

206. (25*S*)-1 α , 25, 26-Trihydroxycholecalciferol, a New Vitamin D₃ Metabolite: Synthesis and Absolute Stereochemistry at C (25)

Preliminary Communication

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Summary

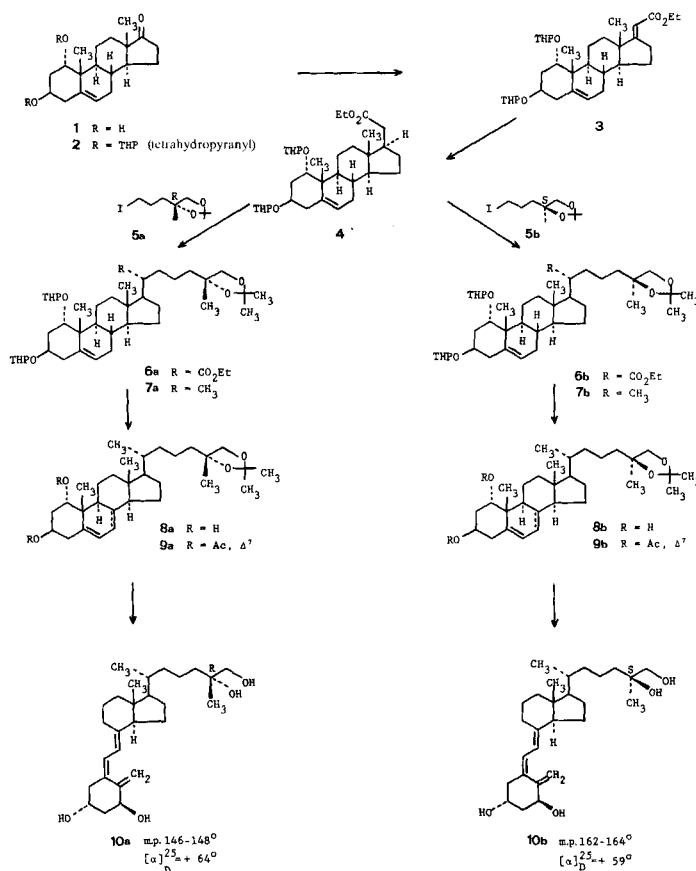
The 1 α , 25, 26-trihydroxy metabolite of vitamin D₃, isolated from bovine serum, was shown to possess the (25*S*)-configuration by HPLC. comparison of the 1,3,26-triacetate derivative with authentic (25*R*)- and (25*S*)-samples. The convergent synthesis of (25*R*)-1 α , 25, 26- and (25*S*)-1 α , 25, 26-trihydroxycholecalciferols (**10a**) and (**10b**) has been accomplished.

As part of a synthetic program on the vitamin D₃ metabolites, we recently prepared (25*R*)-1 α , 25, 26- and (25*S*)-1 α , 25, 26-trihydroxycholecalciferols, anticipating that one of these substances could be an undiscovered metabolite [1]. Since the absolute configuration of (25*S*)-25, 26-dihydroxycholecalciferol has been conclusively determined [2] [3], 1 α -hydroxylation would afford (25*S*)-1 α , 25, 26-trihydroxycholecalciferol. However, the 26-hydroxylating enzyme is not well characterized [1]. Thus, hydroxylation of 1 α , 25-dihydroxycholecalciferol could in principle give either (25*R*)- or (25*S*)-1 α , 25, 26-trihydroxycholecalciferol. In 1981, Horst *et al.* isolated a new metabolite of vitamin D₃ from the plasma of vitamin D₃-treated cows [4]. We have compared the cow metabolite with pure (25*R*)-1 α , 25, 26- and (25*S*)-1 α , 25, 26-trihydroxycholecalciferols and have shown, by HPLC. co-migration of the triacetate derivatives, that the natural metabolite possesses the (25*S*)-configuration.

The synthesis of the key intermediates, (25*R*)-1 α , 25, 26- and (25*S*)-1 α , 25, 26-trihydroxycholesterols utilizes the alkylation-reduction method of Wicha & Bal [5] [6], which we have also employed in the synthesis of (25*R*)-25, 26- and (25*S*)-25, 26-dihydroxycholecalciferols [2]. 1 α , 3 β -Dihydroxy-androst-5-en-17-one (1 α -

hydroxy-5-dehydro-3-epiandrosterone) (**1**) was converted to the bis(tetrahydropyranyloxy) compound **2** in over 90% yield with 3,4-dihydro-2*H*-pyran and *p*-toluenesulfonic acid catalyst. This substance was treated with triethyl phosphonoacetate and sodium ethoxide in ethanol to afford cleanly the (*E*)-unsaturated ester **3**. Catalytic hydrogenation with platinum oxide in ethanol then afforded ester **4** in 80% overall yield from **2**. This substance was alkylated (lithium diisopropylamide, THF/HMPTA (hexamethylphosphorotriamide), -40°) [5] [6] with the iodide **5a** of established stereochemistry and optical purity [2] to yield the monoalkylated ester **6a** in 85% yield. The ester **6a** was sequentially reduced (LiAlH_4 , THF, 60°), esterified (TsCl , pyridine, 0°), and hydrogenolyzed (LiAlH_4 , THF, 60°) to oily acetonide **7a**. Exposure of **7a** to acidic methanol/2,2-dimethoxypropane 1:1 at 0° afforded crystalline acetonide **8a**; m.p. $151\text{--}153^{\circ}$, $[\alpha]_D^{25} = -28^{\circ}$ ($c = 1$, CHCl_3) in 54% overall yield from **4**. Treatment of either **7a** or **8a** with acidic methanol gave (*25R*)-1a, 25, 26-trihydroxycholesterol; m.p. $235\text{--}238^{\circ}$, $[\alpha]_D^{25} = -24^{\circ}$ ($c = 1$, DMF).

In similar fashion, the ester **4** was alkylated with iodide **5b** to give monoalkylated ester **6b**. This substance was submitted to the hydrogenolysis sequence to yield oily acetonide **7b**, which was treated with methanol/2,2-dimethoxypropane



1:1 and *p*-toluenesulfonic acid to yield the crystalline acetonide **8b**; m.p. 175–177°, $[\alpha]_D^{25} = -40^\circ$ ($c = 1$, CHCl_3). Both compounds **7b** and **8b** then afforded (25 *S*)-1*a*,25,26-trihydroxycholesterol; m.p. 197–199°, $[\alpha]_D^{25} = -29^\circ$ ($c = 1$, DMF) on stirring with acidic methanol.

The cholesterol acetonides **8a** and **8b** were converted into 5,7-diene derivatives **9a** and **9b** by sequential acetylation (acetic anhydride, pyridine), allylic bromination (1,3-dibromo-5,5-dimethylhydantoin), and dehydrobromination (*s*-collidine). These dienes were individually photolyzed using a mercury lamp to give the corresponding previtamins which were saponified and thermolyzed at 90°. The acetonide groups were then removed with acidic methanol. HPLC-purification and recrystallization afforded pure (25 *R*)-1*a*,25,26-trihydroxycholecalciferol **10a**; m.p. 146–148°, $[\alpha]_D^{25} = +64^\circ$ ($c = 0.5$, CH_3OH) in 30% overall yield from **8a** and (25 *S*)-1*a*,25,26-trihydroxycholecalciferol **10b**; m.p. 162–164°, $[\alpha]_D^{25} = +59^\circ$ ($c = 0.5$, CH_3OH) in 33% overall yield from **8b**.

To prove unequivocally the absolute configuration of the natural bovine metabolite, we compared the HPLC. elution times of the 1,3,26-triacetates of the natural substance and of the synthetic (25 *R*)-1*a*,25,26- and (25 *S*)-1*a*,25,26-trihydroxycholecalciferols. A 1:1 mixture of the synthetic triacetates, individually prepared with acetic anhydride in pyridine, gave the HPLC. trace A of the Figure. This tracing demonstrated that the (25 *S*)-epimer is eluted before the (25 *R*)-epimer. A sample of bovine metabolite was prepared by multi-step purification of eight liters of bovine plasma [4]. This sample was purified further by HPLC. (μ Porasil[®] column; hexane/2-propanol 4:1 as eluant) and then acetylated. The bovine metabolite and its triacetate were identified by their characteristic UV. and high-resolution mass spectra. Addition of the triacetylated bovine metabolite to the *ca.* 1:1 mixture of triacetylated synthetic epimers previously described gave the HPLC. trace B shown in the Figure. The co-migration of the bovine metabolite with the faster-eluting epimer shows that bovine 1*a*,25,26-trihydroxycholecalciferol possesses the (25 *S*)-configuration.

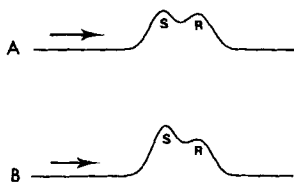


Figure. A) HPLC. tracing of a 1:1 mixture of the synthetic triacetates; B) HPLC. tracing of a 1:1 mixture of the synthetic triacetates upon addition of the triacetylated bovine metabolite.

R = (25 *R*)-1*a*,25,26-Trihydroxycholecalciferol 1,3,26-triacetate

S = (25 *S*)-1*a*,25,26-Trihydroxycholecalciferol 1,3,26-triacetate

Chromatograph: Waters 244

Eluant: 2:1 hexane/ethyl acetate

Flow Rate: 2 ml/min – 5 recycles

Column: 3 × μ Porasil[®]

Detector: UV., 254 nm

REFERENCES

- [1] *A.W. Norman*, 'Vitamin D: The Calcium Homeostatic Steroid Hormone', Academic Press, New York 1979, pp. 93–198.
- [2] *J.J. Partridge, S.-J. Shiuey, N.K. Chadha, E.G. Baggiolini, J.F. Blount & M.R. Uskoković*, *J. Am. Chem. Soc.* **103**, 1253 (1981).
- [3] *R. Barner, J. Hübscher, J.J. Daly & P. Schönholzer*, *Helv. Chim. Acta* **64**, 915 (1981).
- [4] *T.A. Reinhardt, J.L. Napoli, D.C. Beitz, E.T. Littledike & R.L. Horst*, *Biochem. Biophys. Res. Commun.* **99**, 302 (1981).
- [5] *J. Wicha & K. Bal*, *J. Chem. Soc., Chem. Commun.* **1975**, 968.
- [6] *J. Wicha & K. Bal*, *J. Chem. Soc., Perkin Trans. I*, **1978**, 1282.