SHORT PAPER

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Autoxidation of isotachysterol: formation of new epoxides†

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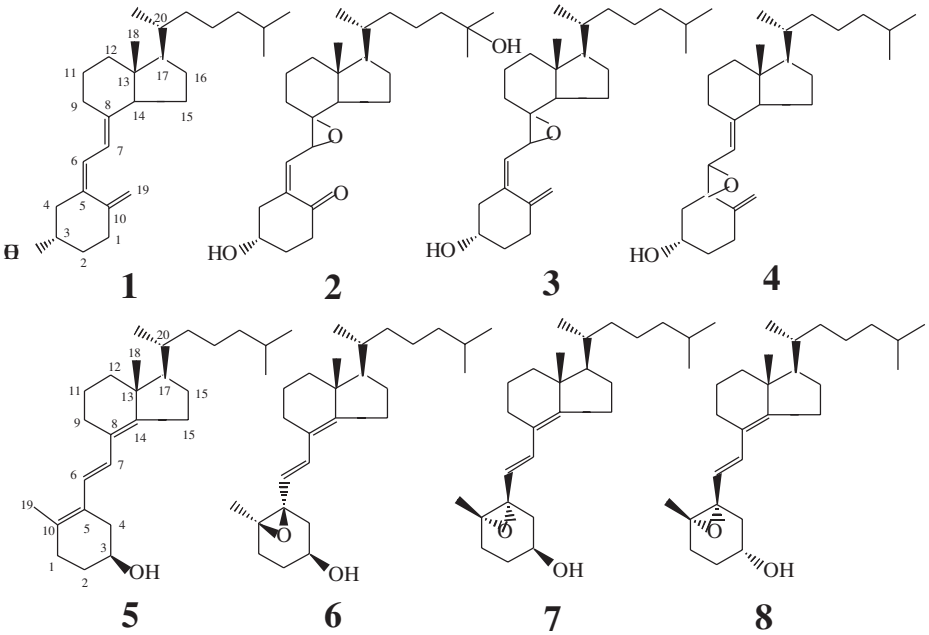
Autoxidation of isotachysterol under atmospheric oxygen in the dark at ambient temperature produces two new epoxides: (*3S,5R*)- and (*3S,5S*)-5,10-epoxy-isotachy sterols.

Keywords: vitamin D_3, isotachysterol, autoxidation, epoxides

The chemistry and biochemistry of cholecalciferol (vitamin D_3, **1**) have been extensively studied for over half a century due to the great diversity of its chemistry and, especially, its important roles in calcium regulation, immunological regulation and inducing cancer cell differentiation.[1] Over 30 natural metabolites of vitamin D_3 have been identified from humans and animals[2] and many more synthetic analogues, especially those of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), have been made to explore their anticancer potential and other biological activities.[3] Structural alterations of vitamin D_3 by metabolism mostly occurred at the 1α-position and the side chain,[2] while oxidation of the conjugated triene part has scarcely been reported.[4-6] The unique epoxide found in natural metabolites of vitamin D_3 is 7,8-epoxy-25- hydroxy-19-nor-10-oxovitamin D_3 (**2**).[4] Takayama and coworkers[5] found that **1** could be regio- and stereoselectively oxidized by *m*-chlorobenzoic acid and *tert*-butyl hydroperoxide catalysed by $VO(acac)_3$, giving (*7R*)-7,8-epoxyvitamin D_3 (**3**) and (*5S*)-5,6-epoxyvitamin D_3 (**4**) respectively. Photosensitised oxidation of vitamin D_3 by singlet oxygen has also been reported.[6] However, autoxidation of vitamin D_3 and its isomers has not been reported previously. It is well-known that vitamin D_3 is relatively stable in the air at ambient temperature,[6a] while its acid-catalysed isomerisation product, isotachysterol (**5**), is very labile in the air even in the dark.[7,8] We report herein the first autoxidation reaction of **5**. Three new epoxides **6**, **7** and **8** (Fig. 1) are produced.

Isotachysterol (**5**) was prepared by HCl-catalysed isomerisation of vitamin D_3 (**1**) in methanol.[8] It was found that the pale yellow oil of **5** was oxidised rapidly in the air at ambient temperature to a very complex mixture from which three pure compounds **6**, **7** and **8** were obtained**.** HR-ESI-MS determination gave M+1 peaks at 401.3413, 401.3422 and 401.3403 for **6**, **7** and **8** respectively, corresponding to the same molecular formula $C_{27}H_{44}O_2$ for the three compounds (requires 401.3419 for M+H), *i.e.*, molecules with one more oxygen than **5**. Comparison of their ^1H and ^{13}C NMR spectra with those of vitamin D_3 and its metabolites[9] and with that of isotachysterol[10] demonstrates clearly that **6**, **7** and **8** are 5,10-epoxides of **5** since remarkable changes on ^{13}C chemical shifts are only observable for 5-C and 10-C (from double bond carbons to epoxy carbons) and on ^{13}C and ^1H chemical shifts for 19-Me, and to a lesser



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extent, for 4-C. The coupling constants of 3-H of **6** are not well resolved, but the coupling constants of its 4-Ha and 4-He are 8.0 and 12.0 Hz, and 4.5 and 12.0 Hz, respectively, suggesting that the 3-H of **6** is axial. The coupling constants of the 3-Hs of **7** and **8** are 9.6, 9.6, 4.7 and 4.7 Hz, and 8.1, 8.1, 5.1 and 5.1 Hz, respectively, demonstrating that the 3-Hs of **7** and **8** are also axial. The facts that the 2-He (δ 1.82), 3-Hs (δ 3.97) and 19-CH_3 (δ 1.18) of **7** are appreciable downfield shifted from those of **6** (δ 1.73, 3.81 and 1.08 respectively) and **8** (δ 1.70, 3.89 and 1.06 respectively), and that the 2-Ha (δ 1.38) of **7** is significantly upfield shifted from those of **6** (δ 1.64) and **8** (δ 1.63), suggest clearly that the epoxy ring and 3-Ha is on the same side in **7**, while on the opposite side in **6** and **8**, and that the epoxy ring and 2- and 4-Hs are on the opposite side in **7**, while in the same side in **6** and **8**. That is, the epoxy ring and 3-OH are *anti*- in **7**, while *syn*- in **6** and **8**. These configurations are supported by their NOESY spectra as shown in Fig. 2.

In addition, epoxidation of isotachysterol (**5**) with anhydrous *tert*-butyl hydroperoxide (TBHP) in benzene in the presence of $VO(acac)_2$ (0.01 equiv) at 0°C gave **6** as the sole epoxy product (yield 45 %). It is well known that epoxidation of homoallylic alcohols with TBHP / $VO(acac)_2$ produces stereospecifically

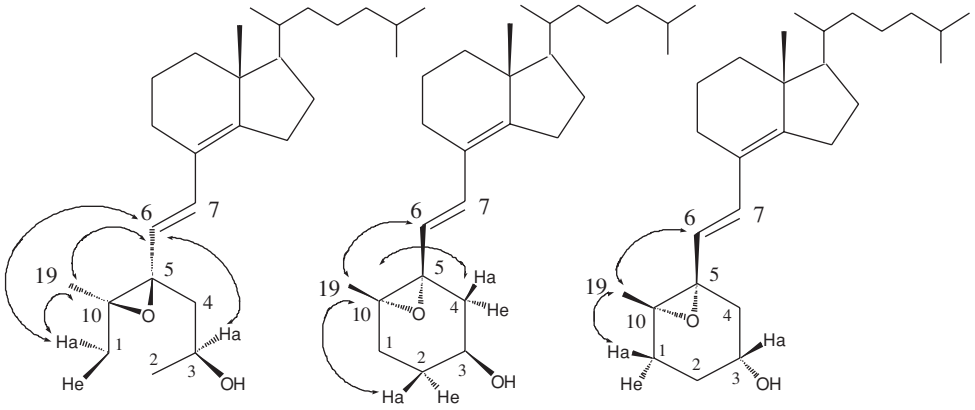

Fig. 2. Principal NOESY correlations of **6, 7** and **8**.

Table 1 ^1H (400MHz) and ^{13}C (100MHz) chemical shifts of compounds **5–8** in acetone-d_6

Carbon	5	6	7	8	Proton	5	6	7	8
1	32.27	33.68	35.44	35.06	1α	1.82	1.42	1.87	1.88
					1β	2.17	1.86	1.46	1.42
2	32.10	30.40	32.73	31.28	2α	1.86	1.73	1.82	1.63
					2β	1.48	1.64	1.38	1.70
3	67.31	67.32	66.75	66.78	3α	3.81[a]		3.97[a]	3.89[a]
					3β		3.81[a]		
4	35.51	32.55	43.34	44.27	4α	2.53	1.95	1.95	1.97
					4β	2.04	1.60	1.60	1.61
5	127.15	77.59	77.09	78.02					
6	124.65	129.54	131.16	132.41	6	6.53[b]	5.73[c]	5.82 [d]	5.96[e]
7	125.89	128.59	127.98	127.67	7	6.36[b]	6.61[c]	6.45 [d]	6.52[e]
8	125.40	124.48	124.54	124.68					
9	26.27	26.13	26.06	26.10	9α	2.38	2.36	2.35	2.45
					9β	2.47	2.47	2.47	2.47
10	131.60	73.09	73.79	72.32					
11	27.62	27.58	27.60	27.60	11α	1.92	1.89	1.90	1.91
					11β	1.46	1.47	1.46	1.42
12	38.63	37.96	37.98	38.02	12α	1.18	1.19	1.18	1.15
					12β	2.01	2.01	1.98	2.00
13	44.56	44.40	44.39	44.27					
14	149.27	148.08	148.82	148.37					
15	24.82	24.98	24.97	25.06	15α	2.04	1.98	1.93	1.97
					15β	2.24	2.12	2.15	2.13
16	19.64	19.60	19.59	19.62	16α	1.90	2.01	2.01	2.03
					16β	1.74	1.75	1.73	1.73
17	57.23	57.13	57.17	57.18	17	1.18	1.19	1.20	1.17
18	18.45	18.37	18.39	18.37	18	0.90	0.90	0.90	0.89
19	18.86	23.92	23.82	24.55	19	1.75	1.08	1.18	1.06
20	35.27	35.31	35.30	35.29	20	1.50	1.48	1.51	1.50
21	19.38	19.34	19.36	19.35	21	0.97	0.97	0.97	0.96
22	36.57	36.56	36.56	36.56	22	1.10[f]	1.43	1.43	1.43
						1.36[f]			
23	24.37	24.32	24.33	24.32	23	1.10[f]	1.36	1.10[f]	1.05[f]
						1.43[f]		1.36 [f]	1.39[f]
24	40.15	40.16	40.15	40.14	24	1.17	1.15	1.11	1.13
25	28.57	28.60	28.58	28.57	25	1.50	1.48	1.52	1.52
26	22.76	22.99	22.99	22.98	26	0.86	0.87	0.86	0.85
27	22.98	22.77	22.77	22.75	27	0.86	0.87	0.86	0.85

[a]*J* values see text; [b]*J* = 16.0 Hz; [c]*J* = 15.9 Hz; [d]*J* = 16.1 Hz; [e]*J*= 16.2 Hz; [f]α or β protons.

syn-epoxy alcohols.[11] Therefore, **6**, **7** and **8** are assigned as all-*trans*-9,10-seco-5β,10β-epoxy-6,8(14)-cholestadien-3β-ol [(*3S,5R*)-5,10-epoxy-isotachysterol], all-*trans*-9,10-seco-5α,10α-epoxy-6,8(14)-cholestadien-3β-ol [(*3S,5S*)-5,10-epoxy-isotachysterol] and all-*trans*-9,10-seco-5α,10α-epoxy-6,8(14)-cholestadien-3α-ol [(*3R,5S*)-5,10-epoxy-isotachysterol], respectively. Total ^{1}H and ^{13}C NMR assignments are listed in Table 1. It is believed that the small amount of **8** was derived from the 3-epimer of **5** which was formed during the preparation of **5** by HCl-catalysed isomerisation of vitamin D_3.

The formation of these epoxides is interesting since they are formed in the dark and in the absence of any other oxidants and/or initiators except for atmospheric oxygen. Other epoxides of vitamin D_3 derivatives reported previously were all prepared by chemical and photochemical oxidations.[4-6] Since isotachysterol is the acid-catalysed isomerisation product of vitamin D_3 similar autoxidation reaction might also take place in living systems and have biological significance. Mordi and Walton[12] have studied in detail the autoxidation of β-carotene in the dark and proposed a self-initiated autocatalytic mechanism for the formation of the 5,6-epoxide of β-carotene and other oxidation products. A similar mechanism may be also applicable to this autoxidation of isotachysterol. Mechanistic studies of this reaction are underway in this laboratory.

Experimental

HR-ESI-MS was determined on a Bruker APEX II FT-MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer in acetone-d_6 with TMS as the internal standard. IR spectra were taken on a Nicolet 170SX IR spectrometer. Optical rotation was measured on a Perkin-Elmer 241 polarimeter.

Isomerisation of vitamin D_3 (**1**): To a solution of vitamin D_3 (**1**, 200 mg) in methanol (30 ml) was added HCl (0.1ml)and the solution was refluxed for 0.5h. The reaction mixture was neutralised with Na_2CO_3, extracted with AcOEt and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure using a rotavapor the residue was column chromatographed on silica gel (20g) with AcOEt-PE (1:5) giving a pale yellow oil (150 mg, 75%) of isotachysterol (all-*trans*-9,10-seco-5(10),6,8(14)-cholestatrien-3β-ol, **5**): HR-ESI-MS: 385.3463 ($C_{27}H_{44}O$ + H requires 385.3465); $[\alpha]^{25}_D$ + 4 (*c* 0.25 in acetone); ν_{max} (neat) / cm^{-1} 3403 (OH), 1671 and 1589 (conjugated triene), 957 (*trans*-CH=)); λ_{max} (MeOH) /nm 288, indicative of an all-*trans*-triene system. For NMR data see Table 1. HPLC analysis showed that **5** contained a tiny amount of its 3-epimer which was not removed.

Autoxidation of isotachysterol (**5**): The pale yellow oil of **5** (150 mg) was taker in a small beaker at ambient temperature in the dark and was oxidised rapidly to a very complex mixture as monitored by TLC, so that after 1-2 days little **5** was left. Oxidation by bubbling air to a CH_2Cl_2 solution of **5** for 4 hrs gave the same result. The mixture was separated by column chromatography (silica gel, AcOEt-PE, 1:1 v/v) and the most polar fraction (20 mg, R_f = 0.2) was subjected to HPLC separation with a semipreparative ODS column (1 × 25 cm) eluted with MeOH/H_2O (90:10 v/v) at flow rate of 2 ml/min and detected at 245 nm. Three pure compounds **6** (10.6 mg, 53 % based on this fraction), **7** (6.3 mg, 31 %) and **8** (1.1 mg, 5 %) with retention time of 15, 12 and 10 minutes, respectively, were obtained. Their purity was further confirmed by HPLC using an analytical ODS column (0.46 × 25 cm) eluted with MeOH/H_2O (95:5 v/v). All-*trans*-9,10-seco-5β,10β-epoxy-6,8(14)-cholestadien-3β-ol [(*3S,5R*)-5,10-epoxy-isotachysterol] (**6**), HR-ESI-MS: 401.3413 ($C_{27}H_{44}O_2$ + H requires 401.3419); $[\alpha]^{25}_D$ + 25.8 (*c* 0.97 in acetone); ν_{max} (neat) / cm^{-1} 3386 (OH), 1278, 859 and 800 (epoxide), 971 (*trans*-CH=); λ_{max} (MeOH) / nm 247. All-*trans*-9,10-seco-5α,10α-epoxy-6,8(14)-cholestadien-3β-ol [(*3S,5S*)-5,10-epoxy-isotachysterol] (**7**), HR-ESI-MS: 401.3422 ($C_{27}H_{44}O_2$ + H requires 401.3419); $[\alpha]^{25}_D$ + 29.1 (*c* 1.03 in acetone); ν_{max} (neat) / cm^{-1} 3388 (OH), 1275, 874 and 836 (epoxide), 970 (*trans*-CH=); λ_{max} (MeOH) / nm 247. All-*trans*-9,10-seco-5α,10α-epoxy-6,8(14)-cholestadien-3α-ol [(*3R,5S*)- 5,10-epoxy-isotachysterol] (**8**), HR-ESI-MS: 401.3403 ($C_{27}H_{44}O_2$ + H requires 401.3419); $[\alpha]^{25}_D$ + 22.8 (*c* 0.35 in acetone); ν_{max} (neat) / cm^{-1} 3393 (OH), 1261, 885 and 810 (epoxide), 973 (*trans*-CH=); λ_{max} (MeOH) / nm 247. For NMR data see Table 1.

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