25-Hydroxycholecalciferol: a Biologically Active Metabolite of Cholecalciferol

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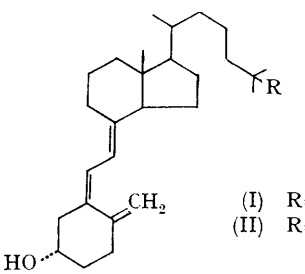
IT has long been suspected that cholecalciferol (I) (vitamin D_3) must first be converted into a biologically active metabolite before it could exert its metabolic effects.[1] However, it was not until 1966 that the existence of biologically active metabolites was unequivocally shown in this laboratory.[2] From these, a major polar metabolite fraction designed as "Peak IV" from its chromatographic behaviour appeared to meet the criteria of the metabolically active form of the vitamin.[1-4] It has now been possible to isolate the biologically active component of this metabolite fraction from the blood plasma of hogs given 250,000 i.u. cholecalciferol daily for $3\frac{1}{2}$ weeks. This substance is identified as 25-hydroxycholecalciferol (II).

The protein precipitated from 6·8 l. of plasma from 4 hogs was extracted with chloroform–methanol and its crude extract submitted to absorption chromatography followed by partition chromatography, yielding 1·3 mg. of pure (II). This was identified as follows. Its u.v. spectrum displayed a maximum at 265 mμ as for (I). G.l.c. at 240° gave two peaks, representing 25-hydroxypyrocholecalciferol and its isopyro-isomer, both of which had u.v. spectra identical to those for the corresponding pyro- and isopyro-cholecalciferols arising from (I) under similar conditons. Thus the similarity in structure between (I) and (II) was established. The mass spectrum of (II) indicated a M.W. of 400, ($C_{27}H_{44}O_2$), *i.e.* a cholecalciferdiol. Further, the mass spectrum was very similar to that of (I), both showing a peak at *m/e* 271 ($C_{19}H_{27}O$) arising from the removal of the side-chain. Hence the extra hydroxyl group in (II) is located on the side-chain. The presence of a peak at *m/e* 59 (C_3H_7O) in the spectrum of (II) but not in the spectrum of (I) suggested the location of the hydroxyl function at C-25. This was confirmed by the 100 Mc./sec. n.m.r. spectrum of (II), which



(I) R=H
(II) R=OH

was identical with the spectrum of (I) except that the doublet at 0·87 p.p.m. (J 6·0 c./sec.) due to the secondary C-26,27 methyl groups in (I) was absent and was replaced by a strong singlet peak at 1·20 p.p.m., identical with the peak due to the C-26,27 methyl groups in 25-hydroxycholesterol.

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