Synthesis of Cholesta-5,8-dien-3β-ol

Mario Anastasia,* Alberto Fiecchi, and Giovanni Galli

Institute of Chemistry, School of Medicine, and Laboratory of Applied Biochemistry, School of Pharmacy, University of Milan, I-20133 Milano, Italy

Received February 13, 1981

Cholesta-5,8-dien-3β-ol was synthesized in two steps by starting from 3β-acetoxycholesta-5,7-diene. Diethyl azodicarboxylate reacts with 3β-acetoxycholesta-5,7-diene to afford 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)-cholesta-5,8-diene and 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8(14)-diene. The former was then reduced with lithium in ethylamine to the title compound.

In a previous paper we have described[1] the isolation and the structure identification of the previously unknown cholesta-5,8-dien-3β-ol (**1a**, Chart I), a sterol accumulating in the liver of rats born from mothers given AY-9944 [*trans*-1,4-bis[[(2-chlorobenzyl)amino]methyl]cyclohexane dihydrochloride] a widely used inhibitor of the last steps of cholesterol biosynthesis. The dienol **1a** was also found in the liver of pregnant rats. Compound **1a** is of interest since its biosynthetic origin is not clear, and it may indicate that the 5,6-dehydrogenase does not require a Δ^7 substrate as commonly accepted in mammalians.[2] In order to carry out studies on the biosynthetic origin of **1a** and on its possible metabolism to cholesterol, we approached the problem of its synthesis.

Since the reported[3] synthesis of **1a** proved to be irreproducible,[4] a reaction sequence was initially devised in our laboratory for the preparation of **1a** by introduction of a Δ^5 double bond in a $\Delta^{8(9)}$ sterol, in analogy with the pathway reported for the synthesis of 22,23-dihydroergosterol from 5α-ergost-7-en-3-one.[5]

However, the presence of the Δ^8 double bond in the starting material lowers the yields of cholesta-4,8-dien-3-one and consequently of **1a** which was obtained in a total 0.2% yield.[6]

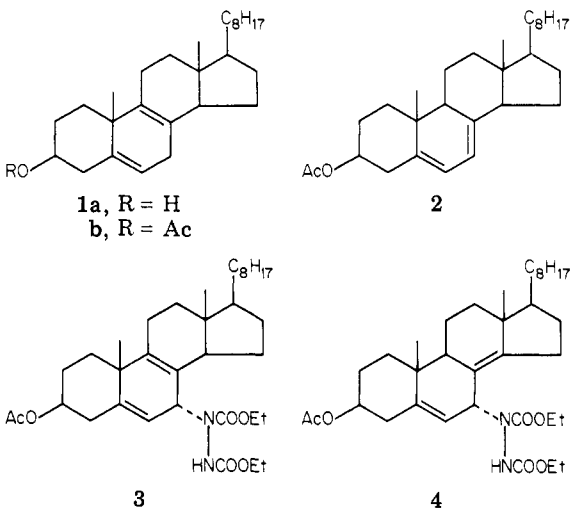
A better synthesis of **1a** was achieved by starting from 3β-acetoxycholesta-5,7-diene (**2**). Treatment of this compound with diethyl azodicarboxylate was described to give[7] 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8-diene (**3**). In our hands the reaction gave a second adduct, 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8(14)-diene (**4**).

The assignment of the structure of **4** is in agreement with mechanistic expectation and with ^{1}H NMR evidence.[8] The ^{1}H NMR spectrum of **4** shows coincident C-18 and C-19 methyl peaks at δ 0.86 in agreement with the values observed for the same methyl groups of steroids having a $\Delta^{5,8(14)}$ diene system.[9]

Alkaline saponification of the dicarbamate moiety of **3** was first attempted in order to obtain a substituted allylhydrazine from which **1a** could be obtained by air oxidation to the allyl diimide and loss of molecular nitrogen. However, the saponification required high temperature (exceeding 80 °C) and afforded a mixture containing no detectable amount of **1a**. On the other hand, steroidic allyldiimides were recently shown to collapse to olefins through a [1,5] sigmatropic shift with transfer of hydrogen to the β carbon bond and π bond migration.[10] Therefore a milder conversion of the dicarbamate to the allyl diimide[11,12] was not pursued.

The problem was solved by treatment of **3** with lithium in ethylamine at −20 °C. A dienol was obtained in good yield, uncontaminated by cholesta-5,7-dien-3β-ol, to which the structure **1** was attributed on the basis of its spectroscopic properties and of its selective hydrogenation to the known 5α-cholest-8-en-3β-ol. Samples of natural[1] and synthetic cholestadienol **1a** proved to be indistinguishable. The synthesis of **1a** opens the way to experiments on the biosynthetic generation of such unusual diene systems.

Chart I



1a, R = H
b, R = Ac

2

3

4

Experimental Section

Spectra were recorded as KBr disks with a Perkin-Elmer 257 spectrometer. ^{1}H NMR spectra were determined with deuteriochloroform as solvent and tetramethylsilane as an internal reference on a Varian HA-100 and Varian XL-100 spectrometers. Routine optical rotations were recorded with a Perkin-Elmer Model 141 spectropolarimeter for 1% solutions in chloroform. The mass spectra were determined on a Varian MAT 112 S spectrometer by direct-inlet methods. The progress of all reactions was monitored by TLC on silica gel G (HF_{254}) microplates or by GLC (2-m sylanized glass column of 3% SE-30 on Gas Chrom Q support, operating at 220–240 °C).

* To whom correspondence should be addressed at the Institute of Chemistry.

(1) Fumagalli, R.; Bernini, F.; Galli, G.; Anastasia, M.; Fiecchi, A. *Steroids* **1980**, *6*, 665.
(2) Mulheirn, L. J.; Ramm, P. *J. Chem. Soc. Rev.* **1972**, *1*, 259.
(3) Arima, K.; *Chem. Pharm. Bull.* **1953**, *1*, 224. Tsuda, K.; Arima, K.; Hayatsu, R. *J. Am. Chem. Soc.* **1954**, *76*, 2933.
(4) Barton, D. H. R.; Corrie, J. E. T.; Widdowson, A.; Bard, M.; Woods, R. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1326.
(5) Brynjolffssen, J.; Hands, D.; Midgley, J. M.; Whalley, W. B. *J. Chem. Soc., Perkin Trans. 1* **1976**, 826.
(6) Anastasia, M.; Bernini, F.; Fiecchi, A.; Fumagalli, R.; Galli, G. *Ital. J. Biochem.* **1980**, *29*, 388.
(7) Van der Gen, A.; Lakeman, J.; Gras, M. A. M. P.; Huisman, H. O. *Tetrahedron* **1964**, *20*, 2521.
(8) The isolation of **4** (unreported previously) forces the rejection of the early impression that the side chain prevents the removal of the proton at C_{14} in the reaction of **2** with diethyl azodicarboxylate.[7,9]
(9) Lakeman, J.; Speckamp, W. N.; Huisman, H. O. *Tetrahedron* **1968**, *24*, 5151.
(10) Shah, J. N. *Indian J. Chem., Sect. B* **1979**, *18B*, 488.
(11) Little, R. D.; Venegas, M. G. *J. Org. Chem.* **1978**, *43*, 2921.
(12) Jung, M. E.; Lyster, M. A. *J. Chem. Soc., Chem. Commun.* **1978**, 315.

0022-3263/81/1946-3421$01.25/0

Reaction of Diethyl Azodicarboxylate with 3β-Acetoxycholesta-5,7-diene (2). To a solution of **2** (1 g) dissolved in sodium-dried benzene (10 mL) was added diethyl azodicarboxylate (1 g), and the solution was refluxed under nitrogen for 4 h. Removal of the solvent and of the excess ester under reduced pressure gave a crude solid which on crystallization from hexane yielded 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8-diene (**3**): 0.770 g; mp 138–139 °C (from hexane; lit.[7] mp 138–139.5 °C); $[\alpha]^{20}_D$–76°; IR 3470, 1755, 1715, 1708 cm^{-1}; ^1H NMR δ 6.12 (1 H, m, NH), 5.45 (1 H, m, 6-H), 5.18 (1 H, m, 7β-H), 4.05–4.85 (5 H, overlapping, 3α-H and 2 $COOCH_2CH_3$), 2.00 (3 H, s, CH_3COO), 1.21 (3 H, s, 19-CH_3) 0.65 (3 H, s, 18-CH_3); mass spectrum, *m/e* 424 (2%, M – $C_6H_{12}N_2O_4$), 365 (100), 349 (15).

Anal. Calcd for $C_{35}H_{56}N_2O_6$: C, 70.0; H, 9.4; N, 4.7. Found: 69.9; H, 9.3; N, 4.7).

Evaporation of the mother liquor under reduced pressure gave a residue which was purified by preparative TLC (20% EtOAc/toluene) to afford **3** (95 mg) and 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8(14)-diene (**4**): 250 mg; mp 74–75 °C (amorphous); IR 3470, 1755, 1705 cm^{-1}; ^1H NMR δ 6.30 (1 H, m, NH), 5.30 (1 H, m, 6 H), 5.06 (1 H, m, 7β-H), 4.05–4.85 (5 H, overlapping, 3α-H and 2 $COOCH_2CH_3$), 2.00 (3 H, s, CH_3COO), 0.86 (6 H, s, 18- and 19-CH_3); mass spectrum, *m/e* 424 (3%, M – $C_6H_{12}O_4N_2$), 365 (100).

Anal. Calcd for $C_{35}H_{56}N_2O_6$: C, 70.0; H, 9.4; N, 4.7. Found: C, 69.8; H, 9.5; N, 4.7.

Synthesis of Cholesta-5,8-dien-3β-ol (1a). 3β-Acetoxy-7α-(1,2-dicarbethoxyhydrazo)-5,8-diene (**3**, 0.500 g) dissolved in ethylamine (20 mL) was treated with lithium (0.200 g), and the mixture was stirred at –20 °C for 30 min longer than required for the initial appearance of a blue color. The usual workup afforded cholesta-5,8-dien-3β-ol (**1a**): 208 mg; mp 106–107 °C (from methanol); $[\alpha]^{20}_D$ –4.5; IR 3400 cm^{-1}; ^1H NMR δ 5.48 (1 H, m, 6-H), 3.55 (1 H, m, 3α-H), 2.54 (2 H, m, 7-H_2), 2.36 (1 H, m, 4α-H), 2.28 (1 H, m, 4β-H), 1.18 (3 H, s, 19-CH_3), 0.66 (3 H, s, 18-CH_3); mass spectrum, *m/e* (relative intensity) 384 (60, M^+), 351 (100, M – (H_2O + Me)), 325 (20), 271 (20, M – C_8H_{17}), 253 (20, M – (C_8H_{17} + H_2O)), 217 (20), 211 (23).

Anal. Calcd for $C_{27}H_{44}O$: C, 84.3; H, 11.5. Found: C, 84.4; H, 11.4.

Acetylation of **1a** with acetic anhydride–pyridine afforded 3β-acetoxycholesta-5,8-diene (**1b**): mp 100–101 °C lit.[1] 98–100 °C; from methanol $[\alpha]^{20}_D$ –17°; IR 1740, 1250 cm^{-1}; ^1H NMR δ 5.48 (1 H, m, 6-H), 4.62 (1 H, m, 3α-H), 2.54 (2 H, m, 7-H_2), 2.42 (1 H, m, 4α-H), 2.35 (1 H, m, 4β-H), 2.02 (3 H, s, CH_3COO), 1.20 (3 H, s, 19-CH_3), 0.66 (3 H, s, CH_3); mass spectrum, *m/e* (relative intensity) 426 (5, M^+), 366 (87, M – AcOH), 351 (100), 253 (20), 211 (30).

Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.6; H, 10.9. Found: C, 81.5; H, 10.8.

Reduction of Cholesta-5,8-dien-3β-ol (1a). A solution of the dienol **1a** (100 mg) in ethanol (10 mL) containing Raney nickel (200 mg) was shaken in hydrogen. The usual workup afforded 5α-cholest-8-en-3β-ol: 80 mg (from methanol); mp 127–128 °C; $[\alpha]^{23}_D$ + 48°; identical by mixture melting point with an authentic sample.[13]

Acknowledgment. We acknowledge support from the Ministero della Pubblica Istruzione.

Registry No. 1a, 70741-38-7; **1b**, 17137-76-7; **2**, 1059-86-5; **3**, 3914-89-4; **4**, 77965-72-1; 5α-cholest-8-en-3β-ol, 566-97-2.

(13) Barton, D. H. R.; Cox, J. D. *J. Chem. Soc.* **1949**, 214.

Structure and Synthesis of 25-Hydroxycholecalciferol-26,23-lactone, a Metabolite of Vitamin D

David S. Morris, Dudley H. Williams,* and Alan F. Norris

University Chemical Laboratory, Lensfield Road, Cambridge, England CB2 1EW

Received February 25, 1981

The aldehyde **6**, prepared from ergosterol, underwent addition with vinylmagnesium bromide. Construction of the carbon side chain of the title compound was completed with a Claisen rearrangement. After conversion to the hydroxy acid **11**, halolactonization and subsequent dehalogenation gave the desired five-membered lactones. Separation of all four possible diastereoisomers was achieved by high-pressure liquid chromatography, and these were carried through to the provitamin stage. By chemical correlation and solution of two X-ray structures, the absolute stereochemistry of all four products was established. Irradiation in the presence of fluorenone as a triplet sensitizer and thermal isomerization gave the four target molecules. The natural product was identified by NMR comparison with the isolated metabolite and consideration of the biochemical pathway which leads to it.

In 1979, the isolation and identification of 23,25-dihydroxycholecalciferol-26,23-lactone (**1**), a new metabolite of vitamin D_3, was reported.[1] The stereochemistry at C-23 and C-25 was undetermined. The lactone was obtained from the plasma of chicks and became a major circulating metabolite of vitamin D_3 under conditions of hypervitaminosis. A synthesis of all four possible diastereoisomeric lactones has recently been reported, and it has been shown that one of these compounds is identical with the natural product, thus confirming the gross structure.[2] However, since the stereochemistries at C-23 and C-25 of the four lactones were not established, the stereochemistry of the natural product at these sites remained unestablished. We now report independent syntheses of the four possible lactones and experiments which establish the stereochemistries at C-23 and C-25 in each case. These experiments establish that the natural product has the 23*R*,25*S* stereochemistry. Biological testing of all four metabolites is also reported.

(1) Wichmann, J. K.; DeLuca, H. F.; Schnoes, H. K.; Horst, R. L.; Shepard, R. M.; Jorgensen, N. A. *Biochemistry* **1979**, *18*, 4775.
(2) Wichmann, J. K.; Paaren, H. E.; Fivizzani, M. A.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1980**, 4667.

Results and Discussion

Our synthetic strategy involved the synthesis of **2** (Scheme I), in which the 3β-OH and ring B diene functionalities would be suitably protected, with both *R* and *S* stereochemistries present at C-25. This last feature should be accessible by oxygenation of an anion (sp^2 hybridization) adjacent to carbonyl. We then planned to

0022-3263/81/1946-3422$01.25/0