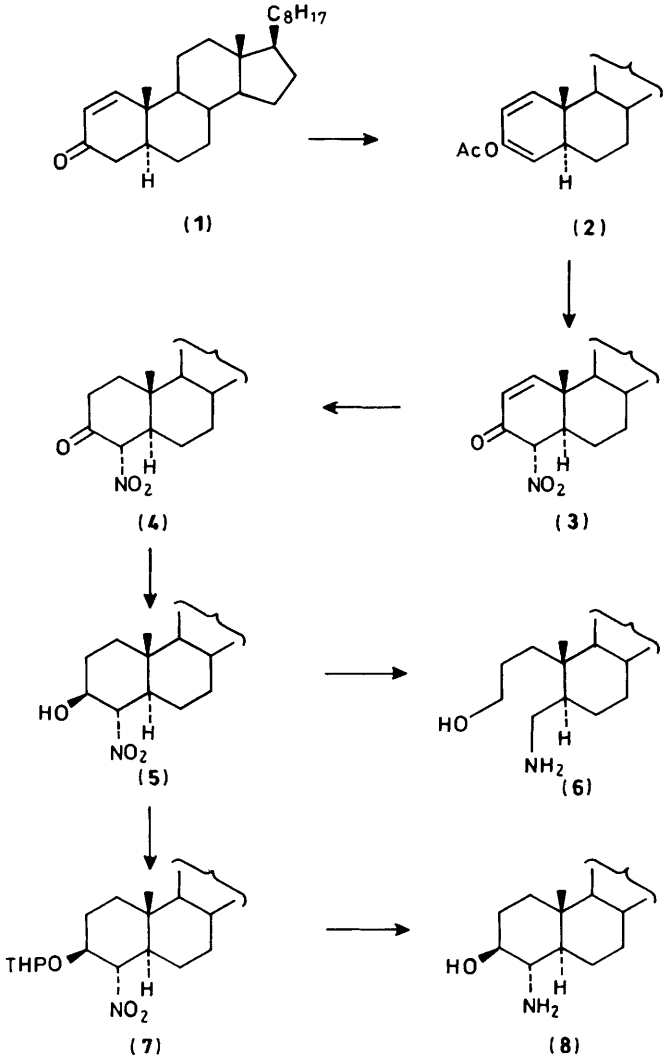


Scheme 1.

As part of an investigation of the sterol 4-demethylation process we have embarked on the development of inhibitors of 4-methyl sterol oxidase. Such inhibitors may be useful probes for the mechanistic study of the enzymes involved in sterol 4-demethylation. This laboratory has earlier reported the design, synthesis, and evaluation of a mechanism-based inactivator of this enzyme system.[3] We report here the synthesis of the hitherto undescribed 4α-nitro-5α-cholestan-3β-ol (**5**) as well as some new amino sterols all of which were designed as potential inhibitors of the 4-methyl sterol oxidase system. The 4α-nitro compound (**5**) is an analogue of a 4α-carboxy-3β-hydroxy steroid and might be expected to inhibit the 4-demethylase process at the methyl oxidase step and/or the decarboxylase stage. The 4α-nitro-3-oxo precursor (**4**) of (**5**) is an analogue of the 4α-carboxy-3-oxo sterol formed in the NAD^+-linked enzymatic decarboxylation process described above. The 4α-aminomethyl compound (**12**) was envisaged as an oxidizable analogue of the 4α-hydroxymethyl intermediate in the 4-methyl oxidase sequence. The 4α-amino compound (**8**) was likewise viewed as a potential mechanism-based inactivator as a result of enzymatic *N*-oxidation.

The preparation of cyclic α-nitro ketones has been reviewed recently,[4] while the synthesis of α-nitrocyclohexanone has been described recently and mechanistic, stereochemical, and conformational aspects of the reaction have been detailed.[5] Of the available literature methods for the preparation of α-nitro ketones, the nitration of a steroidal 3-enol acetate is in principle the simplest route to a 4-nitro-3-keto steroid. However, 3-keto 5α-steroids preferentially enolize towards C-2. In order to generate selectively 4α-nitro-5α-cholestan-3-one (**4**), we imposed the necessary regiocontrol at the enol acetate stage of the synthesis. Thus, 5α-cholest-1-en-3-one was treated with lithium di-isopropylamide, and the resulting enolate was trapped by acetic anhydride to give 3-acetoxy-5α-cholesta-1,3-diene (**2**) (Scheme 2).

Although nitronium tetrafluoroborate has been reported to convert enol acetates and enol trimethylsilyl ethers into the corresponding α-nitro ketones,[5] in our hands the reaction failed when applied to compound (**2**). We then turned to the ammonium nitrate–trifluoroacetic anhydride (TFAA) reagent [5c] which converted the acetate (**2**) into 4α-nitro-5α-cholest-1-en-3-one (**3**) in very good yield. The alpha configuration of the 4-nitro group followed from the large proton coupling constant (13.4 Hz) seen for the C-4 hydrogen. The 1,2-olefin grouping was reduced catalytically (5% Pd/C) to yield quantitatively 4α-nitro-5α-cholestan-3-one (**4**). Again the 4α-configuration is assigned to the nitro group as a result of the 12.8 Hz proton coupling constant seen for the C-4 hydrogen. No enolic form was detected by spectroscopic examination in neutral solution and the existence of compound (**4**) in the ketonic form is to be contrasted with the case of 2-nitro-3-oxo 5α-steroids [6] which exist mainly in the enolic form. On the other hand, a 4β-nitro-3-oxo-5β-androstane has been reported to be entirely ketonic,[6] as are 6α- and 6β-nitro-7-oxo steroids of the 5α-series.[7] The lack of enolization in the 6-nitro-7-oxo steroids, the 4β-nitro-5β-3-oxo

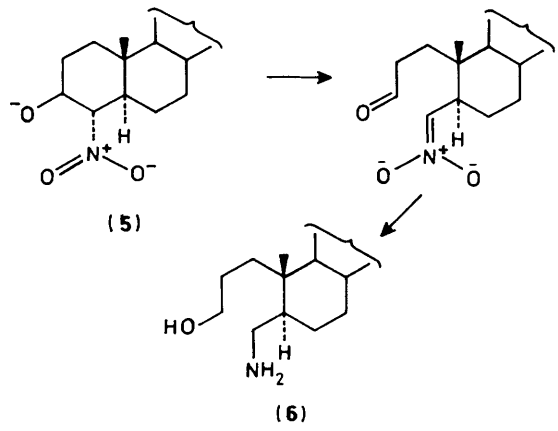


Scheme 2. C_8H_{17} = CH(Me)[CH_2]$_3$CHMe$_2$, THP = [CH_2]$_4$OCH-

steroid, and our 4α-nitro-3-ketone can be attributed in each case, at least in part, to severe nonbonded interactions in the enol forms. These enol-destabilizing effects stem respectively from nitro–4-H,[7] nitro–7-H_α,[6] and nitro–6-H interactions. In addition, enolization of the 6-nitro-6-oxo steroids and the 4α-nitro-3-ketone of this work would generate, respectively, the energetically unfavourable 6,7- or 3,4-double bond.

The 3-carbonyl group was then reduced by sodium borohydride with high stereoselectivity to furnish the desired 4α-nitro-5α-cholestan-3β-ol (**5**). The very high stereoselectivity may be the result of complexation of borohydride with the α-oriented nitro group at C-4 with concomitant α-face delivery of hydride ion at C-3.

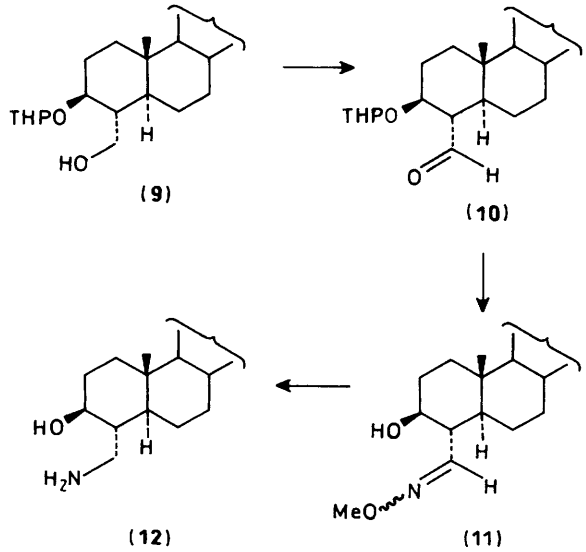
We then attempted the synthesis of 4α-amino-5α-cholestan-3β-ol (**8**) from the above nitro compound (**5**) by reduction with lithium aluminium hydride. However, the reduction of compound (**5**) with lithium aluminium hydride gave instead a product formulated as 4-amino-3,4-seco-5α-cholestan-3-ol (**6**), characterized as the acetoxy acetamido derivative. This product may have arisen by generation of an alkoxide under the reduction conditions, followed by ring fission to give a 3,4-seco-steroid with subsequent reduction to the amino alcohol (**6**) (Scheme 3). To avoid deprotonation of the 3β-hydroxy group in



Scheme 3.

the alcohol (**5**) and thus to avoid cleavage of ring-A, 4α-nitro-5α-cholestan-3β-ol (**5**) was therefore converted into the 3-tetrahydropyran-2-yl (THP) ether (**7**), prior to lithium aluminium hydride reduction. The desired 4α-amino-5α-cholestan-3β-ol THP ether was then obtained in good yield. Removal of the THP group gave the desired amino sterol (**8**). Alternatively catalytic hydrogenation (5% Pd/C) of the protected nitro sterol (**7**) gave the same amine (**8**), after removal of the THP group followed by chromatographic purification.

The homologous amine, 4α-aminomethyl-5α-cholestan-3β-ol (**12**), was made from 4α-formyl-5α-cholestan-3β-ol THP ether (**10**), itself prepared *via* Collins oxidation of 4α-hydroxymethyl-5α-cholestan-3β-ol THP ether (**9**) (Scheme 4).[8] Reaction of



Scheme 4.

compound (**10**), with methoxylamine hydrochloride in pyridine–ethanol gave the methoxime (methoxyimine) which was isolated after silica chromatography as the free 3β-hydroxy compound (**11**). We consider that loss of the THP-ether group did not occur during the chromatography but had already taken place during work-up of the methoxime reaction. Compound (**11**) was then reduced by lithium aluminium hydride to give the amine (**12**), which was characterized as the benzylcarbamate, using benzyl chloroformate.

Experimental

M.p.s were determined on a Kofler hot stage and are uncorrected. ^{1}H N.m.r. spectra were measured in $CDCl_3$ solution using an IBM FT-80 spectrometer at 80 MHz or a Varian XL 200 MHz with TMS as internal standard. Low-resolution mass spectra were measured on an LKB-9000 instrument, and exact mass measurements were obtained using a Kratos VG70S spectrometer. Flash column chromatography, following the method of Still,[9] employed flash chromatography silica gel (J. T. Baker Chemical Co.).

5α-*Cholesta*-1,3-*dien*-3-*yl Acetate* (**2**).—To a stirred solution of di-isopropylamine (1.73 ml) in dry tetrahydrofuran (THF) (25.6 ml) at −20 °C under argon was added butyl-lithium (8.8 ml; 1.55M in hexane). After 30 min the temperature was lowered to −78 °C and a solution of 5α-cholest-1-en-3-one (**1**) (4.0 g) in dry THF (10.3 ml) was added. The mixture was allowed to warm to 0 °C, and was stirred at 0 °C for 1 h. The temperature was again lowered to −78 °C and acetic anhydride (1.16 ml, distilled from anhydrous K_2CO_3) was added. The reaction mixture was allowed to warm to room temperature during 1 h and was poured into cold water and extracted with ether (3 × 300 ml). The combined extract was washed with brine, dried (anhydrous K_2CO_3), and concentrated under reduced pressure. The crude product was chromatographed on silica gel and eluted with ethyl acetate–hexane (5:95) to afford, after crystallization from hexane, 5α-*cholesta*-1,3-*dien*-3-*yl acetate* (**2**) (3.86 g, 87%), m.p. 63—65 °C (Found: C, 81.9; H, 10.8. $C_{29}H_{46}O_2$ requires C, 81.63; H, 10.87%); $\nu_{max.}$($CHCl_3$) 1 745 and 1 650 cm^{-1}; $\lambda_{max.}$(EtOH) 262 nm (ε 2 700); δ_H 5.09 (1 H, dd, *J* 2 Hz, 4-H), 5.61 (1 H, dd, *J* 10.2, 2 Hz, 2-H), and 6.09 (1 H, d, *J* 10.2 Hz, 1-H); *m/z* 426 (M^+), 411, 386, 369, and 306.

4α-*Nitro*-5α-*cholest*-1-*en*-3-*one* (**3**).—To stirred mixture of 5α-cholesta-1,3-dien-3-yl acetate (**2**) (4.0 g, 9.38 mmol), and pre-ground NH_4NO_3 (750 mg, 9.38 mmol) in anhydrous chloroform (10 ml, distilled from P_2O_5 after passage through a column of activated alumina), under argon, was added TFAA (9.36 ml, distilled from $KMnO_4$ and then from K_2CO_3), in one portion. Within 1 h dissolution was attained, methylene dichloride (100 ml) was added and the reaction mixture was poured onto ice–water (150 ml). The organic phase was separated and the aqueous portion was extracted with methylene dichloride (2 × 100 ml). The combined organic phases were washed successively with 1% aqueous $NaHCO_3$, water, and brine, and dried (Na_2SO_4). The crude product was flash chromatographed on silica gel and eluted with ethyl acetate–hexane (5:95). The product was crystallized from hexane to afford 4α-*nitro*-5α-*cholest*-1-*en*-3-*one* (**3**) (2.60 g, 64%), m.p. 134—136 °C (Found: C, 75.3; H, 10.2; N, 3.1. $C_{27}H_{43}NO_3$ requires C, 75.48; H, 10.09; N, 3.26%); $\nu_{max.}$($CHCl_3$) 1 708, 1 690, and 1 555 cm^{-1}; $\lambda_{max.}$(EtOH) 239 nm (ε 9 800); δ_H 5.18 (1 H, d, *J* 13.4 Hz, 4-H), 5.97 (1 H, d, *J* 10.2 Hz, 2-H), and 7.27 (1 H, d, *J* 10.2 Hz, 1-H); *m/z* 429 (M^+), 397, 383, 342, and 300.

4α-*Nitro*-5α-*cholestan*-3-*one* (**4**).—4α-Nitro-5α-cholest-1-en-3-one (**3**) (2.6 g) was dissolved in cyclohexane–THF (2:1; 200 ml) containing 5% Pd/C (600 mg) and was hydrogenated for 3 h at 25 °C and atmospheric pressure. The mixture was filtered through a layer of Celite, concentrated under reduced pressure, and crystallized from hexane at −10 °C to afford 4α-*nitro*-5α-*cholestan*-3-*one* (**4**) (2.6 g, 99%), m.p. 194—196 °C (Found: C, 75.4; H, 10.5; N, 3.15. $C_{27}H_{45}NO_3$ requires C, 75.13; H, 10.51; N, 3.25%); $\nu_{max.}$($CHCl_3$) 1 725 and 1 555 cm^{-1}; δ_H 5.10 (1 H, d, *J* 12.8 Hz, 4-H); *m/z* 431 (M^+), 416, 414, 401, and 386. No high-intensity selective u.v. absorption was observed between 210 and 350 nm in either methanol or hexane.

4α-*Nitro*-5α-*cholestan*-3β-*ol* (**5**).—A solution of 4α-nitro-5α-cholestan-3-one (**4**) (500 mg) in benzene–methanol (3:7; 50 ml) was cooled in an ice–water-bath and sodium borohydride (300 mg) was added in one portion. After the mixture had been stirred for 5 min at 0 °C, excess of borohydride was destroyed by dropwise addition of acetone. The solvent was removed under reduced pressure, and the residue was taken up in ether; the solution was washed successively with 10% HCl (2 × 50 ml) and brine, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (silica gel) and elution with 15% ethyl acetate–hexane afforded, after crystallization from hexane, 4α-*nitro*-5α-*cholestan*-3β-*ol* (**5**), (486 mg, 97%), m.p. 164—165 °C (Found: C, 74.9; H, 11.1; N, 3.1. $C_{27}H_{47}NO_3$ requires C, 74.78; H, 10.92; N, 3.23%); $\nu_{max.}$($CHCl_3$) 3 590 and 1 545 cm^{-1}; δ_H 4.10 (1 H, m, 3-H) and 4.27 (1 H, dd, *J* 10 Hz, 4-H); *m/z* 433 (M^+), 418, 416, 399, and 387.

4-*Amino*-3,4-*seco*-5α-*cholestan*-3-*ol* (**6**).—A solution of 4α-nitro-5α-cholestan-3β-ol (**5**) (320 mg) in ether (230 ml) containing lithium aluminium hydride (150 mg) was refluxed for 3 h. After the mixture had cooled to 35 °C, excess of lithium aluminium hydride was destroyed by dropwise addition of ethyl acetate. The mixture was diluted with methylene dichloride (40 ml), treated with saturated aqueous Na_2SO_4, and filtered. Evaporation under reduced pressure gave the crude product, which was purified by chromatography (silica gel) using $CHCl_3$–CH_3OH–NH_4OH (132:6:1). Crystallization from methanol gave 4-amino-3,4-seco-5α-cholestan-3-ol (**6**) (196 mg), m.p. 84—86 °C. A portion was dissolved in acetic anhydride, and kept for 12 h at 25 °C. Work-up and crystallization from methanol gave 4-*acetamido*-3,4-*seco*-5α-*cholestan*-3-*yl acetate*, m.p. 132—134 °C (Found: M^+, 489.4181. $C_{31}H_{55}NO_3$ requires *M*, 489.4182); $\nu_{max.}$($CHCl_3$) 3 300, 1 740, 1 645, and 1 550 cm^{-1}; δ_H 1.94 (3 H, s, Ac), 2.01 (3 H, s, Ac), 4.0 (2 H, m, 3-H), and 5.47 (1 H, t, N*H*Ac).

4α-*Amino*-5α-*cholestan*-3β-*ol* (**8**).—A solution of 4α-nitro-5α-cholestan-3β-ol (**5**) (150 mg) in dry THF (15 ml) containing freshly redistilled dihydropyran (1.0 ml) and pyridinium toluene-*p*-sulphonate (30 mg) was stirred under N_2 at 25 °C for 18 h. The solvent was removed under reduced pressure and the residue was dissolved in ether; the solution was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dried under high vacuum for 4 h, redissolved in anhydrous ether (20 ml), and treated with lithium aluminium hydride (250 mg). After 3 h under reflux the solution was cooled to 25 °C, excess of lithium aluminium hydride was destroyed by the dropwise addition of ethyl acetate, and saturated aqueous Na_2SO_4 was added with stirring until the aluminium salts coagulated. The supernatant liquid was dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was then deprotected using 10% HCl (2 ml) in refluxing THF (25 ml) for 1 h, cooled to room temperature, and neutralized with saturated aqueous $NaHCO_3$. Ether extraction gave the crude product, which was chromatographed on silica gel. Elution with $CHCl_3$–CH_3OH–NH_4OH (132:12:1) gave 4α-amino-5α-cholestan-3β-ol (**8**) (124 mg), m.p. 188—190 °C.

A solution of the above 4α-amino-5α-cholestan-3β-ol (30 mg) in methylene dichloride (2 ml) was treated with acetic anhydride (5 ml) and the methylene dichloride was removed under reduced pressure. After 18 h at 25 °C the solid which had separated was filtered off and crystallized from methanol to afford 4α-*acetamido*-5α-*cholestan*-3β-*yl acetate* (26 mg), m.p. 144—146 °C (Found: M^+, 487.4031. $C_{31}H_{53}NO_3$ requires *M*, 487.4025); $\nu_{max.}$($CHCl_3$) 1 725, 1 720, and 1 520 cm^{-1}; δ_H 1.92 (3 H, s, Ac), 2.01 (3 H, s, AcO), 3.94 (1 H, q, *J* 10.4 Hz, 4-H), 4.61 (1 H, m, 3-H), and 5.20 (1 H, d, *J* 9.5 Hz, 4-NH).

4α-*Aminomethyl-5α-cholestan*-3β-*ol* (**12**).—A solution of 4α-hydroxymethyl-5α-cholestan-3β-ol 3-THP ether (**9**)[8] (1.0 g) in dry methylene dichloride (50 ml) was treated with Collins reagent (4.11 g), stirred at 25 °C for 20 min, and then filtered through a layer of Celite. The filtrate was passed through a short column of Florisil, the eluant was concentrated under reduced pressure, and the product was crystallized from acetone to give 3β-(tetrahydropyran-2-yloxy)-5α-cholestan-3α-carbaldehyde (**10**) (890 mg), m.p. 128—131 °C; $\nu_{max.}$($CHCl_3$) 2 860 and 1 725 cm^{-1}; δ_H 9.60 (d, *J* 4.8 Hz) and 9.47 (d, *J* 4.8 Hz) [4α-CHO; two signals because of the diastereoisomers of the 3-tetrahydropyranyl ether], 4.81 (br s) and 4.55 (br s) [acetal CH of tetrahydropyranyl ether], 3.96 (m, 3α-H), 3.75—3.43 (signals due to tetrahydropyranyl grouping), and 2.38 (m, 4β-H); *m/z* 500 (M^+), 416, and 400.

A suspension of this aldehyde (760 mg) and methoxylamine hydrochloride (260 mg) in dry pyridine (1.0 ml) and absolute ethanol (20 ml) was heated under reflux (2 h). Solvent was removed under reduced pressure and the residue was filtered through a silica gel column with hexane–ethyl acetate (2:3) to give the methoxime derivative (**11**) (580 mg) as a *Z*:*E* mixture; δ_H 7.15 (d, *J* 8 Hz) and 6.41 (d, *J* 8 Hz) [CH=N of methoxime isomers in the ratio 1.9:1.0], 3.8 (3 H, s, OMe), and 3.4 (1 H, m, 3α-H).

A solution of this material in anhydrous ether (30 ml) containing lithium aluminium hydride (500 mg) was heated under reflux for 4 h. After the mixture had cooled to room temperature, excess of lithium aluminium hydride was destroyed by dropwise addition of ethyl acetate, followed by addition of methylene dichloride (40 ml) then saturated aqueous Na_2SO_4. The resulting mixture was dried (Na_2SO_4), filtered, and chromatographed on silica gel. Elution with chloroform–methanol–ammonia (132:12:1) followed by crystallization from methanol gave 4α-*aminomethyl-5α-cholestan*-3β-*ol* (**12**) (491 mg, m.p. 166—172 °C (Found: M^+, 417.3980. $C_{28}H_{51}NO$ requires *M*, 417, 3971). Although the m.p. was broad, the compound was homogeneous by t.l.c. ($CHCl_3$–MeOH–NH_4OH, 132:12:0.9).

This amine was characterized as the benzylcarbamate derivative by reaction of a stirred solution of compound (**12**) (80 mg) in chloroform (10 ml) with benzyl chloroformate (0.30 ml) at 0—4 °C for 2 h. Dry pyridine (0.50 ml) was then added, and after being stirred for 30 min the solution was washed successively with 1M sulphuric acid, water, and brine, dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography on silica (methylene dichloride–methanol, 49:1) gave the *benzylcarbamate derivative* of amine (**12**) (88 mg, 83%), m.p. 162—164 °C (from MeOH) (Found: C, 78.3; H, 10.7; N, 2.6. $C_{36}H_{57}NO_3$ requires C, 78.35; H, 10.41; N, 2.54%); $\nu_{max.}$ 3 380 and 1 690 cm^{-1}; δ_H 7.35 (5 H, s, Ph), 5.10 (2 H, q, $PhCH_2$), 4.93 (1 H, m, NH), 3.82 (2 H, m, 3α-H and CHN), and 3.05 (1 H, CHN); *m/z* 551 (M^+), 533, and 444.

Acknowledgements

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