206. (25*S*)-1α,25,26-Trihydroxycholecalciferol, a New Vitamin D_3 Metabolite: Synthesis and Absolute Stereochemistry at C(25)

Preliminary Communication

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Summary

The 1α,25,26-trihydroxy metabolite of vitamin D_3, 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*)-1α,25,26- and (25*S*)-1α,25,26-trihydroxycholecalciferols (**10a**) and (**10b**) has been accomplished.

As part of a synthetic program on the vitamin D_3 metabolites, we recently prepared (25*R*)-1α,25,26- and (25*S*)-1α,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], 1α-hydroxylation would afford (25*S*)-1α,25,26-trihydroxycholecalciferol. However, the 26-hydroxylating enzyme is not well characterized [1]. Thus, hydroxylation of 1α,25-dihydroxycholecalciferol could in principle give either (25*R*)- or (25*S*)-1α,25,26-trihydroxycholecalciferol. In 1981, *Horst et al.* isolated a new metabolite of vitamin D_3 from the plasma of vitamin D_3-treated cows [4]. We have compared the cow metabolite with pure (25*R*)-1α,25,26- and (25*S*)-1α,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*)-1α,25,26- and (25*S*)-1α,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]. 1α,3β-Dihydroxy-androst-5-en-17-one (1α-

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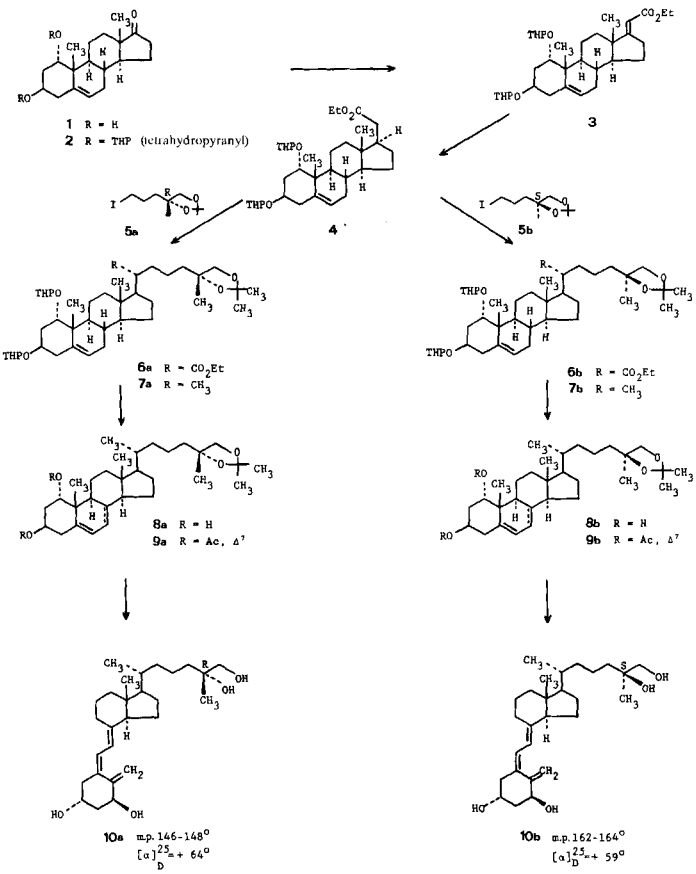
hydroxy-5-dehydro-3-epiandrosterone) (**1**) was converted to the bis(tetrahydropyranyloxy) compound **2** in over 90% yield with 3,4-dihydro-2*H*-pyran and *p*-toluenesulfonic acid catalyst. This substance was treated with triethyl phosphonoacetate and sodium ethoxide in ethanol to afford cleanly the (*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. 151–153°, $[\alpha]_D^{25} = -28°$ ($c = 1$, $CHCl_3$) in 54% overall yield from **4**. Treatment of either **7a** or **8a** with acidic methanol gave (25*R*)-1α,25,26-trihydroxycholesterol; m.p. 235–238°, $[\alpha]_D^{25} = -24°$ ($c = 1$, 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°, $[\alpha]_D^{25} = -40°$ ($c = 1$, $CHCl_3$). Both compounds **7b** and **8b** then afforded (25 *S*)-1α,25,26-trihydroxycholesterol; m.p. 197–199°, $[\alpha]_D^{25} = -29°$ ($c = 1$, 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*)-1α,25,26-trihydroxycholecalciferol **10a**; m.p. 146–148°, $[\alpha]_D^{25} = +64°$ ($c = 0.5$, CH_3OH) in 30% overall yield from **8a** and (25 *S*)-1α,25,26-trihydroxycholecalciferol **10b**; m.p. 162–164°, $[\alpha]_D^{25} = +59°$ ($c = 0.5$, 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*)-1α,25,26- and (25 *S*)-1α,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 1α,25,26-trihydroxycholecalciferol possesses the (25 *S*)-configuration.

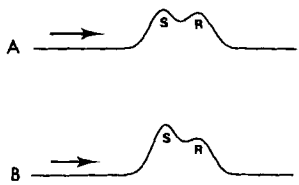


Figure. A) HPLC. tracing of a 1:1 mixture of the synthetic triacetates; B) HPLC. tracing of a 1:1 mixture of the synthetic triacetates upon addition of the triacetylated bovine metabolite.

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

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