25-HYDROXYERGOCALCIFEROL: A BIOLOGICALLY ACTIVE METABOLITE OF VITAMIN D_2

T. Suda, H. F. DeLuca, H. Schnoes and J. W. Blunt

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

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SUMMARY. A polar, biologically active metabolite of vitamin D_2 (ergocalciferol) has been isolated in pure form from the plasma of pigs fed 500,000 I.U. of vitamin D_2 per day for 26 days. By means of its behavior during gas-liquid chromatography, ultraviolet spectra, mass spectrometry and nuclear magnetic spectrometry, its structure has been positively identified as 25-hydroxy-ergocalciferol.

In this laboratory over the course of 5 to 6 years, evidence has accumulated that cholecalciferol is metabolized to a major biologically active metabolite (1, 2). Recently this metabolite was isolated in pure form from hog plasma and identified as 25-hydroxycholecalciferol (3, 4). Strong evidence that this substance represents the metabolically active form of vitamin D_3 has also been obtained (5, 6). Ergocalciferol (vitamin D_2) is also converted to a biologically active metabolite (7) and it has now been possible to isolate it in pure form and identify it as 25-hydroxyergocalciferol.

The blood plasma from 4 hogs fed for 26 days on a ration supplying 500,000 I.U. ergocalciferol/day to each animal was obtained. The α_2 globulin which binds the

vitamin and its metabolites was precipitated by 70% saturation with $(NH_4)_2SO_4$ and extracted with methanol and chloroform. Silicic acid column chromatography of the chloroform phase yielded a purified metabolite fraction (IV) which on rechromatography on silicic acid columns and finally on partition columns yielded 314 μg of pure metabolite. Purity was established by both tlc and glpc. The compound showed the expected absorption, λ_{max} ether = 264 mμ, in its spectrum and it gave characteristic isopyro and pyro forms on glpc. High resolution mass spectra* showed a molecular weight of 412.3341 establishing a formula of $C_{28}H_{44}O_2$ (calc. 412.3341). A characteristic fragment of mass 271 ($C_{19}H_{27}O$, M- side chain) for both the metabolite, ergocalciferol, 25-hydroxycholecalciferol, and cholecalciferol demonstrated the additional oxygen in the side chain. A peak at m/e 59 (C_3H_7O) and the loss of 58 m.u. (m/e 354, $C_{25}H_{38}O$) from M^+ in the mass spectrum of the metabolite but not in that of ergocalciferol strongly suggested a hydroxyl function at C-25. Confirmation was obtained when the trimethylsilyl ether of the metabolite was prepared, purified and subjected to mass spectrometry. The spectrum of this derivative (MW = 556, as expected for the disilyl ether) exhibited an intense peak of mass 131 ($C_6H_{15}SiO$), only possible for a C-25-O-silyl function.

The nmr spectrum of the metabolite, obtained with a Varian Associates HA-100 instrument and time averaging

*Determined on an MS-9 mass spectrometer (A.E.I.) coupled on-line to a Sigma-7 computer (Scientific Data Systems). For a description of the system, see A. L. Burlingame in "Advances in Mass Spectrometry," E. Kendrick, Ed., The Institute of Petroleum, London, 1968, p. 15; A. L. Burlingame, D. H. Smith, and R. W. Olson, Anal. Chem., 40, 13 (1968).

computer, showed a strong singlet at δ 1.24 ppm which was not present in the original ergocalciferol spectrum while two doublets at δ 0.87, (J = 7.0 cps) and at δ 0.98 ppm (J = 6.0 cps) originally present in the ergocalciferol spectrum were absent from that of the metabolite. Substitution of a hydroxyl group at only one position, namely the 25 position, could eliminate two doublets and give a strong singlet in this fashion. Two doublets remained at the δ 0.79 ppm (J = 6.5 cps) and at the δ 0.81 ppm (J = 7.0 cps) in the metabolite spectrum corresopnding to the 21 and 28 methyl groups. The singlet due to the 18 angular methyl group was present in both. Clearly then the structure of the metabolite is established as 25-hydroxyergocalciferol (Figure 1).

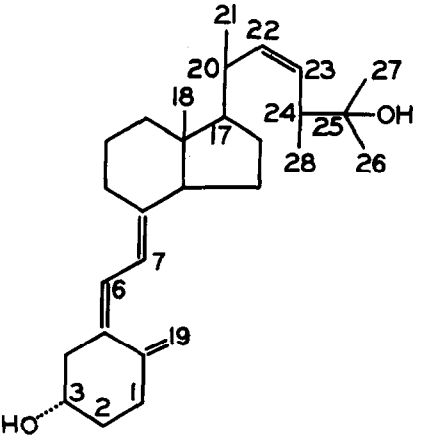

Figure 1. Structure of 25-hydroxyergocalciferol.

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