Tetrahedron. Vol. 30, pp. 2701 to 2705. Pergamon Press 1974. Printed in Great Britain

A NEW SYNTHETIC METHOD OF 1α-HYDROXY-7-DEHYDROCHOLESTEROL

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(*Received in Japan* 16 *February* 1974; *Received in the UK for publication* 19 *March* 1974)

Abstract—Cholesta - 1,4,6 - trien - 3 - one (1) was converted to 3β - hydroxycholesta - 1,5,7 - triene (3) *via* the deconjugation procedure using t-BuOK in DMSO followed by the subsequent reduction with $Ca(BH_4)_2$. The compound (3) readily reacted with 4-phenyl-1,2,4-triazoline-3,5-dione to yield the corresponding 1,4-addition product (4). Epoxidation of 4 with *m*-chloroperbenzoic acid resulted in the formation of the 1α,2α-epoxide (5) and the 1β,2β-epoxide (6) in the ratio 2:3. Reduction of 5 with LAH under reflux in THF afforded the titled compound (7). The same reduction of 6 gave 2β-hydroxy- and 1β - hydroxy - 7 - dehydrocholesterol (8 and 9) in the ratio 8:1.

The compound (4) can be obtained in 25% yield from 1 without any purification of the intermediate compounds; cholesta - 1,5,7 - trien - 3 - one (2, a very unstable compound) and 3. Since 1 is obtained readily from cholesterol in high yield, the present study provides a simple and efficient synthetic method of 1α-hydroxycholecalciferol and is reasonably expected to be applicable in the synthesis of 12,25-dihydroxycholecalciferol and the other metabolites of vitamin D_3.

In the light of recent findings that cholecalciferol (vitamin D_3) must be hydroxylated at C-25 by the liver and then at C-1α by the kidney to 1α,25-dihydroxycholecalciferol (1α,25-$(OH)_2$-D_3) before it can induce either intestinal calcium transport or bone mineral mobilization,[1,2] various analogues of the biologically active vitamin D_3 metabolite (1α,25-$(OH)_2$-D_3) have been synthesized and tested for biological activity in various ways.[3,4] The most brilliant result obtained by these efforts has been the finding of 1α-hydroxycholecalciferol (1α-OH-D_3). This compound was synthesized by DeLuca *et al.* for the first time[5] and then by several research groups including ours.[6-9] Three groups further showed independently that 1α-OH-D_3 had comparable biological activity to 1α,25-$(OH)_2$-D_3 in anephric rats[5,8] and was more active than 25-OH-D_3 in thyroparathyroidectomized rats,[6,8] and was much more potent than vitamin D_3 with an extremely rapid onset of its physiological action in normal rats.[5,6,8]

These works suggest not only that 1α-OH-D_3 is expected to be extremely useful in clinical medicine but also that searching for new preparative methods of 1α-OH-D_3 and the related 1α-hydroxy derivatives of vitamin D_3 (1α,25-$(OH)_2$-D_3, 1α,24,25-$(OH)_3$-D_3,[10] etc) is an urgent need.

With one exception[9] which followed essentially the same method originally employed by Lythgoe *et al.* in the non-photochemical synthesis of vitamin D_3,[11] the synthesis of 1α-OH-D_3 so far reported[5-8] have used 1α-hydroxycholesterol as a key compounds.† Therefore, the latter syntheses[5-8] necessarily include acetylation of 1α-hydroxycholesterol followed by bromination, dehydrobromination, and hydrolysis‡ in order to obtain 1α-hydroxy-7-dehydrocholesterol (7) ready for the subsequent photochemical ring opening in the B ring.

It seems desirable to prepare 7 in a different way so as to skip these four steps. The efforts along this line have led us to find a new preparative method of 7 which does not include 1α-hydroxycholesterol in the sequence. Furthermore, the method does not use either acidic or pyrolytic conditions in any step

†Synthesis of 1α-hydroxycholesterol was also reported by two other groups. The physical constants reported by Pelc and Kodicek differ markedly from those reported by other groups.[6-8] See, M. Morisaki, K. Bannai and N. Ikekawa, *Chem. Pharm. Bull.* 21, 1853 (1973); B. Pelc and E. Kodicek, *J. Chem. Soc.* 1624 (1970).

‡The hydrolysis step can also be after the ring opening step.[5-7]

and thus seems to be applicable to the synthesis of the related vitamin D analogues, such as 1α,25-$(OH)_2$-D_3 having rather labile OH groups.

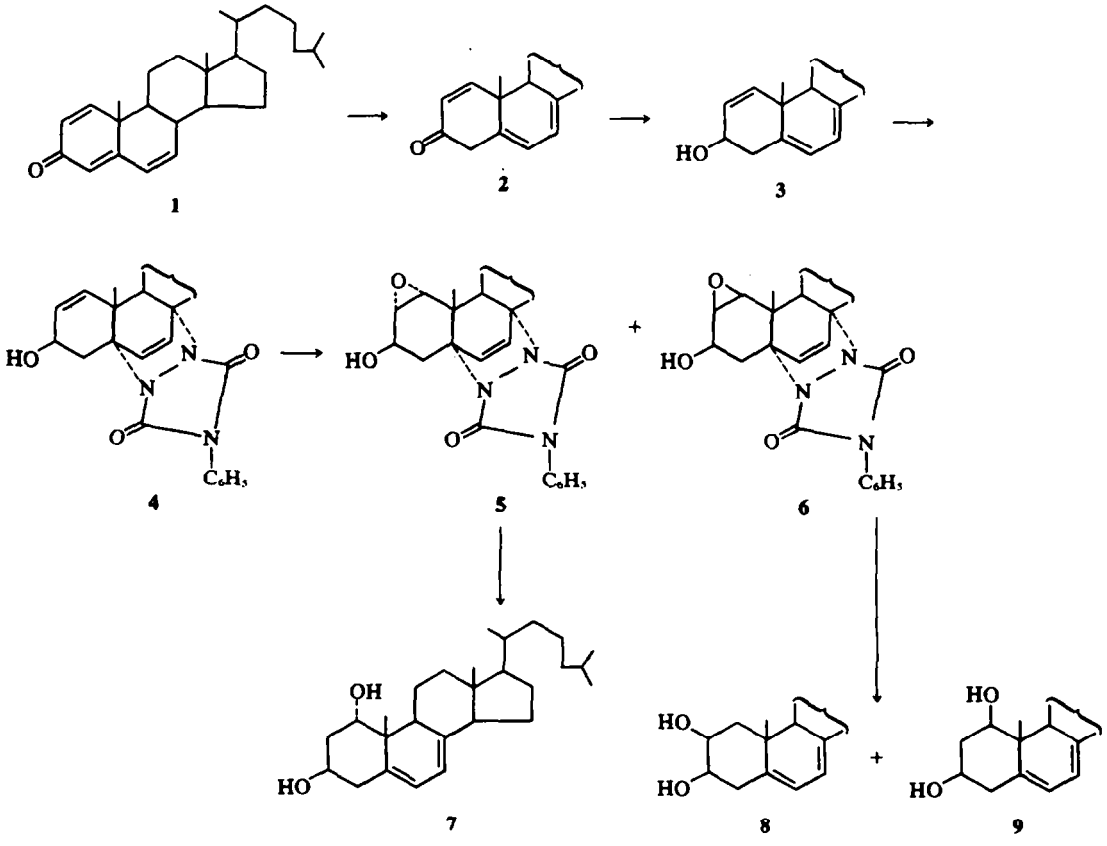
Our method consists of five steps from cholesta-1,4,6 - trien - 3 - one (1) available from cholesterol in good yield.[12]

Treatment of 1 with t-BuOK in DMSO followed by a rapid addition into ice-water (saturated with CO_2) and extraction with benzene-ethyl acetate led to cholesta - 1,5,7 - trien - 3 - one (2).* After a rapid and careful recrystallization from methanol of the product mixture, crude crystals of 2 were obtained. Though its m.p. has a wide range (136–146°), its spectroscopic data were in good accordance with the assigned structure. The compound (2) was so unstable that though it survived several weeks in a refrigerator, it decomposed within a few days if it was stored in a sample tube at room temp. Furthermore, though it was fairly stable in an alcohol, it decomposed within a minute by the addition of dilute aqueous KOH into the solution. Marked increasement of the solubility and neutrality as compared with $NaBH_4$, calcium borohydride seemed to be especially fitted to the present reduction and it was actually found that the reduction with this reagent in ethanol at −10° or below resulted in the formation of the desired product (3).† If purification was done at this stage by the use of column chromatography, 3 was obtained in *ca* 25% yield from 1. This compound (m.p. 128–129°) showed typical homoannular diene absorptions (λ_{max}^{EtOH}: 263 (log ε 3·92), 271 (4·08), 281 (4·10) and 292 nm (3·85)) and an acceptable NMR spectrum. The trien-3-ol (3) reacted with 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione[13] at room temperature to give only one kind of 1,4-addition product (m.p. 178–182°) in 85% yield.

More conveniently, the addition product (4) was

*The deconjugation reactions under these conditions were first used to form the 1,5 - diene - 3 - keto - steroids by Shapiro *et al.* and then applied successfully in the preparation of 5,7 - diene - 3 - keto - steroids by Kruger: see, E. Shapiro, L. Weber, E. P. Oliveto, H. L. Herzog, R. Neri, S. Tolksdorf, M. Tanabe and D. F. Crowe, *Steroids* **8**, 461 (1966); G. Kruger, *J. Org. Chem.* **33**, 1750 (1968).

†This reagent was originally used by Hójos and Füchs. We have also used it in the reduction of 3-keto-$\Delta^{1,5}$-steroids and found that the yield of 3β-hydroxy-$\Delta^{1,5}$-steroids was higher than that obtained by $NaBH_4$ reduction.[8] If $NaBH_4$ was used instead of $Ca(BH_4)_2$, the reduction did not afford the desired product but gave the product having typical heteroannular diene absorptions (λ_{max}: 233, 240, and 248·5 nm). See, A. Hójos and O. Füchs, *Acta Chim. Acad. Science Hung.* **21**, 137 (1959).



1 2 3 4 5 6 7 8 9

obtained in the following way. The whole $Ca(BH_4)_2$ reduction product obtained as above was treated with 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione (addition was continued until discharge of the red color terminated) and **4** was separated from the other reduction products by chromatography on silica gel. Thus, the addition product (**4**) can be obtained without any purification of the intermediate compounds (**2** and **3**) from **1** in the yield of 25%.

The structure of **4** was confirmed by hydrogenation (5%-Pd/C in methanol) to the tetrahydrocompound (**10**), mp 164–166°, identical in all respects with the dihydro derivative obtained from the same catalytic hydrogenation of the addition product (mp 151–153°) of 7-dehydrocholesterol with 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione.* This experiment also confirmed unequivocally the β-configuration of the hydroxy function in the compound 3.

The addition product (**4**) was then treated with 2 mole equivalent of *m*-chloroperbenzoic acid in $CHCl_3$ at room temperature for 2 days and the epoxides (**5** and **6**) were resolved by chromatography on silica gel with ether.

The fraction more strongly adsorbed on alumina was the 1α,2α-epoxide (**5**), m.p. 152–154°, whose yield was 30–35%. The other fraction (the less polar fraction) was the 1β,2β-epoxide (**6**), m.p. 172–173° (50–55%).

The structure of **5** was confirmed by LAH reduction (in reflux THF for 1 h) affording 1α - hydroxy - 7 - dehydrocholesterol (**7**), m.p. 155–158° (55–65% yield), whose identity with the authentic sample prepared in our previous work[8] was assured by the direct m.m.p. determination and the comparison of spectral data. This step corresponds to the final step in the present synthesis and also demonstrates the presence of 1α,2α-epoxy function in **5**.

By the same reduction, **6** afforded two diols (**8**: m.p. 176–180° and **9**: m.p. 176–180°) in the ratio 8:1 in very high yield (80–85%). The structure of both diols were supported by the presence of the absorption maxima of homoannular diene and by the reasonable NMR spectra. The acetonide formation (checked by the presence of M^+ at *m/e* 440) further confirmed the structure of **8** as 2β - hydroxy - 7 - dehydro - cholesterol.

The synthesis of **7** from **1** was thus accomplished by five steps in approximately 5% overall yield. The present study seems to provide a new preparative method of 1α - hydroxy - 7 - dehydrocholesterol (**7**) from cholesterol having comparable (if not the best) efficiency to any of the previously published methods.[5-8] Present method seems to be especially suited to the conversion of cholesterols having a labile OH group to the corresponding 7-analogues, since this method uses neither acidic nor pyrolytic condition in any of its steps.

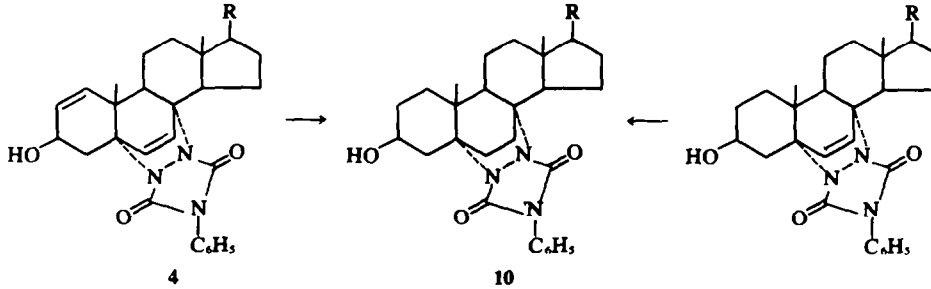
The newly synthesized hydroxy - 7 - dehydrocholesterols (**8** and **9**) were converted to the corresponding cholecalciferols, whose biological activity is now under investigation.

EXPERIMENTAL

M.ps are uncorrected and were determined in a capillary tube. The IR spectra were recorded in KBr pellets on DS-403G and IR-S JASCO spectrometers. The UV spectra were determined on a Hitachi Model-323. The NMR spectra were obtained using a C-60 HL JEOL (60 Mc.p.s) and the chemical shifts are given in τ-units. The multiplicities of the NMR signals are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Mass spectra were recorded on a Hitachi model RMU-7M double focus spectrometer using all cases a direct sample insertion into the ion source. Optical rotation values were measured by Yanagimoto-Model OR-10 direct reading polarimeter. Satisfactory analytical results were obtained for all crystalline compounds, except **2**.

Preparation of the 1,4-*adduct* (**4**) *of* 3β - *hydroxycholesta* - 1,5,7 - *triene with* 4 - *phenyl* - 1,2,4 - *triazoline* - 3,5 - *dione from cholesta* - 1,4,6 - *trien* - 3 - *one* (**1**). To the soln of **1** (2 g) in abs ether (40 ml) and DMSO (40 ml; distilled before use after dehydration with CaH_2) was added finely powdered *t*-BuOK (prepared from 1 g of K) and the soln was stirred vigorously for 12 min at 10°. The mixture was poured into ice-water (previously saturated with CO_2 by the addition of dry ice) and then extracted with 800 ml of benzene–EtOAc (1:2 v/v; cooled previously at *ca* 5°). The organic layer was rapidly separated from the aqueous layer and washed with CO_2 saturated ice-water several times. Evaporation of the solvent

*This reagent was known to react with ergosterol in the same way.[14,15] Barton *et al.* further showed that the adduct was reconverted into ergosterol in a quantitative yield by LAH reduction.[15]



4 10

after drying over Na_2SO_4 below 35° *in vacuo* gave 1·6 g of oil containing unstable **2**. This fraction was then subjected to reduction by $Ca(BH_4)_2$ without further purification.

To a finely powdered $CaCl_2$ (1·5 g) in 35 ml of MeOH was added 750 mg $NaBH_4$ dissolved in 40 ml EtOH with stirring at −10° or below. After the addition was complete, the deconjugation product obtained as above (1·6 g) in 50 ml of ether was added slowly with stirring at the same temp as above and the stirring was continued for 1 h and then for 40 min at 0°. After the excess of the reagent was decomposed by addition of excess 50% aq acetone, acetone was removed under reduced pressure. Forty percent aq AcOH was added to the residue until clear soln was obtained and the whole was extracted with CH_2Cl_2. After being dried over $MgSO_4$, the solvent was evaporated under reduced pressure to afford an oily residue, 1·5 g. This residue was dissolved in CH_2Cl_2 (30 ml) and 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione (freshly sublimed before use) was added in small portions (285 mg) until discharge of the red color terminated. After stirring for 1 h at room temp, solvent was evaporated and the residue was chromatographed on silica gel. The fraction which was eluted with ether–hexane (7 : 3 v/v) was largely the desired addition product **4** (775 mg). Recrystallization of this fraction from ether afforded pure **4**, yield; 735 mg (25% based on **1**), m.p. 178–182°. λ_{max}^{EtOH}: 255 nm (log ε 3·63). ν_{max}: 3400, 1755 and 1700 cm^{-1}; NMR ($CDCl_3$): 2·67 (broad s, 5H), 3·60 (d, J = 8 Hz, 1H), 3·83 (d, J = 8 Hz, 1H), 4·32 (s, 2H), 5·0 (t, J = 7 Hz 1H), and 6·66 (d.d, J = 14 and 7 Hz, 1H). Mass m/e 557 (M^+).

Synthesis of cholesta - 1,5,7 - trien - 3 - one (**2**) *from cholesta - 1,4,6 - trien - 3 - one* (**1**). The whole deconjugation product (1·6 g) obtained as above from 2 g of **1** was recrystallized from MeOH (refluxing MeOH and high concentration of **2** caused its decomposition and should be avoided) to afford crude crystals of **2** (250 mg), m.p. 136–146°. λ_{max}^{EtOH}: 230, 268, 277, 288 nm; λ_{min}^{EtOH}: 251 nm, NMR ($CDCl_3$): 3·17 (d, J = 11 Hz, 1H), 4·07 (d, J = 11 Hz, 1H), 4·30 (d, J = 6 Hz, 1H), 4·53 (d, J = 6 Hz, 1H), 6·60 and 7·03 (an AB q, J = 17 Hz, 2H). This material decomposed slowly at room temp. The compound **2** was reduced to **3** in 75–80% yield by the reduction with $Ca(BH_4)_2$ as described above.

Preparation of cholesta - 1,5,7 - trien - 3β - ol (**3**) *from* **1**. Compound **1** was treated as described in the conversion of **1** to **4** to afford 1·6 g of crude oily residue after $Ca(BH_4)_2$ reduction. The residue was chromatographed on alumina with ether–hexane mixture. The fraction eluted with ether–hexane (1 : 1 v/v) was largely the trien-3β-ol and recrystallized from MeOH to afford pure **3**, m.p. 128–129° (500 mg). λ_{max}^{EtOH}: 263 (log ε 3·92), 271 (4·08), 281 (4·10) and 292 nm (3·85). Mass m/e: 382 (M^+), 364, 349. ν_{max}: 3400, 1620 cm^{-1}; NMR ($CDCl_3$): 4·37 (broad s, 2H), 4·37 (m, 1H), 4·60 (d, J = 5 Hz, 1H), 5·70 (d.d, J = 10 and 6 Hz, 1H).

This compound (**3**) was converted to **4** by the treatment with 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione (1 mole equiv) in nearly quantitative yield.

Catalytic reduction of the cycloadduct (**4**) *with 5% Pd/C*. The cycloadduct (**4**), 96 mg, was reduced in MeOH in the presence of 20 mg of 5% Pd/C. After H_2 uptake was terminated, the catalysts were filtered off and the residue obtained from filtrate was recrystallized from ether to afford **10**, m.p. 164–166° (70 mg); ν_{max}: 3400, 1700 cm^{-1}. Mass m/e 561 (M^+). UV: shoulder peak at 215 nm. NMR ($CDCl_3$): 2·60 (broad s, 5H) 5·50 (m, 1H), 6·45 and 6·65 (q, J = 7 Hz, 2H).

The same compound was also obtained from the cycloadduct (m.p. 151–153°; m/e 559 (M^+)) of 7-dehydrocholesterol with 4 - phenyl - 1,2,4 - triazoline - 3,5 - dione by the same catalytic reduction.

Epoxidation of the cycloadduct (**4**) *with m-chloroperbenzoic acid*. To the soln of **4** (1·25 g) in 50 ml of $CHCl_3$ was added *m*-chloroperbenzoic acid (560 mg). After stirring for 24 h at room temperature, further amount (200 mg) of the peracid was added and the whole was stirred for 20 h. Chloroform was added and the whole was washed with 10% K_2CO_3 aq twice. The organic layer was separated and dried over $MgSO_4$. The residue was chromatographed on silica gel. The fraction eluted with ether was collected and recrystalized from MeOH to afford 685 mg of **6**, m.p. 172–173° (53%) ν_{max}: 3400, 3215, 1760, 1710 cm^{-1}. Mass m/e 398, 380, 365, 354, 338. NMR ($CDCl_3$): 2·67 (broad s, 5H), 3·67 (d, J = 8 Hz, 1H), 3·87 (d, J = 8 Hz, 1H), and 5·10 (m, 1H).

The fraction eluted after **6** by the same solvent was recrystallized from MeOH to afford 430 mg of **5**, m.p. 152–154° (34%). ν_{max}: 3400, 1750, 1690 cm^{-1}. Mass m/e 398, 380, 365, 351, 338. NMR ($CDCl_3$): 2·66 (broad s, 5H), 3·65 (d, J = 8 Hz, 1H), 3·90 (d, J = 8 Hz, 1H), and 5·08 (m, 1H).

Lithium aluminum hydride reduction of α-epoxidated cycloadduct (**5**). The adduct **5** (500 mg) in 40 ml of THF was added to LAH (500 mg) in 40 ml of THF and the whole was refluxed gently for 1 h. After destroying the excess of reagent with sat. Na_2SO_4 aq, the organic layer was separated and dried over $MgSO_4$. The residue was purified by chromatogrpahy on silica gel (ether–hexane 7 : 3 v/v) to afford crude **7**, Recrystallization from MeOH afforded 210 mg of **7**, m.p. 155–158° (60%). $[\alpha]_D$ −45° ($CHCl_3$). Mass m/e 400 (M^+), 382, 364, 341, 326, 312, 251, 157, 145. NMR ($CDCl_3$): 4·36 (d, J = 6 Hz, 1H), 4·66 (d, J = 6 Hz, 1H), 6·05 (m, $W_{1/2}$ = 25 Hz, 1H), and 6·30 (m, $W_{1/2}$ = 8 Hz).

The identity of **7** with authentic material prepared in our previous work[8] was assured by m.m.p. determination and by the comparison of other spectral data (UV and IR).

Lithium aluminum hydride reduction of β-epoxidated cycloadduct (**6**). The cycloadduct, **6** (250 mg) was reduced with 250 mg of LAH in THF and worked up as described above. Upon silica gel column chromatography with ether–hexane (7 : 3 v/v), two fractions were eluted. The more polar fraction was the 2β,3β-dihydroxycholesta - 5,7 - diene, which by recrystallization with MeOH afforded pure **8**, m.p. 176–180° (130 mg: 74%). λ_{max}^{EtOH}: 262 (log ε 3·92), 271 (4·07), 282 (4·11), 294 (3·87). Mass m/e 400 (M^+), 382 364, 341, 326, 312, 251, 157, 145. NMR ($CDCl_3$): 4·40 (d, J = 6 Hz, 1H), 4·70 (d, J = 6 Hz, 1H), 6·15–6·60 (m, 2H).

This diol (**8**) formed an oily acetonide by the usual way.[16] Due to its low yield, its formation was detected by the presence of m/e 440 (M^+) in the mass spectrum.

The other fraction (the less polar fraction) afforded the other diol which was recrystallized to give **9**, m.p. 176–180° (18 mg, 10%). Mass m/e 400 (M^+).

This compound did not form the acetonide and thus its structure was assigned as 1β,3β - dihydroxycholesta-5,7 - diene.

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