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ACTION AND METABOLISM OF DIHYDROTACHYSTEROL$_2$

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Summary—Dihydrotachysterol$_2$ (DHT_2) is a synthetic analogue of vitamin D_2. DHT_2 is used extensively in the treatment of renal osteodystrophy and hypoparathyroidism. It is equally efficacious as 1α,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3. Moreover, it offers interesting therapeutical advantages and it is surprising that until recently little was known of its metabolism and sites of action. This paper deals with studies on the pharmacology of DHT_2 in rats. Following the synthesis of [^{3}H]DHT_2 and oral administration, evidence was obtained that DHT_2 is metabolized extensively; three of the major metabolites could be identified as 25-hydroxy-DHT_2, 1α,25- and 1β,25-dihydroxy-DHT_2.

INTRODUCTION

Historical background

Dihydrotachysterol$_2$ (DHT_2) is a synthetic derivative of vitamin D_2 which over the last 40 yr has been widely used in the treatment of renal osteodystrophy and hypoparathyroidism. The therapeutic efficacy of DHT_2 is equivalent to the other short-acting vitamin D analogues, i.e. 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and 1α-hydroxyvitamin D_3 ($1(OH)D_3$).

DHT_2 was discovered, in 1939, by Von Werder [1] as a reduction product of tachysterol$_2$. In 1943, Lui and Chu [2] found that this preparation, known under the trade name AT 10, was far more effective than vitamin D in increasing the intestinal absorption of calcium in patients with chronic renal failure. In the following years a large number of papers was published demonstrating the efficacy of AT 10 in the treatment of vitamin D related disorders. However, due to variations in the formulation of AT 10 and the consequent variability in its potency, dose requirements were unpredictable and therefore, the results of treatment were inconsistent.

With the introduction of crystalline DHT_2 (Dihydral®, Hytakerol®), in 1962, this problem was overcome. Crystalline DHT_2 appeared to be more effective than vitamin D_2 on a weight base in the treatment of hypoparathyroidism and renal bone disease. The major advantage of DHT_2 over vitamin D was that in case of overdosage the subsequent hypercalcemia was of shorter duration [3].

Vitamin D is known to undergo a number of metabolic transformations [4]. In the liver vitamin D is hydroxylated at C_{25} to give 25-hydroxyvitamin D_3 [5]. A small portion of the total amount of 25-hydroxyvitamin D produced undergoes further hydroxylations to $1,25(OH)_2D_3$ [6, 7, 8] and 24R,25-dihydroxyvitamin D_3 [9, 10]. It is generally accepted now that $1,25(OH)_2D_3$ is the physiologically active metabolite of vitamin D_3 [11]. This metabolite plays an essential role in the establishment and maintenance of the calcium and phosphorus homeostasis through stimulation of the intestinal absorption of calcium and mobilization of bone mineral. The major site of production of $1,25(OH)_2D_3$ is the kidney [6, 7]. Bone diseases as renal osteodystophy and hypoparathyroidism are associated with a decreased formation of this metabolite and thus it is understandable that many clinicians wished to prescribe the deficient compound in patients with these disorders. Therefore, after the introduction of synthetic $1,25(OH)_2D_3$ into clinical practice, in the middle of the 1970s, the interest in DHT_2 as a therapeutic agent declined rapidly. A second reason why interest in DHT_2 fell off so rapidly must also be sought in the lack of knowledge of its mode of action.

Recently, there has been a revival in interest in the use of DHT_2 in the therapy of renal osteodystropy [12, 13], partly due to alarming reports that treatment with $1,25(OH)_2D_3$ could lead to deterioration in renal function in uremic patients [14]. In addition, a shift in preference could be observed towards therapeutic agents which, in contrast to $1,25(OH)_2D_3$, cause less often quick-onset hypercalcemia and offer a larger safety margin in dose requirements, along with a longer duration of action [13, 15]. In this respect DHT_2 appeared to be the preparation of choice because another interesting compound, $1(OH)D_3$ is not available in all countries of the world.

Unfortunately, in the individual patient, tolerance and dose requirement for DHT_2 may change abruptly with time. Since occasional hypercalcemia occurs commonly, careful monitoring of the serum calcium concentration remains essential. Generally, the daily dose of DHT_2 is adjusted to maintain the serum calcium concentration within normal limits, but, nevertheless, in a number of patients bone histology does not improve. A key to the elimination of these therapeutic problems may be monitoring of the blood concentration of the biologically active form(s) of DHT_2. However, such assays cannot be

developed because a serious lack exists in the understanding of the metabolism and mode of action of DHT_2. This prompted us to start investigations into the pharmacology of DHT_2. This paper focuses on the preparation of radioactive labelled DHT_2, the fate of this tracer in rats and the isolation and identification of the most prominent metabolites.

Chemical structure and presumed pharmacology of DHT_2

Dihydrotachysterol$_2$ is a 5,6-trans-5,7-diene derivate of vitamin D_2 (Fig. 1b). The 5,7,10[19] triene system of vitamin D_2 is reduced to a Δ 5,7-diene and the A ring is rotated 180° around the 5,6-bond [16, 17]. As a result of this rotation, the 3β-hydroxyl group is converted to the geometrical equivalent of a 1α-hydroxyl group, often called the pseudo 1α-hydroxyl configuration. The configuration of the methyl group at C_{10} is S. It should be noted that the carbon atoms in the A-ring of DHT_2 are numbered in reverse with respect to those of vitamin D_2.

Like the vitamins D, the A ring of DHT_2 is dynamically equilibrated between two chair conformers [18]. The axial/equitorial ratio of the 3α-hydroxyl group in DHT_2 is 42:58 [19].

It is generally assumed that DHT_2 requires 25-hydroxylation before it can be effective. This hypothesis has been based on three assumptions:

(1) The metabolite 25-hydroxydihydrotachysterol$_2$ (25(OH)DHT_2) satisfies the structural requirements for vitamin D analogues to produce biological activity in the intestinal cell [20]. This applies, in particular, to the presence of hydroxyl functions at C_{25} and at $C_{(pseudo)1\alpha}$. The requirements for a (pseudo)3β-hydroxyl group or a 5,6-*cis*-triene system are less stringent.

(2) Synthetic 25-hydroxydihydrotachysterol$_3$ (25(OH)DHT_3) is more potent in mobilizing bone mineral in rats than dihydrotachysterol$_3$ (DHT_3) [21]. DHT_3 is the vitamin D_3 analogue of DHT_2. Moreover, some chromatographic evidence was found for the presence of 25-OH-DHT_3 in serum of rats after administration of DHT_3 [22].

(3) It has tentatively been shown that reconstituted liver mitochondrial cytochrome P-450 can carry out 25-hydroxylation of DHT_2 [23]. It should be noted that this conclusion was based solely on the mass spectrometric observation of the putative molecular ion of 25(OH)DHT_2, and not on a complete mass spectrum.

It is supposed that in mammals the metabolic

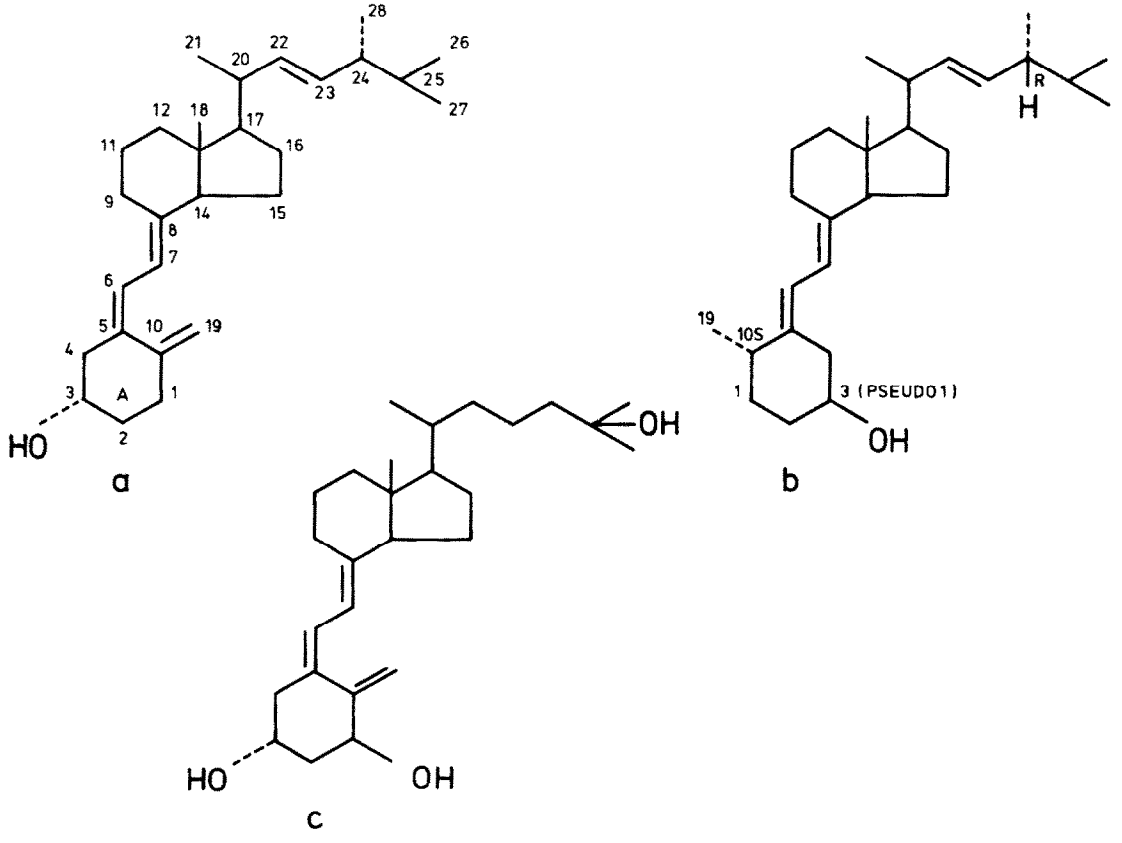

Fig. 1. The chemical structure and the numbering of the carbon atoms of vitamin D_2 (a), dihydrotachysterol$_2$ (b) and 1α,25-dihydroxyvitamin D_3 (c).

routes of DHT_2 and vitamin D are similar and therefore the possibility exists of the *in vivo* formation of 25(OH)DHT_2. It is conceivable that this compound can be metabolized to more polar forms. Evidence for the existence of more polar material came from Gray [24], who demonstrated that plasma of anephric patients treated with DHT_2 contains a substance that co-chromatographed with $1,25(OH)_2D_3$ and that displaced tritiated $1,25-(OH)_2D_3$ from its intestinal receptor.

SYNTHESIS OF TRITIATED DHT_2

Most information on the physiological transformations of vitamins D was obtained in studies of using radioactive-labelled vitamin D sterols in experimental animals. In order to elucidate the pharmacology of DHT_2 a similar approach was chosen, which required, the synthesis of the radioactive labelled compound [25]. Because of its efficiency the two-stage synthesis, introduced by Mourino *et al.*[26], was chosen. With vitamin D_2 as the starting material, the first step in this synthesis applied to the A ring and effectuated a 180° rotation about the 5,6-axis, with 5,6-transvitamin D_2 as the product of the equilibrium reaction. This product may undergo reduction of the double bond between C_{10} and C_{19} leaving the bonds between C_5 and C_8, and between C_{22} and C_{23} unaffected. The reduction of the methylene group at C_{10} generates a chiral centre, and as a result, the yield of dihydrotachysterol$_2$ cannot exceed 50%.

Vitamin D_2 can be converted into 5,6-transvitamin D_2 by iodination under irradiation as well as by triplet-sensitized isomerization [27]. In our hands, the first method led to unwanted side-reaction products which interfered in the purification of 5,6-transvitamin D_2. Triplet-sensitized isomerization, performed with Bengal Rose bound to a bead-formed gel, was not attended with side-reactions. Purification of 5,6-transvitamin D_2 was performed on silica gel and by recrystallization; yield: 44%, pure by HPLC.

Partial, homogeneous catalytic reduction of 5,6-transvitamin D_2 with tritium gas yielded the stereoisomers [10S(19)-^{3}H]dihydrotachysterol$_2$ [^{3}H-DHT_2]- and [10R(19)-^{3}H]dihydro-5,6-transvitamin D_2. After purification on straight-phase HPLC the overall yield of [^{3}H]DHT_2 was 30%. The identification of [^{3}H]DHT_2 was carried out by co-chromatography with unlabelled DHT_2 on HPLC and by u.v.-absorption spectrometry. Since the reduction method applied leads to complete incorporation of hydrogen, the tracer had a high specific radioactivity of 56 kCi/mol. [^{3}H]DHT_2 was stored in solution (*n*-hexane) under dry nitrogen in dark-brown ampoules at −20°C. Four yr after preparation this material still possessed its original chromatographic properties and purity, and did not show any sign of radiolysis.

THE METABOLIC FATE OF ^{3}H-DHT_2 IN RATS

The following step in the elucidation of the pharmacology of DHT_2 was a detailed study of its fate in experimental animals [28]. An experimental design was chosen to yield circumstances under which sufficient material of the metabolites could be obtained enabling structural analyses. [^{3}H]DHT_2 was administered intragastrically to male Wistar rats in amounts of 1.84 nmol or a multiple of that. The fate of the label was followed by means of chromatographic fractionation of the radioactivity in serum. The dose, of 1.84 nmol, was taken as an equivalent of a therapeutic dose in patients (503 nmol), on the basis of the body weight of man (75 kg) and rat (275 g).

Figure 2 shows typical chromatographic distributions of radioactivity in lipid extracts of serum of rats after a dose of 1.84 nmol (1.91 MBq) of [^{3}H]DHT_2, collected 4 h after administration. At 24 h after administration the presence of at least 9 distinct peaks of radioactivity could be established, migrating after chromatography between vitamin D_3 and $1,25(OH)_2D_3$. The peaks, preliminarily designated "b" and "c2", co-chromatographed with DHT_2 and 25(OH)DHT_3. At the 4-h time point the presence of 7 peaks could be ascertained; 24 h after

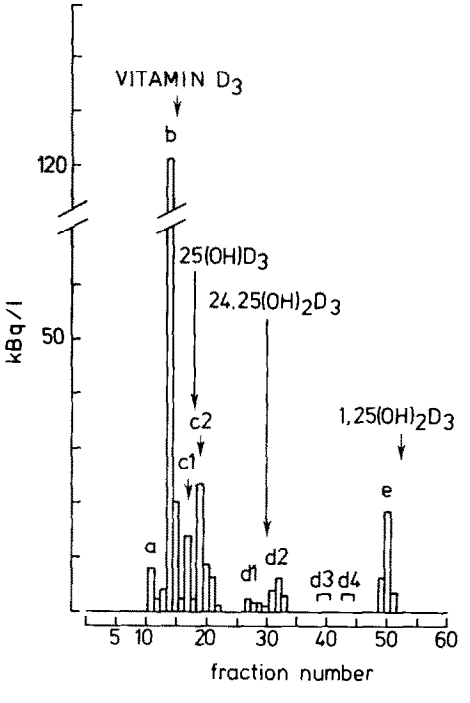

Fig. 2. Typical distribution of radioactivity after chromatography of a lipid extract of rat serum after an oral dose of 1.84 nmol (1.91 MBq) of tritiated dihydrotachysterol$_2$ ([^{3}H]DHT_2). Blood was collected 4 h after administration. Arrows indicate the retention positions of vitamin D_3, its major metabolites and the [^{3}H]DHT_2 derivatives "c1" and "c2". Horizontal brackets indicate the retention positions of two minor [^{3}H]DHT_2 derivatives, "d3" and "d4", which appear not sooner than 24 h after administration.

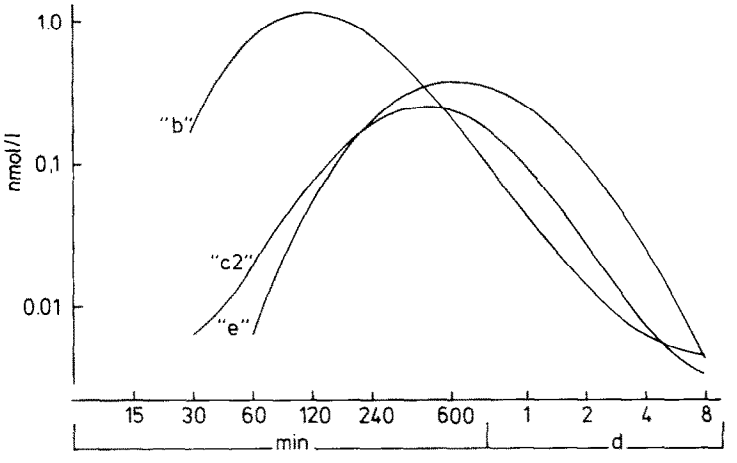

Fig. 3. Time course of the concentration of three major radioactive substances in lipid extracts of serum of rats after an oral dose of 1.84 nmol (1.91 MBq) of tritiated dihydrotachysterol$_2$. Marks (top corners) relate to the peaks in Fig. 2. For details, see text.

administration 2 new, minor peaks "d3" and "d4" had appeared, but peak "c1" had vanished.

It should be noted that the data given in the next two figures have been corrected for analytical recovery after extraction and chromatography. The individual analytical recoveries of the [^{3}H]DHT$_2$ peaks were determined by rechromatography of the individual substances.

Figure 3 shows the concentrations of three of the major [^{3}H]DHT$_2$ metabolites with the time passed since administration. The designations in the top corners of the panels correspond with the peak designations used in Fig. 2.

Detectable amounts of radioactivity were not found under 30 min after administration. The simultaneous appearance of the peak "a", "b", "c1" and "c2", at that time, demonstrates that [^{3}H]DHT$_2$ is rapidly metabolized to more polar substances. It is assumed that "b" represents the starting material. The less polar peak "a" might be attributed to an ester form of [^{3}H]DHT$_2$. The most prominent compound after 24 h is peak "e". Since peak "e" is more polar than "c1" and "c2" and does not appear sooner than after 60 min, one might deduce that it can be formed from "c1" and/or "c2". Another argument in favour of this deduction is the time-point at which the various compounds reach maximum concentrations. Here, the use of a logarithmic time-scale is inconvenient. The maxima of "b", "c2" and "e" really lie at about 2, 4 and 10 h, respectively. The highest concentration of total radioactivity in serum occurred at 4 h; expressed as a percentage of the dose/ml serum it was rather low: 0.11%/ml.

As stated above, one of the purposes of this study was to find experimental conditions under which the largest possible amount of DHT$_2$ metabolites could be obtained. One possible way to attain this purpose was accumulation of the metabolites in the circulation in response to daily administration. The alternative possibility was increasing the administered dose. Since after a single dose of [^{3}H]DHT$_2$ almost negligible amounts of radioactivity were left in the circulation after 24 h, the prospective of a considerable accumulation of material by daily administration was unfavourable. In fact, the actual increments achieved were so small that the concentrations of the metabolites reached a steady state with 96 h after the first administration, at levels hardly higher than after 24 h.

Fortunately, our attempts at raising serum concentrations by increasing the dose were more successful. Figure 4 shows the course of the serum concentrations of the major DHT$_2$ compounds in response to increases in the dose given. Blood was collected 10 h after administration. This point of time was chosen in view of the concentration maximum of the majority of the compounds. Each point in Fig. 4 represents the value measured in one single rat. Logarithmic transformation of both the dose and concentration scale produced highly significant positive correlations. This means that the relative increment of the concentrations are directly proportional to the relative increment of the dose. If the regressions were calculated over the entire dose scale, the slopes were smaller than 1, implying that the concentrations in serum behave as exponential functions of the dose; in fact, if the dose increases 1000-fold, the concentrations of the metabolites increased 400-fold, on the average. The parallelism between the 3 regression lines demonstrates that the mechanisms responsible for the appearance of the compounds in the circulation are closely related. At the two lowest concentrations of the dose the slopes of the regression lines are 1, indicating that at dose levels up to 1.84 nmol DHT$_2$, a feedback control in the formation of metabolites, demonstrable in serum,

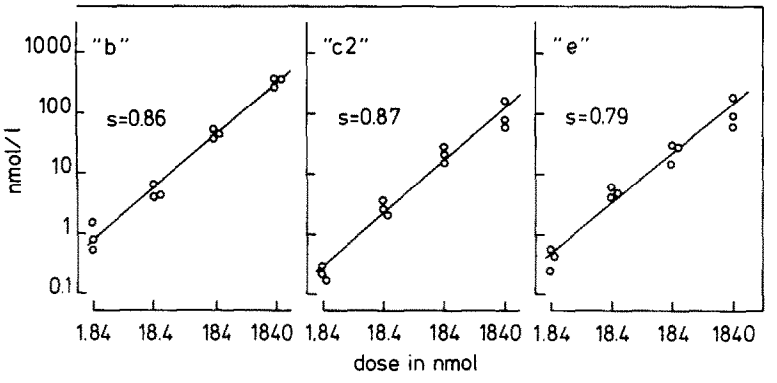

Fig. 4. Cause of the concentrations of radioactive substances in lipid extracts of serum of rats receiving increasing, oral doses of tritiated dihydrotachysterol$_2$. Blood was collected 10 h after administration. Each point relates to a single animal. Marks (top corners) relate to the peaks in Fig. 2. "s" represents the slope of the regressions. For details, see text.

is absent in rats. A similar finding has been reported by Hallick and DeLuca [29], studying the metabolism of DHT_3.

It has been mentioned above that the recovery of serum radioactivity after a dose of 1.84 nmol of [^{3}H]DHT_2 was very low. This result must be due to one or more of the following factors: poor intestinal absorption, rapid decay of radioactivity from the circulation or instability of the tracer. A rapid decay from the blood might be attributed to a fast rate of excretion and/or rapid distribution in the tissues of the body. In response to repeated administration the latter would lead to accumulation of material in the tissues which, in its turn, would be reflected ultimately in an increase of the concentrations in blood. However, in response to daily administration the serum concentrations reached steady-state levels within 96 h, which indicates that the decreases in radioactivity in serum were associated with either peripheral catabolism or excretion. Although it cannot be excluded that the labels at C_{10} and C_{19} in [^{3}H]DHT_2 are lost under *in vivo* conditions, there are strong indications that the tracer itself is stable under the experimental conditions used. At the highest dose level used the serum concentration of compound "e" becomes so high that the u.v. absorption after chromatography of serum lipid extract could be used as a way to measure its concentration. These u.v. results appeared to be very similar to the results of the measurements of radioactivity.

THE ISOLATION AND IDENTIFICATION OF METABOLITES OF DHT_2

In the previous section we reported the occurrence of 9 distinct radioactive substances in serum of rats after an oral dose of tritiated DHT_2. Furthermore, it was shown that the serum concentrations of these compounds could be increased considerably by increasing the dose of DHT_2. Fortunately, the concentration levels realized were high enough to justify attempts aiming at isolation and identification of these compounds. Preliminarily, we have focused our efforts on the compounds designated "b", "c2" and "e" [30]. In the chromatographic systems used peak "b" co-migrated with authentic DHT_2. Therefore, "b" was most likely the starting material.

It is generally assumed that DHT_2 acts through its putative metabolite 25(OH)DHT_2. Since "c2" co-chromatographed with synthetic 25(OH)DHT_3, we expected to have a fair chance to find 25(OH)DHT_2 in peak "c2". We supposed that peak "e", which co-chromatographed with 1,25(OH)$_2D_3$, and the substance described by Gray *et al.*[24]—see Introduction—would be identical. This substance was reported to cause a considerable reduction of the binding of tritiated 1,25(OH)$_2D_3$ to its intestinal receptor, pointing to affinity of this substance to the receptor. In view of this premise it was interesting to isolate and identify "e".

It is not our intension to exhaustively describe the different methods used for isolation. We would confine ourselves to the results of the production and isolation, and deal in more detail with the arguments which led to the structural assignments. We were successful in raising and isolating the metabolites in microgram amounts. However, complete purification was not achieved and thus biological activities could not be determined. It is likely that the problems of purification might be reduced by using different or more extensive forms of chromatography, by changing chromatographic properties through derivation and by using extracts of different body tissues or species.

The structural assignments were supported by liquid chromatographic and u.v. spectral data, but were based mainly on GC–MS analysis. Furthermore, all conclusions shown rested upon the assumptions that the DHT_2 carbon skeleton and the 3α-hydroxyl function (pseudo 1α) remain intact during metabolism in rats. Moreover, for mass spectral analysis it appeared to be desirable to increase

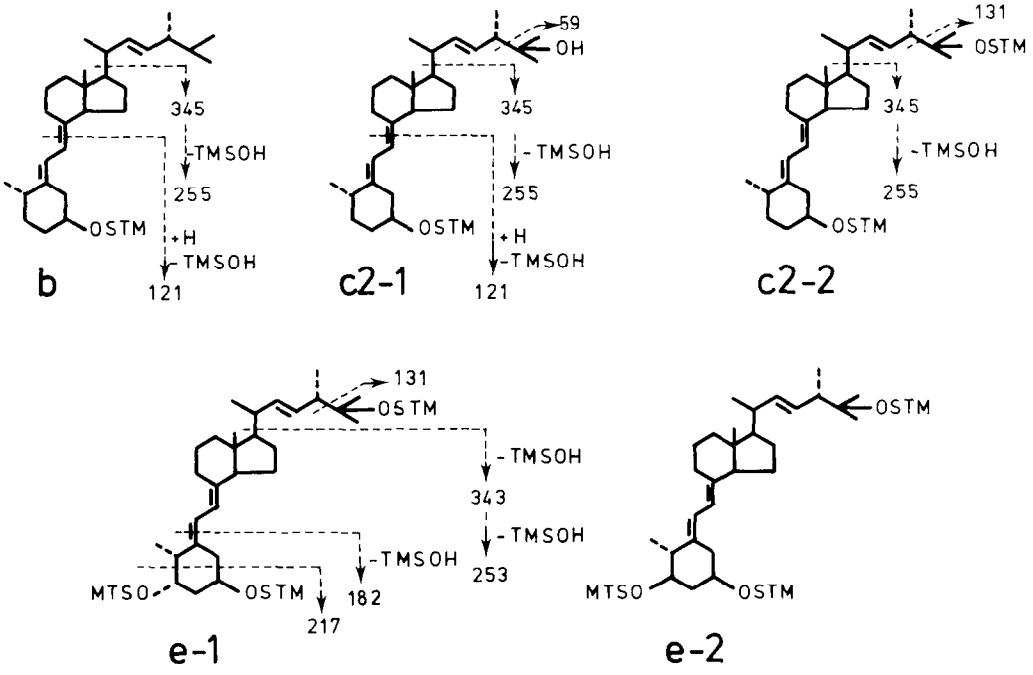

Fig. 5. Chemical structures and mass spectral fragmentation patterns of trimethylsilyl ethers of dihydrotachysterol$_2$ (b), 25-hydroxydihydrotachysterol$_2$ (c2-1: monotrimethylsilylated; c2-2: ditrimethylsilylated), 1β,25-dihydroxydihydrotachysterol$_2$ (e-1) and 1α,25-dihydroxydihydrotachysterol$_2$ (e-2).

the volatility of the compound investigated, which was achieved by trimethylsilylation. The structures of the analyzed compounds and their mass spectral fragmentation patterns have been given in Fig. 5.

Metabolite b

The mass spectrum of silylated "b" was identical to that of the trimethylsilyl ether of authentic DHT_2 showing peaks at m/z (=mass charge ratio) 470 (M+), 380, 255 and 121 (base peak; C_7–C_8 cleavage and concomitant H shift). The chromatographic, u.v. and mass spectral data demonstrated that DHT_2 is present in the circulation after oral administration. This presence in blood after oral administration had not been conclusively demonstrated previously. It is noteworthy that C_7–C_8 cleavage is the main fragmentation reaction in D vitamins [31].

Metabolite c2

Mass spectrometry of silylated "c2" showed a peak at m/z 121 (base peak) characteristic in the spectrum of silylated DHT_2 and a molecular ion at m/z 486, consistent with a monotrimethylsilyl ether of a monohydroxylated DHT_2 derivative. The fragments at m/z 468, 396 and 378 confirmed the presence of a silylated and a free hydroxyl function in the compound. The ion at m/z 255 indicated that the free, additional hydroxyl function was located in the side-chain.

Additional evidence for structural assignment was obtained after prolonged silylation. Now, the mass spectrum of "c2" showed a molecular ion at m/z 558. This corresponds to the molecular ion of a ditimethylsilyl ether of monohydroxylated DHT_2. Ions at m/z 255 and 131 (base peak) indicated the presence of the additional hydroxyl group at C_{25}. Assuming that a hydroxyl group is present at C_3 these mass spectral data together with the data of chromatography and u.v. absorption established the structure of "c2" as 25-hydroxydihydrotachysterol$_2$.

Metabolite e

The total ion current chromatogram of silylated "e" showed that this compound consisted of two components with essentially identical mass spectra. Therefore, the following interpretation of the mass spectral data applies to both compounds. The presence of a molecular ion at m/z 646 suggested the incorporation of two additional hydroxyl groups into DHT_2, it corresponded with the molecular ion of a tritrimethylsilyl ether of DHT_2. The ions at m/z 343 and 253, arising by loss of the entire side-chain, indicated the presence of only one hydroxyl group in the side-chain. A fragment at m/z 131 (base peak) requires C_{25} as its position. The position of the second additional hydroxyl group was apparent from the ions at m/z 182 and 217. The peak at m/z 182 could result from the cleavage of the C_5–C_6 bond, thus suggesting the presence of two hydroxyl groups in the A ring. The ion at m/z 217 confirmed this interpretation since it could only be attributed to the fragment $(CH_3)_2SiO$–CH=CH–CH=$O^+Si(CH_3)_3$

formed by cleavage of the C_1–C_{10} and C_3–C_4 bonds, with a hydroxyl group at C_1 and a hydroxyl group at C_3. These data established that the structure of the two epimeric parent compounds was 1-ambo,25-dihydroxydihydrotachysterol$_2$.

The tentative stereochemistry of the hydroxyl group at C_1 could be deduced by comparison of the relative intensities of the fragments at m/z 646 (M^+) and 556 (M-TMSOH)$^+$ (TMSOH=$(CH_3)_3SiOH$). The molecular ions of the tritrimethylsilyl ethers of the epimers differ considerably in the rate of loss of TMSOH. An axial substituent at C_1 introduces non-bonded interactions into the A ring and these increase the reactivity of the molecular ion. However, due to A ring chair-chair interconversion [19] a trimethylsilyl ether group can take an axial position in both the 1α- and 1β-conformer. Crowding of the hydrogens of the trimethylsilyl ether functions at C_1 and C_3 becomes maximal when both are in the axial position. As a result the molecular ion of the diaxial conformer will have a greater tendency to lose TMSOH compared to its equatorial (C_1)–axial (C_3) counterpart. Thus the structure of the epimer with the highest ratio (M-TMSOH)$^+$/M^+ could be tentatively identified as the tritrimethylsilyl ether of 1α,25-dihydroxydihydrotachysterol$_2$ (1α,25(OH)$_2$DHT$_2$). Consequently, the other compound was 1β,25-dihydroxydihydrotachysterol$_2$ (1β,25(OH)$_2$DHT$_2$).

As a result of the inversion of ring A in DHT_2 the configuration of its 3α-hydroxyl group is geometrically equivalent to the 1α-hydroxyl of 1,25(OH)$_2$D$_3$. The presence of an 1α-hydroxyl group is of paramount importance for the biological activity of the vitamin D [20]. Besides 1α-hydroxylation the vitamins D must undergo 25-hydroxylation before acquiring their full biological activity [4].

It has been proposed that the presence of a hydroxyl group at a geometrically equivalent of the 3β-hydroxyl of 1α,25-dihydroxyvitamin D_3 is not an absolute requirement for biological activity in the intestinal cell once a pseudo 1α-hydroxyl and a 25-hydroxyl group are in the molecule [20]. Indeed, 1,25(OH)$_2$D$_3$ is only eight times more effective than 3-deoxy-1,25-dihydroxyvitamin D_3 in reducing the binding of tritiated 1,25(OH)$_2$D$_3$ to its intestinal receptor. Nevertheless, the identified metabolites "e" suggest that both 1α,25(OH)$_2$DHT$_2$ and 1β,25(OH)$_2$DHT$_2$ might be biologically more active than 25(OH)DHT$_2$. Biological activity depends also on the equatorial/axial ratio of the pseudo 1α-hydroxyl function. The chair conformer in which the 1α-hydroxyl is equatorial is the preferred biologically active form [20]. It is likely that due to steric factors the A ring of 1α,25(OH)$_2$DHT$_2$ will be forced almost entirely into the diequitorial chair. However, it is also conceivable that biological activity is related to the geometric position of the hydroxyl group at pseudo C_3 [32]. Therefore, it is difficult to predict which of the two epimeric forms represents the biologically most active form of DHT_2.

CLOSING REMARKS

It has been mentioned above that at present the clinical interest in DHT_2 makes a new start. The reasons for this revival are the efficacy of DHT_2, the favourable duration of therapeutic action and the relatively slight risk of hypercalcemia. Additional advantages may be the comparatively low production costs and the fact that DHT_2 is a vitamin D_2 derivative. There is a growing piece of evidence that vitamin D_2 sterols are less active in resorbing bone mineral than their vitamin D_3 counterparts [33, 34]. The advantageous therapeutic properties of DHT_2 appeal for further pharmacological investigations. Such studies will no doubt deepen the insights into the biology of D vitamins as a whole and involve DHT_2 to be decided not on its seniority but on its merits.

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