SHORT PAPER

J. Chem. Research (S),
2003, 691–693

Thermal isomerisation of vitamin D_3 in dimethyl sulfoxide†

Xiaoling Jin[a], Xinping Yang[a], Li Yang[a]*, Zhong-Li Liu[a] and Fa Zhang[b]

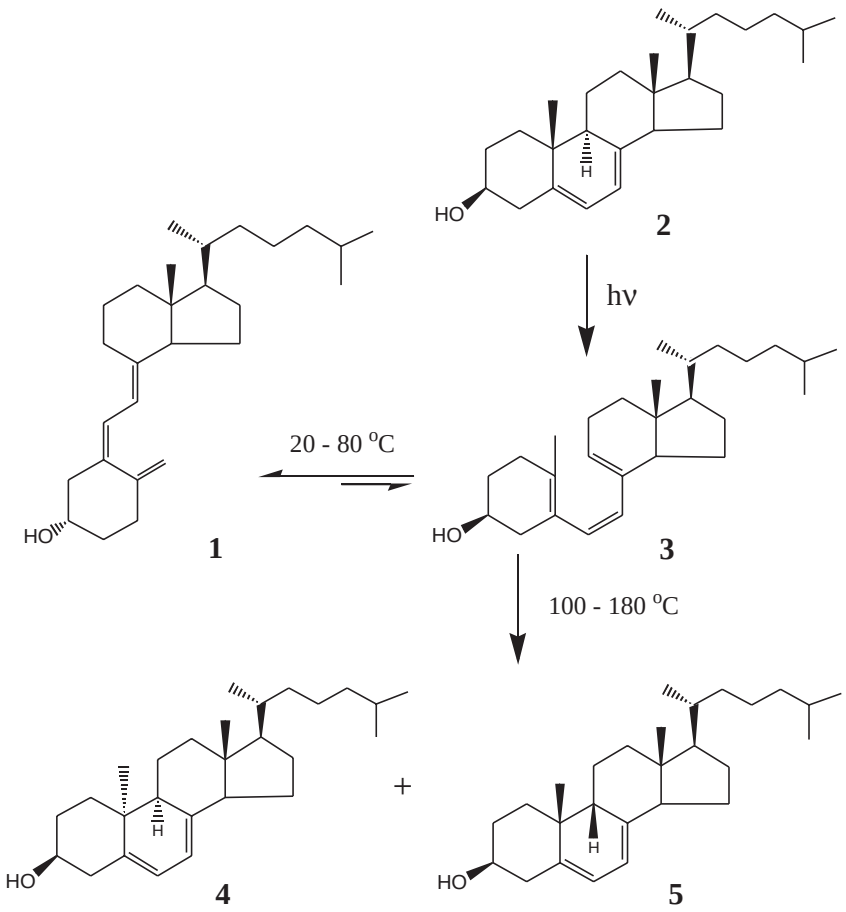
[a]*National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu 730000, China*

[b]*Johnson & Johnson, Skillman, NJ 08558-9418, USA*

Thermal isomerisation of vitamin D_3 at 140 °C under nitrogen in the dark gave isovitamin D_3 and isotachysterol as the principal products, identified by ^1H and ^{13}C NMR spectroscopy.

Keywords: vitamin D_3, isovitamin D_3, isotachysterol, thermal isomerisation.

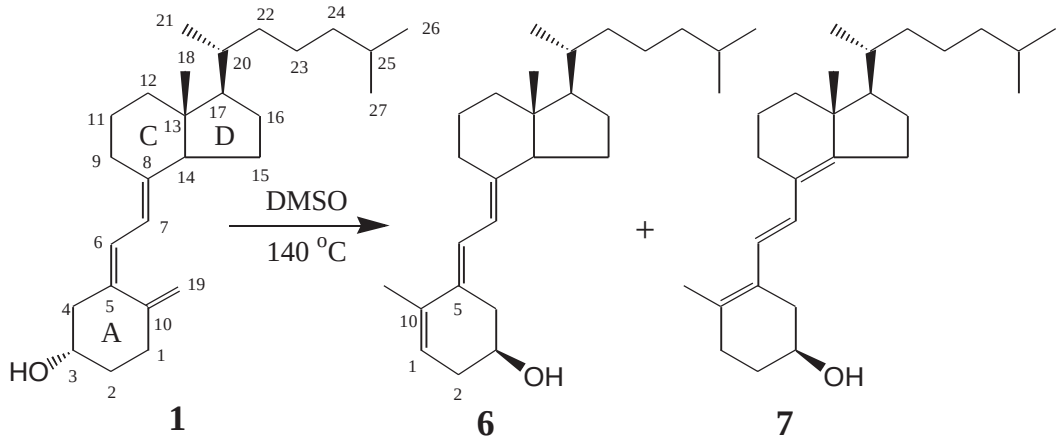
The chemistry and biochemistry of vitamin D_3 (cholecalciferol, **1**) have been extensively studied for over half a century due to the great diversity of its chemistry and, especially, due to its important roles in calcium and phosphorus regulation, immunological regulation and inducing cancer cell differentiation.[1] Vitamin D_3 is formed biosynthetically from provitamin D_3 (7-dehydrocholesterol, **2**) by irradiation followed by isomerisation of the resulting previtamin D_3 (**3**).[1c, 2] It is also known that thermal equilibrium exists in solution between vitamin D_3 (**1**) and previtamin D_3 (**3**) at room temperature and intermediate temperatures (20–80 °C), whilst at higher temperatures (100–180 °C) vitamin D_3 is transformed irreversibly to pyrocholecalciferol (**4**) and isopyrocholecalciferol (**5**) (Scheme 1).[2,3] We found, however, that heating vitamin D_3 in dimethyl sulfoxide (DMSO) solution at 140 °C under nitrogen in the dark did not produce **3, 4** or **5**, but instead gave isovitamin D_3 (**6**) and isotachysterol (**7**) (Scheme 2).



Scheme 1

* To receive any correspondence. E-mail: yangl@lzu.edu.cn

† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



Scheme 2

Table 1 ^{1}H (400.1 MHz) and ^{13}C (100.6 MHz) chemical shifts of **3**, **6** and **7** in acetone-d_6

Carbon	**3**	**6**	**7**	Proton	**3** (*J*/Hz)	**6** (*J*/Hz)	**7** (*J*/Hz)
1	31.2	125.3	32.3	**1**α	1.52 m	5.54 m	1.82 m
				1β	2.12 m		2.17 m
2	32.3	36.1	32.1	**2**α	1.85 m	2.35 m	1.86 m
				2β	1.50 m	2.12 m	1.48 m
3	67.3	67.2	67.3	**3**α	3.75 dt (4.8, 8.8)	3.79 dt (4.3, 9.0)	3.81 dt (3.6, 9.0)
4	38.5	36.1	35.5	**4**α	2.35 m	2.95 m	2.53 m
				4β	2.06 m	2.15 m	2.04 m
5	127.4	122.8	127.2				
6	129.7	120.0	124.7	**6**	5.91 d (11.9)	6.38 d (12.0)	6.53 d (16.0)
7	129.2	117.4	125.9	**7**	5.68 d (11.9)	5.97 d (12.0)	6.36 d (16.0)
8	137.0	144.1	125.4				
9	125.1	29.4	26.3	**9**α	5.52 d (3.1)[a]	1.75 m	2.38 m
				9β		2.89 m	2.47 m
10	129.5	134.7	131.6				
11	25.4	24.5	27.6	**11**α	2.15 m	1.70 m	1.92 m
				11β	2.15 m	1.60 m	1.46 m
12	36.9	41.3	38.6	**12**α	1.38 m	1.35 m	1.18 m
				12β	2.06 m	2.10 m	2.01 m
13	42.7	46.6	44.6				
14	51.5	57.2	149.3	**14**	2.18 m	2.08 m	
15	24.1	24.2	24.8	**15**α	1.26 m	1.56 m	2.04 m
				15β	1.74 m	1.56 m	2.24 m
16	29.0	28.3	19.6	**16**α	1.96 m	2.00 m	1.90 m
				16β	1.30 m	1.80 m	1.74 m
17	55.3	57.4	57.2	**17**	1.22 m	1.31 m	1.18 m
18	11.6	12.3	18.5	**18**	0.74 s	0.57 s	0.90 s
19	20.0	19.7	18.9	**19**	1.61 s	1.81 s	1.75 s
20	36.9	36.6	35.3	**20**	1.44 m	1.38 m	1.50 m
21	19.2	19.2	19.4	**21**	0.98 d (6.5)	0.95 d (6.3)	0.97 d (6.3)
22	36.9	36.6	36.6	**22**	0.95 m[b]	1.04 m[b]	1.10 m[b]
					1.37 m[b]	1.35 m[b]	1.36 m[b]
23	24.5	24.5	24.4	**23**	1.14 m[b]	1.10 m[b]	1.10 m[b]
					1.35 m[b]	1.40 m[b]	1.43 m[b]
24	40.2	40.2	40.2	**24**	1.15 m	1.15 m	1.17 m
25	28.3	28.6	28.6	**25**	1.56 m	1.52 m	1.50 m
26	23.1	22.8	22.8	**26**	0.88 d (6.6)	0.86 d (6.2)	0.86 d (6.2)
27	22.9	23.0	23.0	**27**	0.88 d (6.6)	0.86 d (6.2)	0.86 d (6.2)

[a]Olefinic proton. [b]α or β protons.

A DMSO solution of vitamin D_3 (**1**) was heated at 140 °C under nitrogen and in the dark for 1 hour. HPLC separation of the reaction mixture revealed a 60 % conversion of **1** into two new products **6** (33 % based on the conversion of **1**) and **7** (42 % based on the conversion of **1**). The M+1 peaks in the HR-ESI-MS of **6** and **7** corresponded to the same molecular formula $C_{27}H_{44}O$, *i.e.* isomers of **1**. The UV spectrum of **6** and **7** each exhibited strong absorption at 287 and 288 nm respectively, indicating the presence of an all-*trans*-triene chromophore in the two molecules.

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **6** with those of vitamin D_3 (**1**)[4] revealed that the two compounds possess almost identical chemical shifts except for the ring A carbons. The structure of ring A was established by 2D NMR spectroscopic experiments. The ^{1}H–^{1}H COSY spectrum of **6** showed correlations between the hydroxymethine proton H-3 (δ 3.79) and two methylene protons H-2 (δ 2.35 and 2.12) and H-4 (δ 2.95, 2.15), as well as between H-2 and the olefinic proton H-1 (δ 5.54). In the HMBC spectrum the olefinic carbon C-1 (δ 125.3) correlated with H-2 and the methyl protons H-19 (δ 1.81). Moreover, the quaternary carbon C-10 (δ 134.7) correlated with H-1, H-4, H-19 and another olefinic proton H-6 (δ 6.38). These indicate a 1,10-double bond and the connection of 19-methyl to C-10.

The coupling constants of 3-H (4.3, 4.3, 9.0 and 9.0) suggest its axial conformation. The NOESY spectrum shows clear cross peaks between H-6, H-9 and H-19, demonstrating the presence of an all-*trans*-triene structure. Therefore, **6** is assigned as all-*trans*-9,10-seco-1(10),5,7-cholestatrien-3-β-ol (isovitamin D_3). The total ^{1}H and ^{13}C NMR assignments of **6**, which have not been reported previously, are listed in Table 1.

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **7** with those of vitamin D_3 (**1**) [4] and of previtamin D_3 (**3**)[6] revealed that **7** possesses a similar structure to **3** except for ring C. The olefinic proton H-9 (δ 5.52) in **3** is replaced by methylene protons (δ 2.38 and 2.47) in **7** which are correlated in the ^{1}H–^{1}H COSY spectrum with H-11 (δ1.92 and 1.46), and the latter correlated with H-12 (δ 1.18, 2.01), suggesting the presence of a $–CH_2–CH_2–CH_2–$ moiety in the ring C of **7**. In addition, the chemical shifts of H-18 (δ 0.90) and H-15 (δ 2.04 and 2.24) in **7** are significantly further downfield than those in **3** (δ 0.74, 1.26 and 1.74 respectively) demonstrating that these protons are deshielded in **7** by the 8,14-double bond. Correlations of H-6 and H-19, H-6 and H-9, as well as H-7 and H-4 in the NOESY spectrum confirm the all-*trans*-triene structure in **7**. Therefore, compound **7** is assigned as all-*trans*-9,10-seco-5(10),6,8(14)-cholestatrien-3-β-ol (isotachysterol). The total ^{1}H and ^{13}C NMR assignment of **7**, which have not been reported previously, are listed in the Table.

It was reported previously that isotachysterol (**7**) was formed quantitatively by acid catalysed isomerisation of vitamin D_3 (**1**),[7] and **6** and **7** were detected on an aerosil surface loaded with **1**.[8] Therefore, it was considered probable that the formation of **6** and **7** in the present case was due to the weak acidity of the solvent DMSO. Indeed, addition of a couple of drops of NEt_3 to the reaction system produced previtamin D_3 (**3**) as the principal product rather than **6** and **7**.

In conclusion, this work demonstrates that the thermal isomerisation of vitamin D_3 depends on the solvent. Isovitamin D_3 and isotachysterol are the principal isomerisation products in DMSO, due to the acidity of the solvent.

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 with a 5 mm gradient inverse probe in acetone-d_6 with TMS as the internal standard. UV spectra were recorded with a Hitachi 557 spectrophotometer in methanol. HPLC was performed using a Gilson model 303 programmable pump with a Whatman partisil 10 μm ODS-3 column (10×250 mm) with a UV detector.

A solution of vitamin D_3 (**1**, 100 mg) in DMSO (2 ml) was flushed with nitrogen and stirred in the dark at 140 °C for 1 hour. Then the solution was cooled and subjected to HPLC separation using a 10 μm semipreparative ODS-3 column (250°×10 mm) eluted with acetonitrile/water (95/5 v/v) at a flow rate of 2 ml/min and detected at 270 nm. This gave isovitamin D_3 (**5,** 20 mg), isotachysterol (**6,** 25 mg) and unreacted vitamin D_3 (**1,** 40 mg) with retention times of 18.6, 21.5 and 26.3 minutes respectively.

Previtamin D_3 (9,10-seco-5(10),6,8-cholestatrien-3β-ol, **3**), HR-ESI-MS: 385.3467 ($C_{27}H_{44}O$+H requires 385.3465); λ_{max} (MeOH)/nm 261. For ^{1}H and ^{13}C NMR data see Table 1 which are consistent with those reported previously.[6]

Isovitamin D_3 (all-*trans*-9,10-seco-1(10),5,7-cholestatrien-3β-ol, **6**), HR-ESI-MS: 385.3443 ($C_{27}H_{44}O$+H requires 385.3465); λ_{max} (MeOH)/nm 287. For ^{1}H and ^{13}C NMR data see Table 1.

Isotachysterol (all-*trans*-9,10-seco-5(10),6,8(14)-cholestatrien-3β-ol, **7**), HR-ESI-MS: 385.3463 ($C_{27}H_{44}O$ + H requires 385.3465); λ_{max} (MeOH)/nm 288. For ^{1}H and ^{13}C NMR data see Table 1.

We thank the National Natural Science Foundation of China for financial support.

Received 10 March 2003; accepted 29 July 2003
Paper 03/1829

References

1 (a) D. Feldman, F.H. Glorieux and J.W. Pike, (eds.), *Vitamine D*, Academic Press, San Diego, 1997; (b) R. Kumar, Ed., *Vitamin D: Basic Clinical Aspects*, Martinus Nijhoff Publishings, Boston, 1984; (c) A. W. Norman, *Vitamin D: The Calcium Homeostatic Steroid Homomone*, Academic Press, London, 1979.
2 B. Yeung and P. Vouros, *Mass Spectr. Rev.*, 1995, **14**, 179.
3 (a) E. Havinga, *Experientia*, 1973, **15**, 1181; (b) B. Pelc and D.H. Marshall, *Steroids*, 1978, **31**, 23.
4 M.D. Mizhiriskii, L.E. Konstantinovskii and R. Vishkautsan, *Tetrahedron* 1996, **52**, 1239.
5 F. Boomsma, H.J.C. Jacobs, E. Havingga and A. van der Gen, *J. R. Netherlands Chem. Soc.*, 1977, **96**, 104.
6 W.G. Dauben and D.J.H. Funhoff, *J. Org. Chem*. 1988, **53**, 5376.
7 V.K. Agarwal, *J. Steroid Biochem.*, 1990, **35**, 149.
8 O.G. Dmitrenko, G.M. Telbiz, T.A. Kikteva, A.M. Eremenko and A.A. Chuiko, *Proc. Ind. Acad. Sci. (Chem. Sci.*) 1997, **109**, 333.