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SHORT COMMUNICATION

SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE DIHYDROTACHYSTEROL$_2$ METABOLITE 25-HYDROXYDIHYDROTACHYSTEROL$_2$

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Summary—25-Hydroxydihydrotachysterol$_2$ has been stereoselectively synthesized from 3-*tert*-butyldimethylsiloxy-25-methoxymethyloxyvitamin D$_2$ (4 steps, 23% overall yield) which enabled us to prove that it is one of the metabolites of dihydrotachysterol$_2$ in rats, and allowed its biological properties to be evaluated.

INTRODUCTION

Dihydrotachysterol$_2$ (DHT$_2$, *1*), first isolated by von Werder [1], is successfully used in the treatment of renal osteodystrophy [2], hypoparathyroidism [3, 4] and tetanus [5]. Its metabolism is nevertheless not completely understood and one of us has previously reported, after analyzing the serum of DHT$_2$ treated rats, the formation of several metabolites. One of them, isolated in impure form, was tentatively identified on the basis of u.v. absorption and gas chromatography-mass spectrometry (GC-MS) data as 25-hydroxydihydrotachysterol$_2$ [*2*, 25-(OH)-DHT$_2$] [6]. We have now synthesized 25-(OH)-DHT$_2$ *(2)* in order to verify the identity of the compound isolated from rats and to obtain enough pure material to allow determination of its biological properties. (Scheme 1.)

RESULTS

Desilylation of 3-*tert*-butyldimethylsiloxy-25-methoxymethyloxyvitamin D$_2$ *(3)* [7] (tetra-*n*-butylammonium fluoride, THF, rt) afforded 25-methoxymethyloxyvitamin D$_2$ (*4*, 78%). This compound was isomerized (I_2, fluorescent light, Et_2O, 5 h) [8] to its (5*E*) isomer, (5*E*)-25-methoxymethyloxyvitamin D$_2$ *(5)*, to afford a 45:55 mixture of *4* and *5*, that was resolved by HPLC (Whatman Partisil column, 10 × 250 mm, eluent: 50% EtOAc–hexane). *5* was subjected to the hydrotitanation-protonation procedure recently developed by ourselves [9]. Reaction of $LiAlH_4$–Cp_2TiCl_2 with *5* followed by addition of water led to a mixture of 25-methoxymethyloxydihydrotachysterol$_2$ *(6)* and its (10*R*) epimer 25-methoxymethyloxydihydrovitamin D$_2$-III *(7)* (65%, *6*–*7* ratio 5:1). After chromatographic separation, compound *6* was deprotected by treatment with the ion exchange resin AG50W-X4 in MeOH, to afford 25-(OH)-DHT$_2$ [*2*, 99%; ^{1}H NMR (250 MHz, $DCCl_3$, δ) 6.15 and 5.89 (2H, AB system, J = 11.1 Hz, H7 and H6), 5.33 (2H, m, H22 and H23), 3.60 (1H, m, H3α) 1.17 (3H, s, CH_3-C26 or CH_3-C27), 1.13 (3H, s, CH_3-C27 or CH_3-C26), 1.09 (3H, d, J = 6.6 Hz, CH_3-C28), 1.04 (3H, d, J = 7.7 Hz, CH_3-C19), 1.01 (3H, d, J = 7.8 Hz, CH_3-C21), and 0.56 (3H, s, CH_3-C18); ^{13}C NMR (75.5 MHz, $DCCl_3$, δ) 142.0, 139.8, 139.1, 129.1, 116.9, 115.7, 72.3, 70.9, 56.4, 56.2, 48.2, 45.6, 40.4, 38.2, 37.7, 34.9, 33.2, 28.9, 27.8, 27.0, 26.4, 23.5, 22.3, 21.0, 17.8, 15.7; IR (CCl_4) 3605 (w, free OH), 3445 (br, associated OH), 3010 (s, C = H), 2960 (s, C-H), 2930 (s, C-H), 2875 (s, C-H), 1615 (w), 1465 (m), 1375 (m), 1265 (s), 1100 (s), 1015 (s) cm^{-1}; u.v. (EtOH 95%) λ_{max} 242 nm (ϵ 29500), 252 nm (ϵ 33000), 260 nm (ϵ 21500); LREIMS (20 eV, *m/z*, %) 415 ($M^+ + 1$, 26), 414 (M^+, 90), 396 ($M^+ - H_2O$, 15), 378 ($M^+ - 2H_2O$, 14), 356 (14), 273 (51), 255 (58), 161 (25), 147 (38), 145 (12), 135 (40), 133 (38), 122 (11), 121 (100), 95 (27); HREIMS Calc. for $C_{28}H_{46}O_2$: 414.3498. Found: 414.3485].

The synthetic compound was silylated and analyzed by GC-MS using conditions identical to those described previously [6]. Figure 1 shows the mass spectra of the bis-trimethylsilyl derivatives of (a) the metabolite isolated from rats and (b) the synthetic compound. As the peak at *m/z* 456 of spectrum (a) was due to a contaminant [6], we may conclude that the two samples had identical mass spectra.

The biological activity of 25-(OH)-DHT$_2$ *(2)* was determined in vitamin D deficient chicks. In this assay, graded doses of the test analog (650, 3250, 16250 pmol) were administered intraperitoneally to groups of 6 birds each. Twelve hours later 5 μCi of calcium-45 was placed in the lumen of the duodenum and after 30 min the chicks were sacrificed. The responses of intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were determined as described previously [10, 11]. The dose of the test analog necessary to achieve the ICA or BCM response of 100 pmol of 1α,25-dihydroxyvitamin D$_3$ [1α,25-(OH)$_2$-D$_3$, *8*] was determined and the result expressed as per cent of the response achieved by the 1α,25-(OH)$_2$-D$_3$ (*8*). Thus for the 25-(OH)-DHT$_2$ *(2)*, the ICA response was 0.5% and the BCM 0.6%. In addition the ability of the 25-(OH)-DHT$_2$ to compete with 1α,25-(OH)$_2$-D$_3$ *(8)* for the binding to the chick intestinal receptor was determined. The relative competitive index [RCI, 100% for 1α,25-(OH)$_2$-D$_3$ by definition] for 25-(OH)-DHT$_2$ was 3.8%. In comparison, for DHT$_2$ *(1)* the RCI is 0%, while the ICA and BCM values are 0 and 0.1%, respectively [both in respect to 1α,25-(OH)$_2$-D$_3$].

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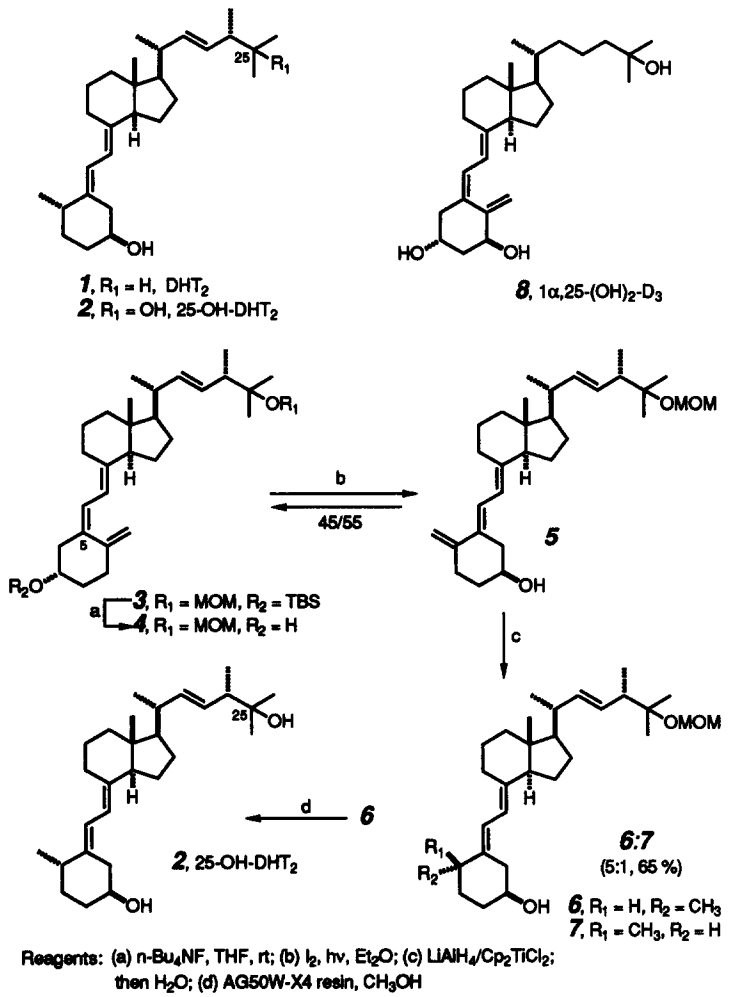

Scheme 1.

So we may conclude that the DHT_2 metabolite 25-(OH)-DHT_2 is more active than DHT_2 itself, and could well be responsible for the therapeutical effectiveness of this agent.

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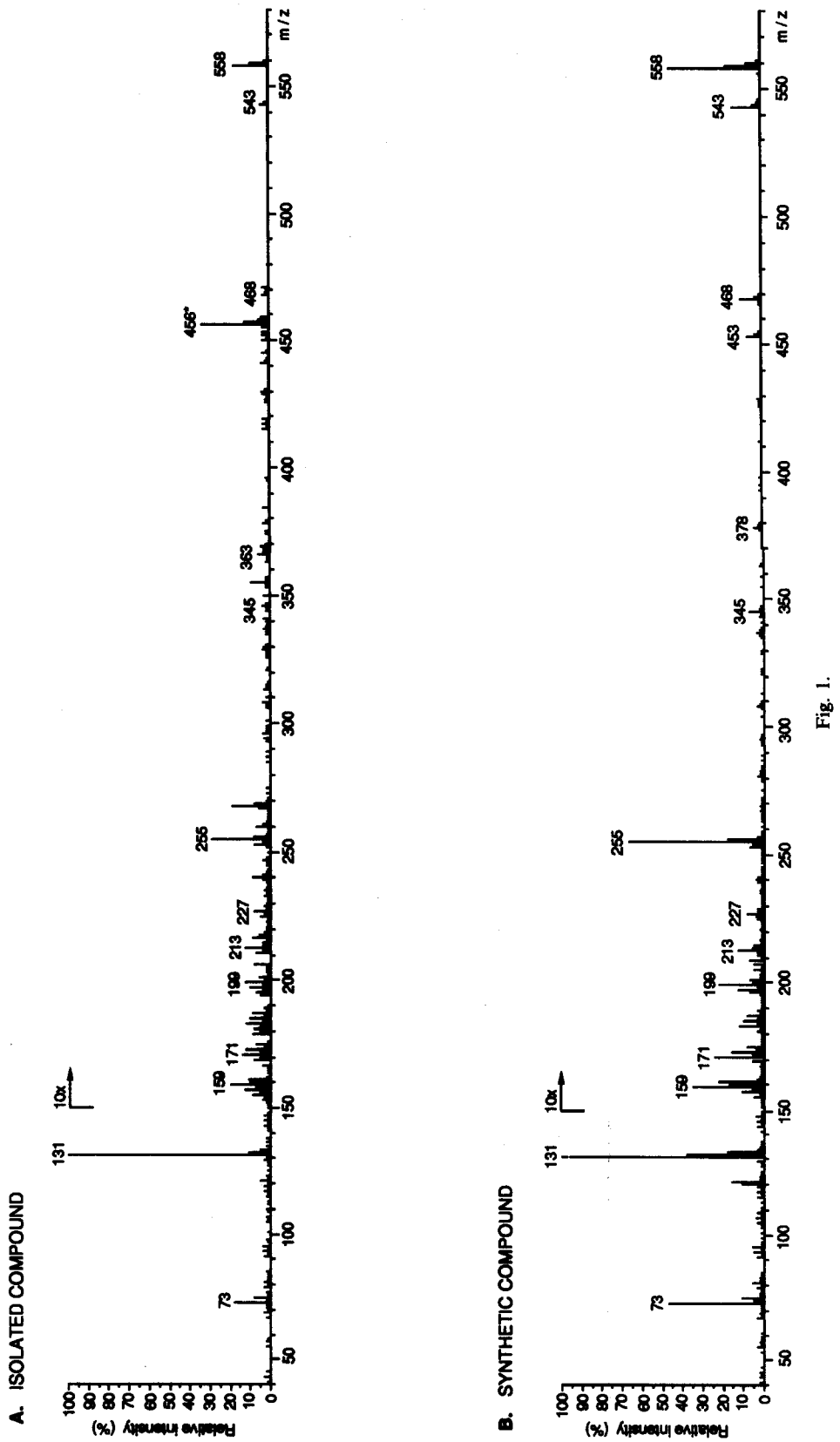
A. ISOLATED COMPOUND
B. SYNTHETIC COMPOUND
Relative Intensity (%)
m/z
10x
73
131
159
171
199
213
227
255
345
363
378
453
456*
468
543
558

Fig. 1.