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Autoxidation of isotachysterol

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Abstract—Isotachysterol, the acid-catalyzed isomerization product of vitamin D_3, produces seven previously unknown oxygenation products in a self-initiated autoxidation reaction under atmospheric oxygen in the dark at ambient temperature. They are (5*R*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (**6a**), (5*S*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (**6b**), (10*R*)-9,10-secocholesta-5,7,14-trien-3β,10-diol (**7a**), (10*S*)-9,10-secocholesta-5,7,14-trien-3β,10-diol (**7b**), (7*R*,10*R*)-7,10-epoxy-9,10-secocholesta-5,8(14)-dien-3β-ol (**8**), 5,10-epidioxyisotachysterol (**9**) and 3,10-epoxy-5-oxo-5,10-*seco*-9,10-secocholesta-6,8(14)-dien-10-ol (**10**). The formation of these products is explained in terms of free radical peroxidation chemistry.

1. Introduction

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 human beings and animals[2] and much more synthetic analogues, especially those of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), have been made to explore their anticancer potentials and other biological activities.[3] Structural alterations of vitamin D_3 by metabolism mostly occur at the 1α-position and the side chain,[2] while the 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 co-workers[5] found that **1** could be regio- and stereoselectively oxidized by *m*-chlorobenzoic acid and *tert*-butyl hydroperoxide catalyzed by $VO(acac)_2$, giving (7*R*)-7,8-epoxyvitamin D_3 (**3**) and (5*S*)-5,6-epoxyvitamin D_3 (**4**) respectively. Photosensitized 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-catalyzed isomerization product, isotachysterol (**5**), is very labile in the air even in the dark.[7,8] However, no effort has been made previously to identify the complex autoxidation products of isotachysterol. We report herein the isolation and identification of the principal autoxidation products of isotachysterol, including (5*R*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (**6a**), (5*S*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (**6b**), (10*R*)-9,10-secocholesta-5,7,14-trien-3β,10-diol (**7a**), (10*S*)-9,10-secocholesta-5,7,14-trien-3β,10-diol (**7b**), (7*R*,10*R*)-7,10-epoxy-9,10-secocholesta-5,8(14)-dien-3β-ol (**8**), 5,10-epidioxy-isotachysterol (**9**) and 3,10-epoxy-5-oxo-5,10-*seco*-9,10-secocholesta-6,8(14)-dien-10-ol (**10**). The formation of these products is discussed in terms of free radical peroxidation chemistry.

2. Results

Isotachysterol (**5**) was prepared by HCl-catalyzed isomerization of vitamin D_3 (**1**) in methanol.[8] The pale yellow oil of **5** was laid in a small beaker at ambient temperature in the dark. **5** was found oxidized rapidly to a very complex mixture as monitored by TLC and after 1–2 days only a little **5** was left. Oxidation by bubbling oxygen to a benzene solution of **5** at ambient temperature gave the similar result together with some polymeric/oligomeric materials which deposited out of the solution, particularly in the later stage of the oxidation. Addition of 2,2′-azobisisobutyronitrile (AIBN) to the benzene solution of **5** significantly accelerated the reaction, suggesting that the reaction proceeded by a free radical chain mechanism. The soluble materials were separated by reverse phase HPLC. Figure 1 shows the chromatogram of the reaction mixture obtained at the early stage (8 h) of the autoxidation of **5** at room temperature in benzene solution, corresponding to ca. 50% conversion of **5**. UV–Vis spectra in the range of 190–400 nm were obtained for all major products. The mixture was also examined at

Keywords: Vitamin D_3; Isotachysterol; Autoxidation; Epoxide; Dioxetane.
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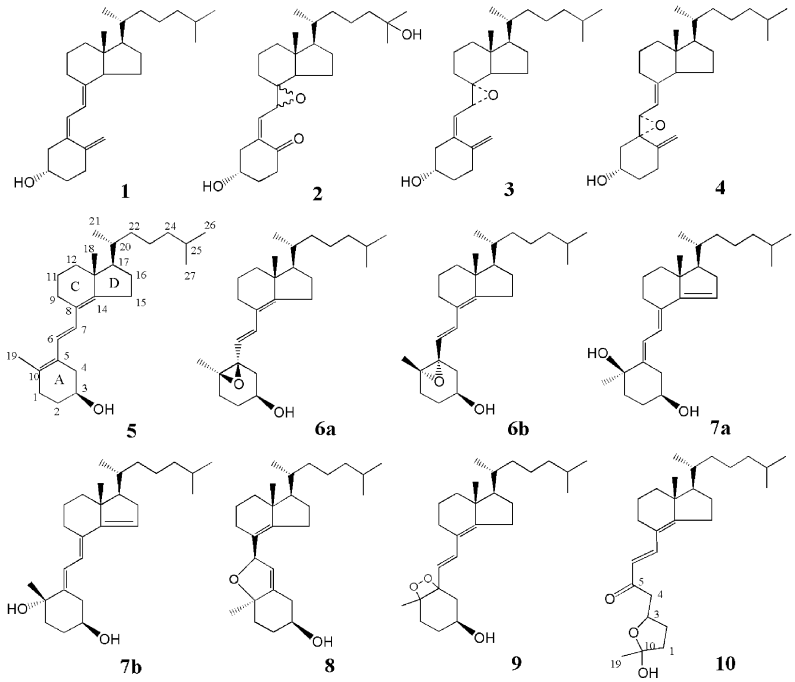


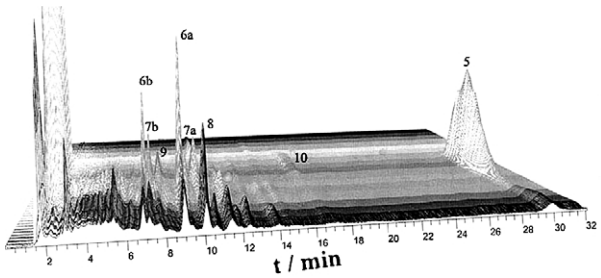


Figure 1. 3D HPLC diagram recorded from the reaction mixture of the autoxidation of isotachysterol in benzene at room temperature for 8 h on a Phenomenex Nucleosil C18 column (5 μm) eluted with MeOH–H_2O (88:12 v/v) at a flow rate of 1 ml/min. *x*-axis: retention time (min), *y*-axis: UV absorption; *z*-axis: intensity of the UV absorption. The peak numbers correspond to the numbers of compounds.

various stages of oxidation by coupled LC–MS using the same column and solvent system. The total ion current (TIC) chromatograms were similar to those obtained in the analytical HPLC, except of different peak intensities.

The peak 5 with the longest retention time (R_t) of 29.9 min and the molecular ion peak of 385.3463 ($C_{27}H_{44}O$+H requires 385.3470) was identified as unreacted isotachysterol (**5**) by comparing its R_t and UV spectrum with that of the authentic sample. The peaks 6a and 6b with R_t of 9.0 and 6.8 min respectively, gave molecular ion peaks of 401.3413 and 401.3422 respectively, corresponding to a same molecular formula with one more oxygen than **5** ($C_{27}H_{44}O_2$+H requires 401.3420). The UV spectra of **6a** and **6b** were almost identical, showing a band at 248 nm which is characteristic of conjugated double bonds. The comparison of their ^{1}H and ^{13}C NMR spectra (Table 1) with those of vitamin D_3 and its metabolites[9] and with that of isotachysterol[10] clearly demonstrates that **6a** and **6b** are 5,10-epoxides of **5** since the remarkable changes on ^{13}C chemical shifts are only observable for 5- and 10-Cs (from double bond carbons to epoxy carbons) and on ^{13}C and ^{1}H chemical shifts for 19-Me, and to a less extent, for 4-C. The coupling constants of 3-H are 8.0, 8.0, 4.5 and 4.5 Hz for **6a**, and 9.6, 9.6, 4.7 and 4.7 Hz for **6b**, respectively, demonstrating that the 3-H is axial in both **6a** and **6b**. The NOESY spectrum of **6a** showed clear cross peaks between 1α-H, 3α-H and 19-CH_3 and between 6-H, 1α-H and 19-CH_3 (Fig. 2), indicating that the epoxy ring and the 3-hydroxyl locate at the same side of the molecule. On the other hand, clear NOESY correlations between 6-, 4β-, 2β-, 1β-Hs and 19-CH_3 of **6b** (Fig. 2) demonstrates that the epoxy ring and the 3-hydroxyl are at the opposite sides of the molecule. 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 **6a** as the sole epoxy product (yield 45%). It is well known that epoxidation of homoallylic alcohols with TBHP/$VO(acac)_2$ produces stereospecifically *syn*-epoxy alcohols.[11] Therefore, **6a** and **6b** are assigned as (5*R*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (5β,10-epoxy-isotachysterol) and (5*S*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol (5α,10-epoxy-isotachysterol) respectively.

The peaks 7a and 7b with R_t of 10.6 and 7.1 min respectively, gave molecular ion peak of 401.3416 and 401.3411 respectively, corresponding to a same molecular formula with one more oxygen than **5** ($C_{27}H_{44}O_2$+H requires 401.3420). Both of them exhibited an UV absorption maximum at 278 nm, suggesting the existence of a conjugated triene chromophore. The comparison of their ^{1}H and ^{13}C NMR spectra (Table 1) with those of isotachysterol (**5**)[10] showed remarkable differences on ^{13}C chemical shifts of 5-, 10- and 15-Cs, and to a less extent, on 16 and 19-Cs, which suggests that the 5(10), 6, 8(14)-triene structure in **5** might change to a 5,7,14-triene system in both **7a** and **7b**. The A-ring structure of **7a** and **7b** was confirmed by their gCOSY spectra which exhibited spin coupling network between the hydroxymethine proton 3-H and 4α-,

Table 1. ^{1}H and ^{13}C NMR chemical shifts of compounds **5**–**10** (acetone-d_6)[a]

Carbon	5	6a	6b	7a	7b	8	10	Proton	5	6a	6b	7a	7b	8	10
1	32.3	33.7	35.4	38.7	38.8	35.0	39.0	1α	1.82	1.42	1.87	1.42	1.51	2.13	1.68
								1β	2.17	1.86	1.46	1.89	1.79	1.53	1.98
2	32.1	30.4	32.7	31.5	32.1	31.2	31.7	2α	1.86	1.73	1.82	1.86	1.90	1.62	2.08
								2β	1.48	1.64	1.38	1.74	1.51	1.80	1.75
3	67.3	67.3	66.7	69.7	69.7	66.5	77.5	3α	3.81	3.81[b]	3.97[b]	3.62[b]	3.52[b]	4.06[b]	4.48
4	35.5	32.5	43.3	35.2	35.4	34.4	48.9	4α	2.53	1.76	1.95	2.65	3.01	2.28	2.95
								4β	2.04	1.88	1.60	2.55	2.04	2.51	2.69
5	127.1	77.6	77.1	144.2	143.8	142.9	198.7								
6	124.6	129.5	131.2	118.0	116.4	122.3	124.4	6	6.53	5.73	5.82	6.41	6.59	5.05	6.12
7	125.9	128.6	128.0	120.4	119.3	83.2	142.2	7	6.36	6.61	6.45	6.39	6.38	5.48	7.51
8	125.4	124.5	124.5	136.5	135.1	122.3	124.5								
9	26.3	26.1	26.1	28.2	27.7	25.3	26.2	9α	2.38	2.36	2.35	1.80	1.83	2.21	2.57
								9β	2.47	2.47	2.47	2.80	2.81	2.42	2.62
10	131.6	73.1	73.8	71.6	71.7	88.1	108.2								
11	27.6	27.6	27.6	22.8	21.9	28.1	27.3	11α	1.92	1.89	1.90	1.77	1.82	1.92	1.97
								11β	1.46	1.47	1.46	1.64	1.68	1.45	1.53
12	38.6	38.0	38.0	41.6	42.8	38.3	37.3	12α	1.18	1.19	1.18	1.64	1.45	1.20	1.23
								12β	2.01	2.01	1.98	2.02	2.00	1.98	2.07
13	44.6	44.4	44.4	47.9	47.0	44.9	45.5								
14	149.3	148.1	148.8	153.9	153.1	144.4	161.4								
15	24.8	25.0	25.0	119.9	118.9	23.2	24.4	15α	2.04	1.98	1.93	5.55	5.55	1.98	2.20
								15β	2.24	2.12	2.15			2.09	1.99
16	19.6	19.6	19.6	36.5	35.6	20.0	19.2	16α	1.90	2.01	2.01	2.16	2.20	1.96	1.80
								16β	1.74	1.75	1.73	1.90	1.97	1.65	2.03
17	57.2	57.1	57.2	59.5	58.8	56.7	56.8	17	1.18	1.19	1.20	1.65	1.64	1.17	1.25
18	18.4	18.4	18.4	17.6	16.7	18.8	18.3	18	0.90	0.90	0.90	0.90	0.88	0.86	0.95
19	18.9	23.9	23.8	27.3	27.3	23.4	22.1	19	1.75	1.08	1.18	1.33	1.33	1.25	1.33
20	35.3	35.3	35.3	39.7	38.8	35.4	35.2	20	1.50	1.48	1.51	1.50	1.48	1.50	1.58
21	19.4	19.3	19.4	21.4	20.4	19.4	19.3	21	0.97	0.97	0.97	1.04	0.94	0.97	0.98
22	36.6	36.6	36.6	36.6	36.6	36.7	36.5	22	1.10[c]	1.43	1.43	1.41	1.42	1.14[c]	1.42
									1.36[c]					1.44[c]	
23	24.4	24.3	24.3	24.3	24.3	24.4	24.4	23	1.10[c]	1.36	1.10[c]	1.08	1.10	1.10	1.12
									1.43[c]		1.36[c]				
24	40.1	40.2	40.1	43.6	42.8	40.2	40.2	24	1.17	1.15	1.11	1.16	1.17	1.18	1.19
25	28.6	28.6	28.6	28.6	28.6	28.8	28.6	25	1.50	1.48	1.52	1.50	1.52	1.53	1.54
26	22.8	23.0	23.0	23.2	23.0	23.0	23.0	26	0.86	0.87	0.86	0.87	0.87	0.87	0.86
27	23.0	22.8	22.8	22.8	23.0	22.8	22.8	27	0.86	0.87	0.86	0.87	0.87	0.87	0.86

[a] Data for compound **9** not included, see text.
[b] *J* values see text.
[c] α or β protons.

4β-, 2α-, 2-β, 1α- and 1β-Hs, and by their HMBC spectra which showed correlations between 19-CH_3 and 1-, 5- and 10-Cs. The structure of the C ring was confirmed by their gCOSY spectra which showed correlations between the allylic 9β-H and 9α-, 11α-, 11β-, 12α- and 12β-Hs, and by their HMBC spectra which show correlations between the olefinic 7-H and 8-, 9- and 14-Cs. The structure of the D ring was confirmed by their gCOSY spectra which showed correlations of the olefinic 15-H with 16α- and 16β-Hs, and 17-H with 16α-H, together with the HMBC correlation of 18-CH_3 with 13- and 14-Cs. The structure of the seco-B ring was confirmed by their HMBC spectra which showed correlations between the olefinic 6-H and 5-, 7-, 8- and 10-Cs. The coupling constants of 3-H (8.0, 8.0, 4.4 and 4.4 Hz for **7a**, and 9.2, 9.2, 4.6 and 4.6 Hz for **7b**, respectively) suggest that the 3-H is axial and the A-ring of **7a** and **7b** might be partitioned between a 30/70 and 24/76 equilibrium mixture of chair conformers favoring an α chair with the 3β-OH equatorially oriented.[9,12] The NOE enhancement was observed for the 7-H with 4α-H and 15-H, and for the 6-H with 9β-H and 19-CH_3 in both of **7a** and **7b**, indicating the triene configuration of the two compounds to be (5*E*, 7*E*, 14*E*), which were also supported by the coupling constant of the olefinic protons ($J_{6,7}$=12.0 Hz). The difference between **7a** and **7b** were observed only in their NOESY spectra which showed clear correlations between 3α-H and 4α-, 2α- and 1α-Hs, and between 19-CH_3 and 6-H in **7a**, while no such correlations occurred in **7b**. Instead, clear NOESY correlations were observed between 19-CH_3 and 4β-, 2β- and 1β-Hs in **7b** (Figure 2). This demonstrates that **7a** and **7b** are 10-epimers and the 19-CH_3 is equatorial and α-oriented in **7a**, while is axial and β-oriented in **7b**. The comparatively downfield shift of the chemical shifts of 2β-H and 4β-H(δ 1.74 and 2.55, respectively) in **7a** compared to those of **7b** (δ 1.51 and 2.04, respectively) also indicated that the 10-OH is axial and β-oriented in **7a** and equatorial and α-oriented in **7b**. Therefore, **7a** and **7b** were assigned as (10*R*)-9,10-secocholesta-5,7,14-trien-3β,10-diol and (10*S*)-9,10-secocholesta-5,7,14-trien-3β,10-diol, respectively.

The peak 8 with R_t of 10.1 min and the molecular ion peak of 401.3411 corresponds to a molecule with one more oxygen than **5**, same as **6** and **7** ($C_{27}H_{44}O_2$+H requires 401.3420). The UV absorption maximum of 206 nm indicates the absence of conjugated double bonds in the compound. The comparison of its ^{1}H and ^{13}C NMR spectra (Table 1) with those of isotachysterol (**5**)[10] showed remarkable differences on ^{13}C chemical shifts of 7- and 10-Cs, from olefinic carbons in **5** to oxygen-connecting

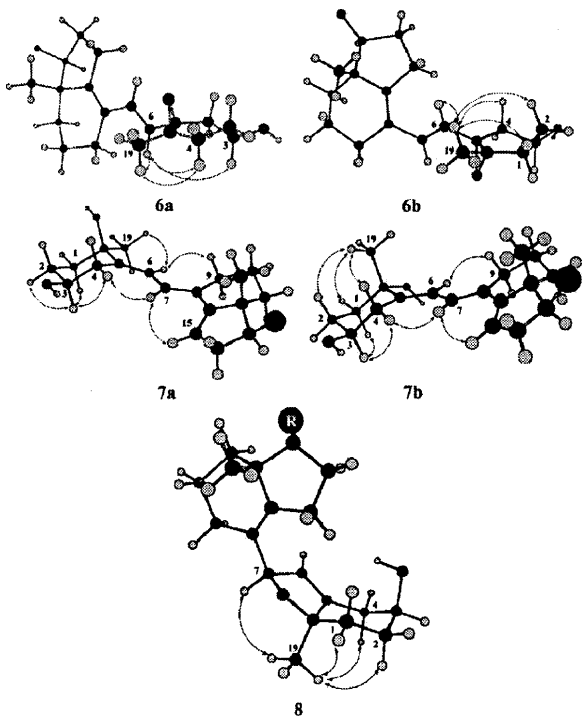


Figure 2. Principal NOE correlations of **6a**, **6b**,**7a**,**7b** and **8** presented with ball-and stick representations of the MM2-optimized structures.

quaternary carbons in **8**, suggesting that **8** is a 7,10 epoxide of **5** containing a dihydofuran ring. The structure of **8** was fully assigned by its 2D NMR spectroscopy. The structure of the A-ring was confirmed by its gCOSY spectrum (Fig. 3) in which the hydroxymethine proton 3α-H (δ 4.06) correlated with 2α-, 2β-, 4α- and 4β-Hs, and the 2β-H correlated with 1α- and 1β-Hs, and by its gHMBC spectrum (Fig. 4) which shows correlations between 19-CH_3 and 1-, 5- and 10-Cs. The dihydrofuran ring was supported by the HMBC correlations of the olefinic 6-H with 4-, 5-, 7- and 10-Cs. The structure of the C- and D-rings was confirmed by its H,H-COSY correlations of 9β-, 9α-, 11α-, 11β-, 12α- and 12β-Hs, of 15α-, 15β-, 16α-, 16β- and 17α-Hs, and by its HMBC correlations between 18-CH_3 and 12-, 13- and 14-Cs. The connection between the tetrahydrofuran ring and C-ring was supported by the HMBC correlations between the oxygen-connecting 7-H and 6-, 8- and 14-Cs. The NOESY 1D spectrum showed clear correlations between 19-CH_3 and 1α-, 2α- and 4α-Hs, indicating that the 19-CH_3 is axial and the 3-H is equatorial which is consistent with the coupling constant of 3-H (3.2, 3.2, 2.4 and 2.4 Hz). The NOESY 1D spectrum also exhibited a correlation between the 19-CH_3 and 7-H, demonstrating that they are located on the same side of the dihydrofuran ring. Thus **8** was assigned as (7*R*,10*R*)-7,10-epoxy-9,10-secocholesta-5,8(14)-dien-3β-ol.

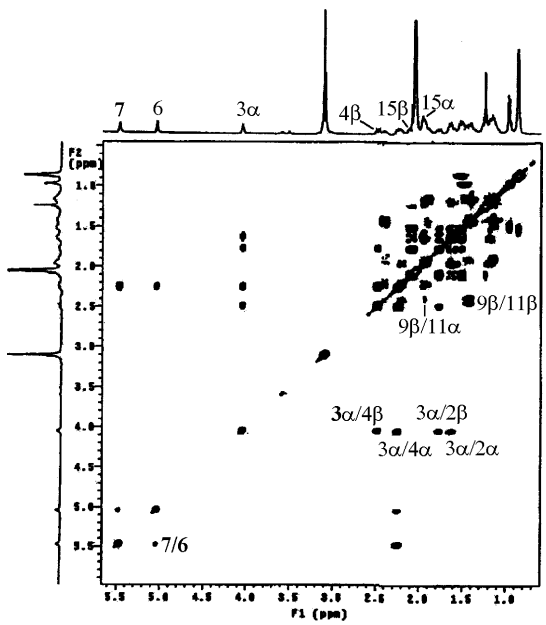


Figure 3. The gCOSY spectrum of **8**.

The peak 9 with R_t of 7.7 min and the molecular ion peak of 439.3211 corresponds to a molecule with two more oxygens than **5** ($C_{27}H_{44}O_3$+Na requires 439.3188). The UV absorption maximum at 296 nm suggested the presence of an extended conjugated system. However, this compound **9** was unstable and gradually converted to a new compound **10**, i.e., peak 10 with R_t of 17.0 min and λ_{max} at 305 nm, during the process of semipreparative HPLC separation of **9**. **10** gave the molecular ion peak of 417.3373 corresponding to a same molecular formula of **9** ($C_{27}H_{44}O_3$+H requires 417.3369). Its UV spectrum showed strong absorption maximum at 305 nm, suggesting the existence of an extended conjugated system. The ^{13}C NMR spectrum revealed the presence of a −C═O, a −OCH− and a −O−C−O− moieties. The comparison of its ^{13}C NMR chemical shifts with those of **5** demonstrated that, besides of the A-ring carbons as well as 7- and 14-Cs, other chemical shifts are almost identical. In the HMBC spectrum the olefinic 6- and 7-Hs and the methylenic 4-Hs correlated with the carbonyl carbon (δ 198.7), indicating that the −C═O is located at the 5-position, that also rationalizes the downfield shift of 4-, 7- and 14-Cs in comparison with those of **5**. The chemical shift of 10-C (δ 108.2) suggested it bonded to two oxygens. Its HMBC showed correlations between the 10-C and 19-CH_3 and 2-Hs, and between the oxygen-connecting 3-C (δ 77.5) with 2- and 4-Hs. Therefore, **10** was assigned as 3,10-epoxy-5-oxo-5,10-*seco*-9,10-secocholesta-6,8(14)-dien-10-ol and **9** was assigned as 5,10-epidioxy-isotachysterol. Total ^1H and ^{13}C NMR assignments are listed in Table 1.

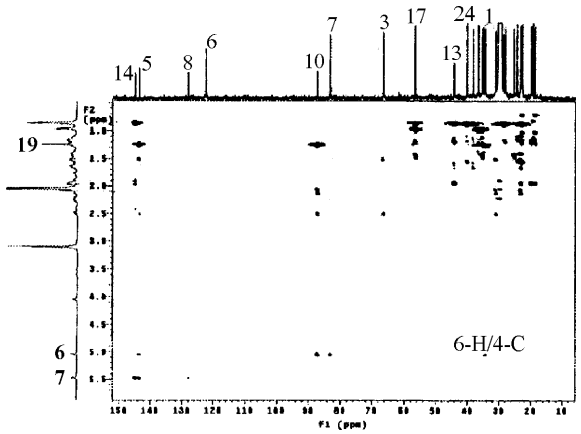
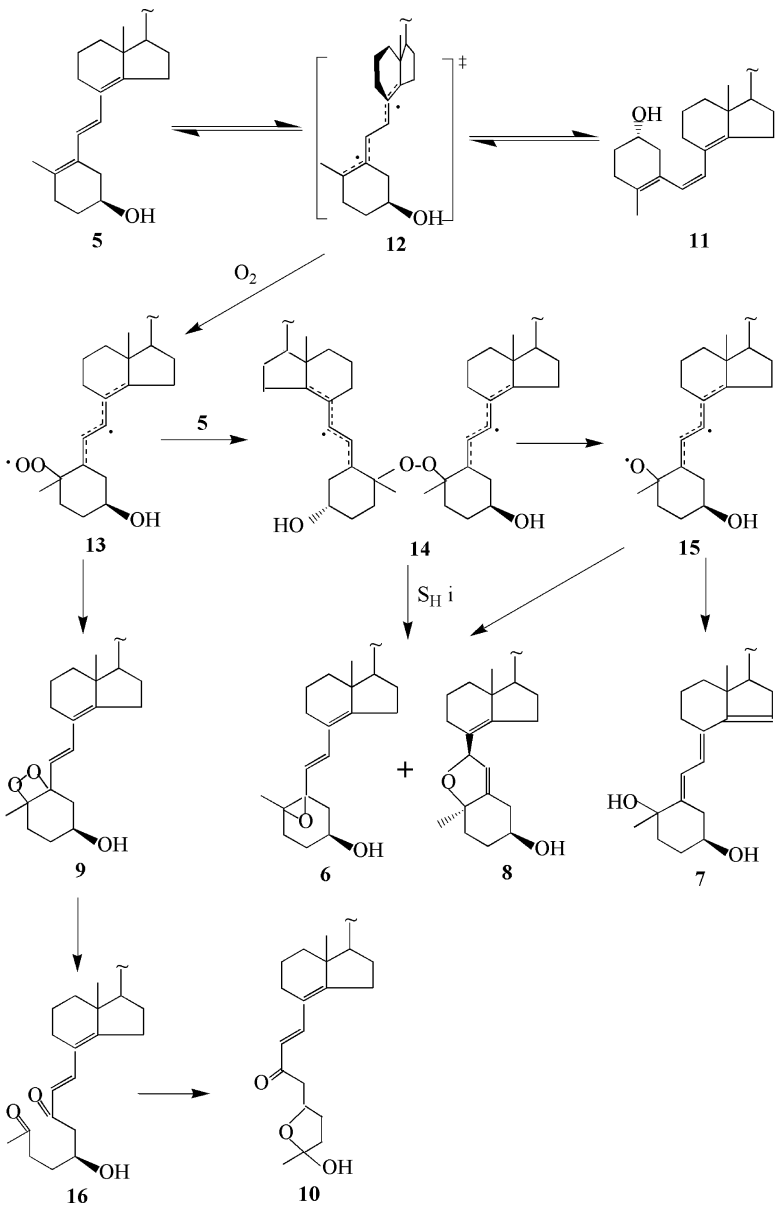


Figure 4. The gHMBC spectrum of **8**.

3. Discussion

Mordi and Walton[13] 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. Similar mechanism might also be applicable to this autoxidation of isotachysterol as shown in Scheme 1. That is, the *all-trans*-triene structure in **5** isomerizes to the corresponding 6,7-*cis*-isomer (**11**) *via* the singlet biradical transition state (**12**), similar to the case of β-carotene[13] which has been proved by Doering and co-workers to be able to take place at temperatures <40 °C.[14] As a matter of fact, a small peak close to the peak of **5** with absorption maximum at 253 nm corresponding to 6,7-*cis*-isotachysterol[15] (**11**) could be observed if a hexane solution of isotachysterol (**5**) was put in the dark and free of oxygen for 6 h as shown in Figure 5. This demonstrated the unambiguous formation of **11**, hence the occurrence of such *trans*/*cis*-isomerization process. It has been reported previously that isotachysterol (**5**) could isomerize to *cis*-isotachysterol (**11**) photochemically.[15a] The present work demonstrates clearly that the *trans*/*cis*-isomerization of isotachysterol can also take place thermally because the singlet biradical **12** is thermodynamically stabilized by delocalization of the two unpaired electrons to the two allylic moieties. This thermal *trans*/*cis*-isomerization of isotachysterol *via* the biradical (**12**) provides a ready explanation for the lability of isotachysterol and the self-initiated autoxidation of the substrate. That is, during twisting of the central carbon–carbon bond of isotachysterol the unpaired spin density would develop in each half of the molecule, reaching a maximum (one free spin in each half) in the perpendicular transition state (**12**). It is reasonably to assume that the unpaired spin can be 'captured' by oxygen to produce a carbon-peroxyl triplet biradical (**13**). Oxygen should preferably attack 10-C to enable the extensive delocalization of another unpaired electron. Being a triplet **13** would be relatively long-lived



Scheme 1. Proposed mechanism for the autoxidation of isotachysterol.

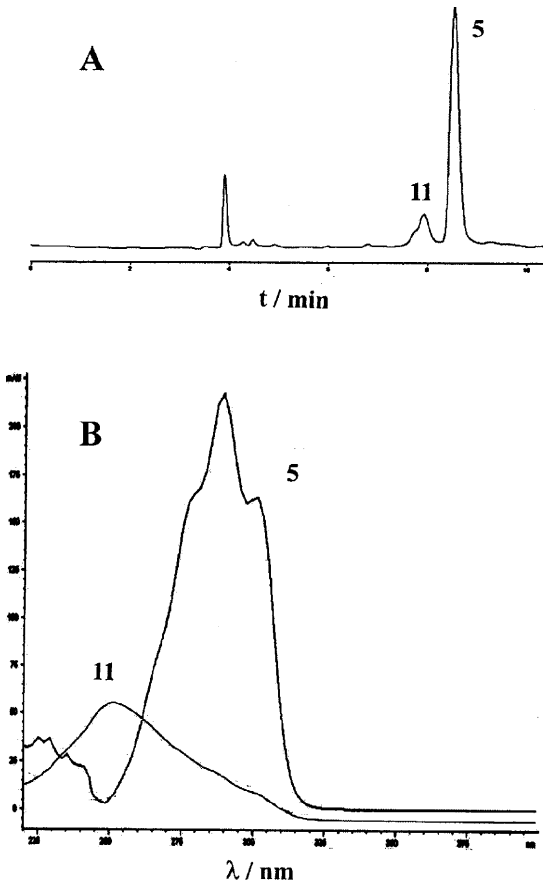
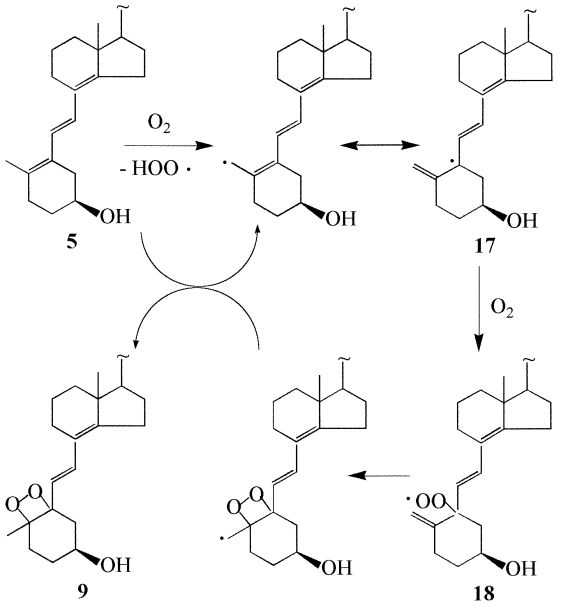


Figure 5. (A) HPLC diagram recorded from a hexane solution of isotachysterol which was stored free from oxygen in the dark for 6 h with a Zorbax Sil column (5 μm) eluted with hexane–AcOEt (85:15 v/v) at a flow rate of 1 ml/min. The UV detector was put at 260 nm. The peak numbers correspond to the numbers of the compounds. (B) The UV spectra of **5** and **11** recorded from the same solution. The intensity of **11** was magnified by 3 times.



Scheme 2. An alternative mechanism for the autoxidation of isotachysterol.

and be able to add to a second molecule of **5** to form a new biradical **14**, again at 10-C. Obviously, **14** can subject to the well-precedented intramolecular homolytic substitution (S_Hi)[16] producing the 5,10-epoxides **6** and the 7,10-epoxide **8** by 5,10- and 7,10-ring closure, respectively, of the intermediate alkoxyl biradical **15**. Compound **7** was also possibly derived from the alkoxyl biradical **15** by consecutive 1,5-sigmatropic rearrangement of the allylic 15-H to 6-C and 1,4-sigmatropic rearrangement of the 6-H to 10-O. On the other hand, the peroxyl biradical **13** may collapse to the thermally unstable dioxetane **9** which would easily subject to peroxide scission to produce the dicarbonyl intermediate **16** followed by acetalation, yielding the cyclic semiketal **10** (Scheme 1). Another possible initiation step might be the direct hydrogen abstraction by oxygen from allylic positions,[17] preferably at C-19 to form the allylic radical **17**, which reacts with oxygen to form the peroxy radical **18**. Being similar to **13** it can follow similar follow-up processes as mentioned above to give the products as exemplified in Scheme 2.

In conclusion, this work demonstrates that despite the relative stability of vitamin D_3 at ambient temperatures, its acid-catalyzed isomerization product, isotachysterol, is liable to autoxidation to form a variety of oxidation products. The formation of these oxidation products is interesting since they are formed in the dark and in the absence of any other oxidants and/or initiators apart from atmospheric oxygen. Other oxides of vitamin D_3 derivatives reported previously were all prepared by chemical and photochemical oxidations.[4–6] Since isotachysterol is the acid-catalyzed isomerization product of vitamin D_3 and also can be formed in the presence of acidic vitamins such as ascorbic acid and folic acid,[18] similar autoxidation reaction might also take place in living systems and have biological significance.

4. Experimental

4.1. General methods

HR-ESI-MS was determined on a Bruker APEX II FT-MS spectrometer. ^1H, ^{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. UV spectra were recorded with a Hitachi 557 spectrophotometer in methanol. Optical rotation was measured on a Perkin–Elmer 341 polarimeter. HPLC was carried out with a Hewlett Packard 1100 system and a diode array detector. Best separations were achieved with a 250×4.6 mm^2 Phenomenex Nucleosil 5 μm C18 and 250×10 mm^2 Whatman Partisil 10 μm ODS-3 columns. Coupled LC–MS was carried out with the same HPLC system and the Bruker APEX II FT-MS spectrometer in the electrospray ionization mode.

4.1.1. Preparation of isotachysterol (5). To a solution of vitamin D_3 (**1**, 200 mg) in 30 ml methanol was added 0.1 ml HCl and the solution was refluxed for 0.5 h. The reaction mixture was neutralized 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 (20 g) with AcOEt–PE (1:5) giving a pale yellow oil (150 mg, 75%) of isotachysterol (all-*trans*-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (**5**): HR-ESI-MS: 385.3463 ($C_{27}H_{44}O$+H requires 385.3470); $[\alpha]_D^{25}$=+4 (*c* 0.3 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.

4.2. Autoxidation of isotachysterol (5)

The pale yellow oil of **5** (150 mg) was laid in a small beaker at ambient temperature in the dark which was oxidized rapidly to a very complex mixture as monitored by TLC, and after 1–2 days little **5** was left. Oxidation by bubbling air to a benzene solution of **5** at 40 °C for 4 h gave the same result. The reaction mixture was separated by column chromatography (silica gel, AcOEt–PE, 1:1 v/v) followed by HPLC to give **6a** (10.6 mg), **6b** (6.3 mg), **7a** (4.0 mg), **7b** (5.8 mg), **8** (8.0 mg) and **10** (3.2 mg) respectively. Compound **9** could not be collected because it was unstable and changed to **10** during HPLC separation.

4.3. Stereospecific epoxidation of isotachysterol (5)

To a solution of **5** (100 mg, 0.31 mmol) and $VO(acac)_2$ (2 mg, 7.3 mmol) in dry benzene (2 ml) was added slowly anhydrous benzene solution of TBHP (0.21 ml, 0.62 mmol) at 5 °C. The solution was then stirred for 30 min at 5 °C. After addition of aqueous Na_2SO_3, the mixture was extracted with benzene, the extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed (silica gel, AcOEt–PE 1:1) to give **6a** (43 mg, 43%) as the predominant product.

4.3.1. (5*R*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol [5β,10-epoxy-isotac-hysterol] (6a). HR-ESI-MS: 401.3413 ($C_{27}H_{44}O_2$+H requires 401.3420; $[\alpha]_D^{25}$=+25.8 (*c* 1.0 in acetone); ν_{max} (neat, cm^{-1}) 3386 (OH), 1278, 859 and 800 (epoxide), 971 (*trans*-CH=); λ_{max} (MeOH, nm) 247. For NMR data see Table 1.

4.3.2. (5*S*)-5,10-epoxy-9,10-secocholesta-6,8(14)-dien-3β-ol [5α,10-epoxy-isotac-hysterol] (6b). HR-ESI-MS: 401.3422 ($C_{27}H_{44}O_2$+H requires 401.3420); $[\alpha]_D^{25}$=+29.1 (*c* 1.0 in acetone); ν_{max} (neat, cm^{-1}) 3388 (OH), 1275, 874 and 836 (epoxide), 970 (*trans*-CH=); λ_{max} (MeOH, nm) 247. For NMR data see Table 1.

4.3.3. (10*R*)-9,10-secocholesta-5,7,14-triene-3β,10-diol (7a). HR-ESI-MS: 401.3416 ($C_{27}H_{44}O_2$+H requires 401.3420); $[\alpha]_D^{25}$=−144 (*c* 0.7 in acetone); λ_{max} (MeOH, nm) 278. For NMR data see Table 1.

4.3.4. (10*S*)-9,10-secocholesta-5,7,14-triene-3β,10-ol (7b). HR-ESI-MS: 401.3411 ($C_{27}H_{44}O_2$+H requires 401.3420); $[\alpha]_D^{25}$=−112 (*c* 0.6 in acetone); λ_{max} (MeOH, nm) 278. For NMR data see Table 1.

4.3.5. (7*R*,10*R*)-7,10-epoxy-9,10-secocholesta-5,8(14)-dien-3β-ol [7,10-epoxy-isotac-hysterol] (8). HR-ESI-MS: 401.3411 ($C_{27}H_{44}O_2$+H requires 401.3420); $[\alpha]_D^{25}$=+134 (*c* 0.3 in acetone); λ_{max} (MeOH, nm) 206. For NMR data see Table 1.

4.3.6. 3,10-epoxy-5-oxo-5,10-*seco*-9,10-secocholesta-6,8(14)-dien-10-ol (10). HR-ESI-MS (417.3378 for $C_{27}H_{44}O_3$+H, requires 417.3369); λ_{max} (MeOH, nm) 305. For NMR data see Table 1.

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