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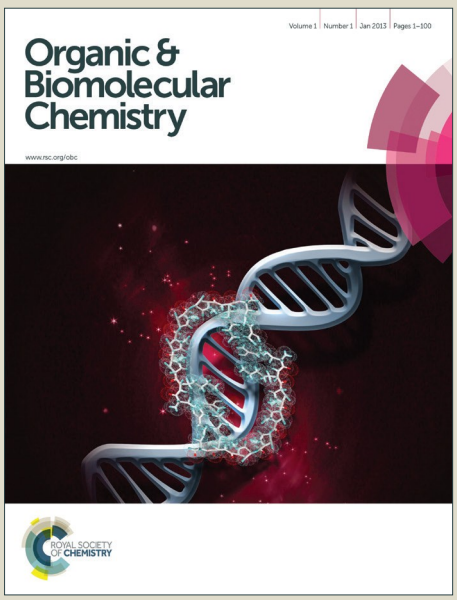

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A new suprasterol by photochemical reaction of 1α,25-dihydroxy-9-methylene-19-norvitamin D_3

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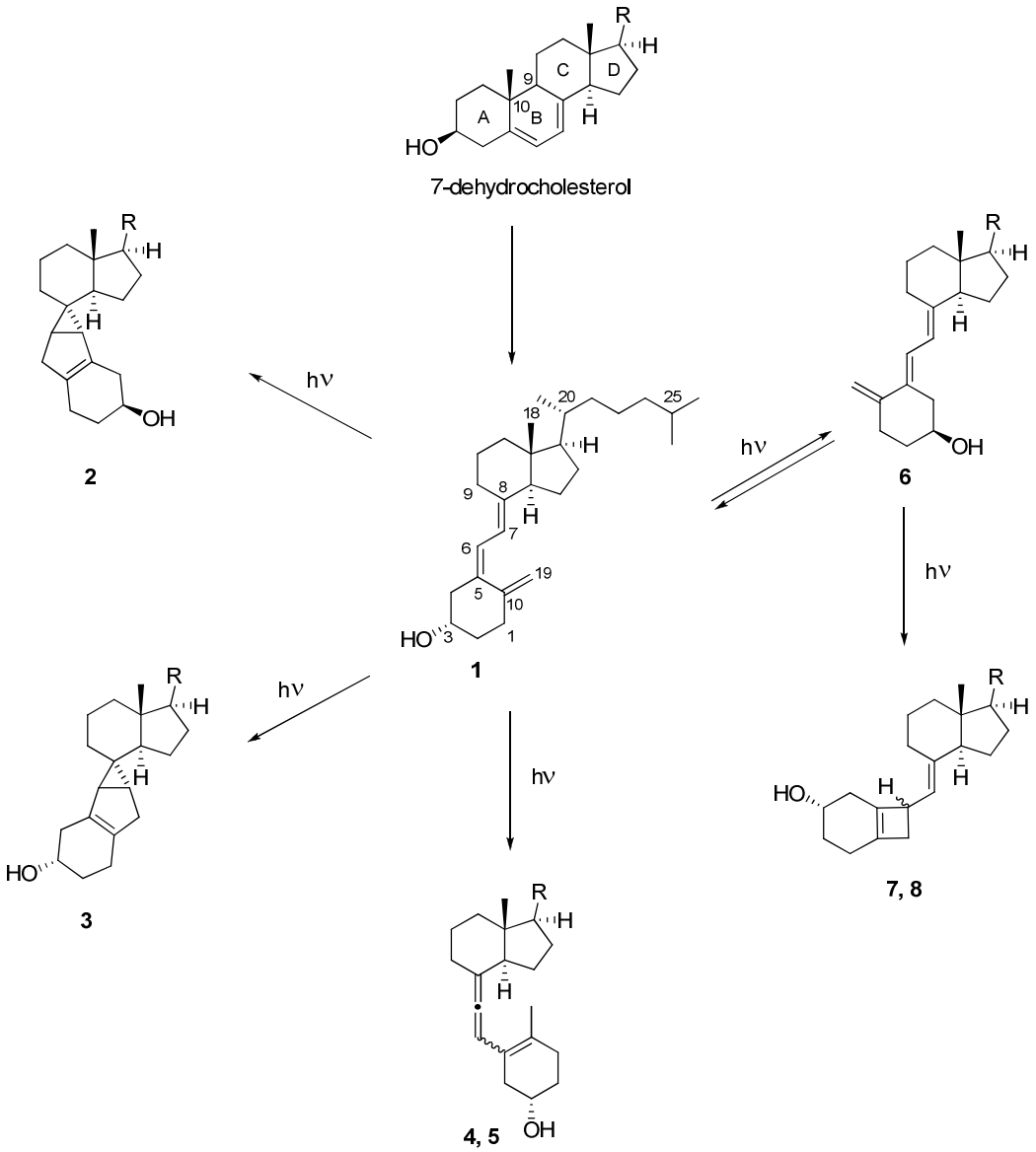
Abstract

UV-induced photochemical reaction of 1α,25-dihydroxy-9-methylene-19-norvitamin D_3 has been investigated. The pentacyclic structure of the isolated product has been unequivocally established by X-ray crystallographic analysis. The possible reaction paths of the examined photochemical transformation are discussed. Biological *in vivo* and *in vitro* tests proved that the photoproduct is devoid of calcemic activity.

Introduction

Reactions of organic compounds containing a conjugated double bond system in their structures have been the subject of intensive studies for many decades.[1] There are many reasons for such interest, among others, the wide occurrence of unsaturated fragments in the structures of natural products, as well as the crucial role that chemical and photochemical reactions of polyenes play in many biological processes.

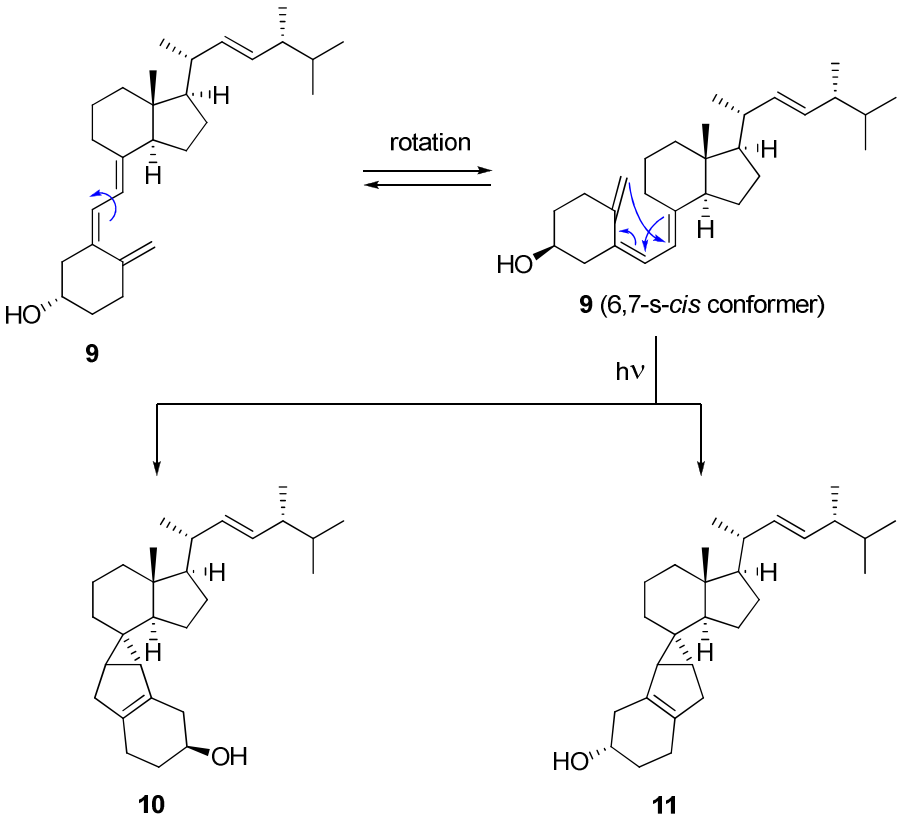
Vitamin D_3 (**1**, Scheme 1) possesses three conjugated double bonds, derived from the photochemical cleavage of the B-ring C(9)-C(10) bond of 7-dehydrocholesterol.[2] The presence of 5,7,10(19)-triene moiety in the B-*seco* steroid **1** enables the occurrence of a number of



Scheme 1 Photochemical transformations of vitamin D_3.

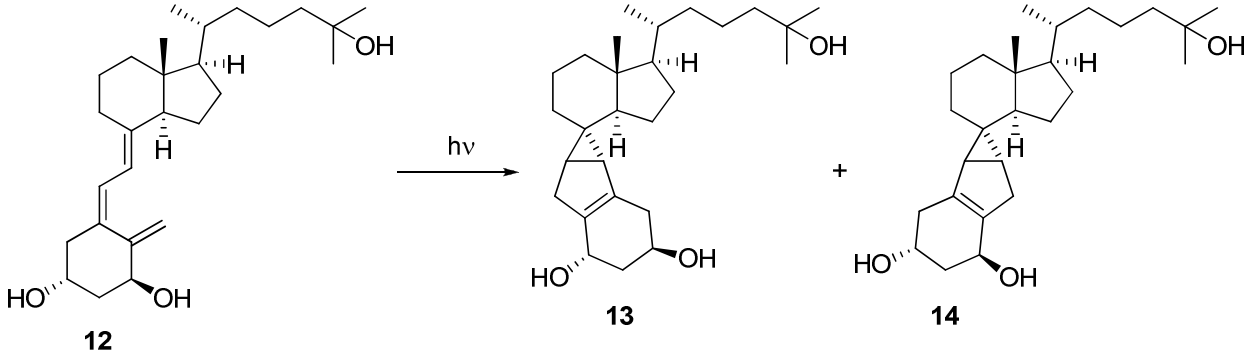
interesting photochemical and thermal rearrangements.[3] Although these processes have been studied since the 1930s, they still attract the attention of chemists. Thus, Windaus,[4] Westerhof,[5] Dauben,[6] and Havinga[7,8] studied the reactions occurring during irradiation of vitamin D_3 with ultraviolet light. The main products of these transformations were identified as suprasterols I (**2**) and II (**3**) which were formed in 80-90% yield. At least five minor photoproducts were also isolated, including stereoisomeric vinylallenes (**4** and **5**) and cyclobutenes (**7** and **8**); the latter originating mainly, if not exclusively from an isomeric 5*E*-vitamin D_3 (**6**).[9]

The mechanism of ultraviolet light-induced formation of suprasterol I (**10**) and II (**11**), derived from vitamin D_2 (**9**, Scheme 2), was suggested in 1972 by Dauben and Kellogg.[10] They assumed that the first stage of this process could be a closure of the cyclopropane ring, followed by the formation of a five-membered ring. In parallel, an alternative mechanism for this photoreaction involving an intramolecular crossed Diels-Alder reaction was also proposed. Thus, this process can be considered as a [4+2] cycloaddition with a tether consisting of a single bond that connects the diene and dienophile. The reaction could proceed in a concerted fashion, with two rings created in a single step (Scheme 2).[11]



Scheme 2 Photochemical transformations of vitamin D_2.

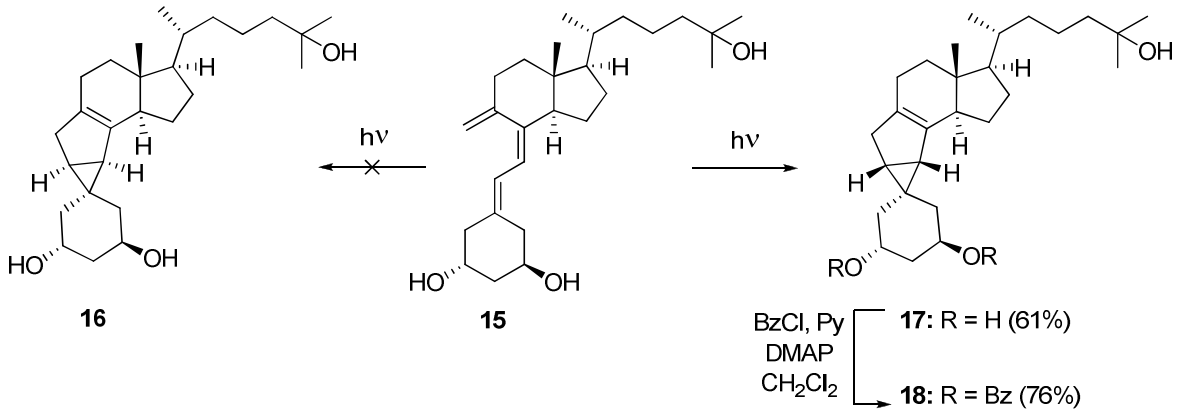
Similar studies on the ultraviolet irradiation of 1α,25-$(OH_2)D_3$ (**12**, calcitriol, Scheme 3) were also performed by Okamura's group.[12] Based on the ^{1}H NMR spectrum of the obtained crude material (signals of Me-18 group), they established the presence of two major photoproducts in the reaction mixture, namely, 1α,25-dihydroxy suprasterols I (**13**) and II (**14**), being in a similar ratio to that reported by Havinga. Aside from these compounds no additional photochemical products have been identified in this experiment.



Scheme 3 Photochemical transformations of 1α,25-dihydroxyvitamin D_3.

Results and discussion

We have recently described the synthesis of vitamin D analogs possessing an unnatural triene system, "reversed" in comparison to the native hormone **1**.[13,14] Among them, 1α,25-dihydroxy-9-methylene-19-norvitamin D_3 (**15**, Scheme 4)[14] seemed to be an interesting substrate for UV irradiation experiment. Thus, compound **15** was irradiated in ethanol with a medium-pressure mercury lamp equipped with a Vycor filter. We expected formation of two "inverted" suprasterols **16** and **17**. However, the photochemical reaction resulted in formation of a single product, which was converted to its dibenzoate for structural characterization. The homogeneity of both compounds as single products was confirmed by HPLC, ^{1}H NMR and ^{13}C NMR spectra.



Scheme 4 Photochemical reaction of 1α,25-dihydroxy-9-methylene-19-norvitamin D_3.

However, the NMR data were not enough to assign the configuration of the newly formed stereogenic centers. Fortunately, crystallization of the diol and its X-ray analysis allowed to establish its structure as **17** (Figure 1).

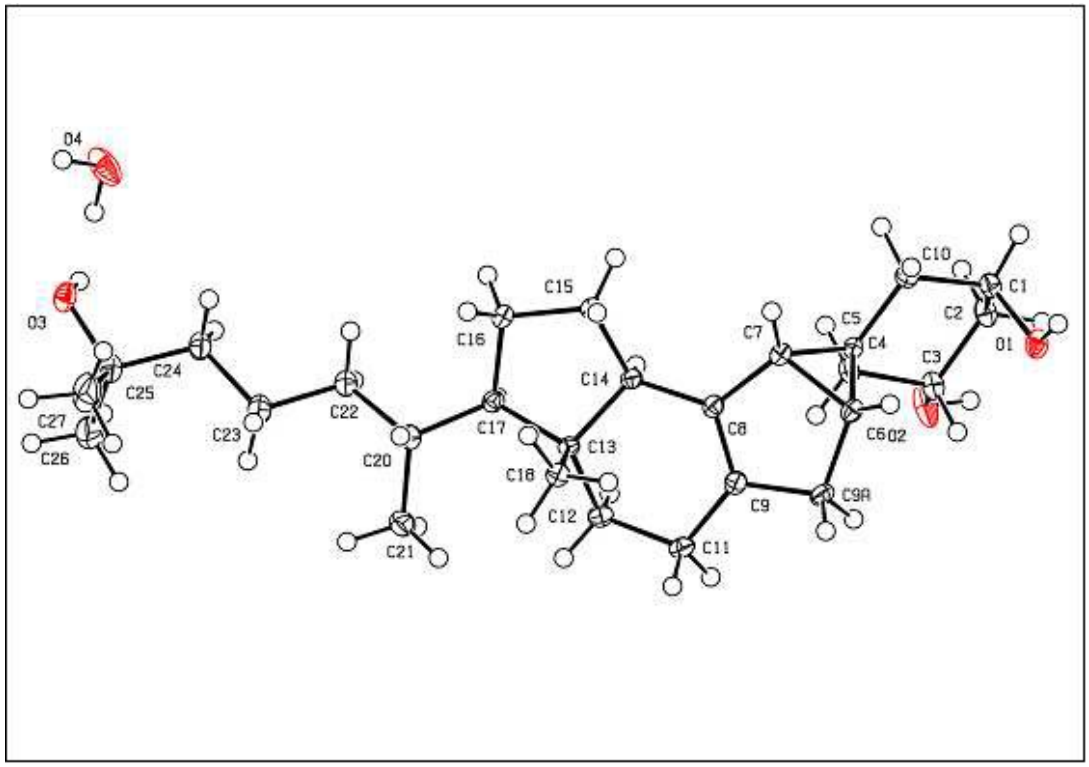
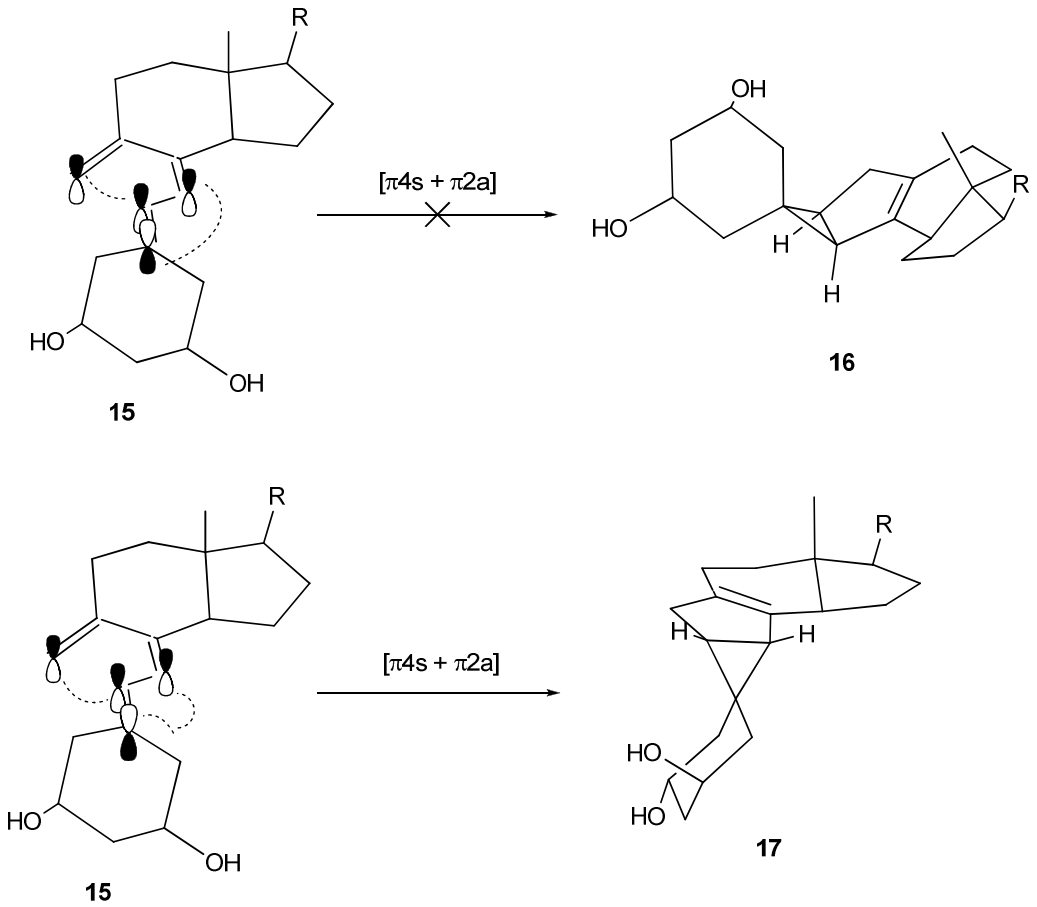


Figure 1. ORTEP drawing derived from the single-crystal X-ray analysis of the suprasterol analog **17**.

When this photochemical isomerization is considered as an intramolecular Diels-Alder reaction, then according to Woodward-Hoffmann rules, it could proceed via $[_{\pi}4_{s}+_{\pi}2_{a}]$ or $[_{\pi}4_{a}+_{\pi}2_{s}]$ mechanism.[15] The interesting studies on the photochemistry of substituted 1,3,5-hexatrienes, described by Padwa et al. in 1972,[12] indicated that formation of the bicyclo[3.1.0]hex-2-ene products could involve the former reaction path. The results of the parallel investigation on photoreactions of conjugated trienes reported by Dauben and Kellogg[11] did not support the concerted nature of this transformation. Thus, they concluded that such intramolecular $[_{\pi}4+_{\pi}2]$ cycloaddition could occur in the two consecutive steps, the first consisting of a conrotatory cyclopropane ring closure, and the second being a non-stereospecific electrocyclization of the formed vibrationally excited zwitterion. This conclusion was confirmed by quantum chemical calculations of Salem,[16] as well as the studies of Jacobs[17] on the formation of bicyclohexene

products from the irradiation of the vitamin D compounds labeled with deuterium at 6- and 19-positions, involved in cyclopropane and cyclopentene ring formation, respectively. The kinetic isotope effect of 1.19 was found only for the former 6-deutero analog, whereas no effect was observed in the compound labeled with deuterium at C-19, involved in the 5-membered ring closure. Clearly, these results strongly supported the hypothesis of a two-step mechanism.



Scheme 5 Possible mechanisms of photoisomerization of the triene **15**.

Scheme 5 depicts a probable mechanism of the photochemical reaction of triene **15** leading to product **17**, that is consistent with the mechanism of photochemical concerted Diels-Alder reaction [$_{\pi}4_{s}+_{\pi}2_{a}$] postulated by Padwa[12], as well as with the two-step process suggested by Dauben[11], assuming conrotatory closure of the cyclopropane ring in s-*trans* part of the triene system, followed by formation of the bond between C-6 and C-9a. Inspection of models confirms

that the transition state leading to the closure of three-membered ring and formation of the sterically more hindered isomeric product **16** is less favorable.

First, biological activity of the tetracyclic photoproduct **17** was assayed *in vitro* and its lack of binding affinity to the vitamin D receptor (VDR) was established. Also, it did not show antiproliferative activity effects on HL-60 cells and displayed negligible VDR dependent transcriptional activity (induction of the 24-hydroxylase). As might be expected from the *in vitro* measurements, compound **17** was devoid of either bone calcium mobilization activity or intestinal calcium transport activity *in vivo* (data not shown).

Conclusions

Lack of substitution of 9-exomethylene group in compound **15**, combined with symmetrical nature of its ring A, precludes establishing of antara- or suprafacial mode of the triene cyclization that leads to formation of three- and five-membered rings. An important difference between the structures of the irradiated compounds **1**, **9**, **12** and **15** depends on the character of their *cis*-diene moiety: in the case of vitamin D compounds, an easy interconversion between their two A-ring chair forms is possible,[18] whereas ring C in the latter compound, being a part of a rigid *trans*-hydrindane system, exists in the one strongly favored conformation with the 9-exomethylene group directed to the α-side. Whatever mechanism of the examined photo-rearrangement operates, it is evident that in this case, due to the steric constraints, the UV light-induced photocyclization preferentially occurs (in concerted or two-step zwitterionic mode) with C-5 approaching C-7 from the bottom (α-side) of the hydrindane ring system. Lack of conformation flexibility of the *cis*-diene fragment in **15** must be responsible for the exclusive formation of the single diastereoisomeric photoproduct **17**.

Experimental section

Chemistry. The melting point (uncorrected) was determined on a SMP10 Stuart Scientific capillary melting point apparatus. Optical rotation were measured in chloroform using a Perkin-Elmer model 343 polarimeter at 24 °C; $[\alpha]_D$ values. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ solutions using Bruker DMX-500 instrument. Chemical shifts (δ) are reported in parts per million relative to $(CH_3)_4Si$ (δ 0.00) or solvent signal as an internal standard. Signals in ^1H NMR spectra are described using the following abbreviations: s - singlet, d - doublet, t -

triplet, m – multiplet. High-resolution mass spectra were recorded on LCT (TOF) or Mass Quattro LC spectrometers using electrospray ionization (ESI) technique. High-performance liquid chromatography (HPLC) purifications were performed on Shimadzu UFLS liquid chromatograph equipped with SPD-20A tunable absorbance detector.

Photoisomerization of the vitamin 15

9a,6;5,7-Dicyclo-9,10-secocholest-8-en (17). A solution of the vitamin D analog **15** (76 mg, 0.177 mmol) in deoxygenated absolute EtOH (600 mL) was irradiated using 450 W Hanovia medium pressure mercury lamp with a Vycor filter (50% cut-off below 230 nm) for 40 min until all starting material disappeared (TLC control). Formation of a slightly less polar product was observed. The reaction mixture was concentrated and the residue was purified by HPLC (10 mm × 25 cm Phenomenex Luna Silica column, 4 mL/min) using a hexane/2-propanol (8:2) solvent system. The suprasterol analog **17** was collected at Rv 53 mL (46 mg, 61%): $[\alpha]^{24}_{D}$ +42.1 (*c* 0.9 in $CHCl_3$); m.p. 100-103 ^{o}C (hexane/acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.653 (3H, s, 18-H_3), 0.948 (3H, d, J = 6.5 Hz, 21-H_3), 1.213 (6H, s, 26- and 27-H_3), 2.39 (1H, br dd, J ~ 17 and 6 Hz), 4.09 and 4.12 (1H and 1H, each m, 1β- and 3α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.3 (CH_3), 18.8 (CH_3), 20.8 (CH_2), 22.9 (C), 23.2 (CH_2), 24.0 (CH_2), 25.2 (CH), 29.0 (CH_2), 29.2 (CH_3), 29.3 (CH_3), 29.9 (CH_2), 34.4 (CH_2), 35.7 (CH), 36.0 (CH), 36.3 (CH_2), 36.5 (CH_2), 42.6 (CH_2), 43.5 (C), 44.36 (CH_2), 44.43 (CH_2), 49.8 (CH), 54.1 (CH), 66.3 (CH), 67.1 (CH), 71.1 (C), 134.8 (C), 134.9 (C); HRMS (ESI): calcd for $C_{27}H_{44}O_3Na$ $(M + Na)^+$: 439.3188, found: 439.3189.

Protection of hydroxyl groups in compound 17

1α,3β-Dibenzoyloxy-25-hydroxy-9a,6;5,7-dicyclo-9,10-secocholest-8-en (18). DMAP (3 mg) was added to a solution of the vitamin D compound **17** (36 mg, 0.084 mmol) in CH_2Cl_2 (3 mL) and pyridine (54 μL, 0.672 mmol) and the resulting mixture was cooled to 0 ^{o}C. After 10 min benzoyl chloride (39 mg, 0.336 mmol) was slowly added and the reaction mixture was stirred at 0 ^{o}C for 3 h. The reaction was quenched by addition of water, extracted with CH_2Cl_2, washed with saturated $CuSO_4$, dried (Na_2SO_4) and concentrated. The residue was purified by HPLC (10 mm × 25 cm Phenomenex Luna Silica column, 4 mL/min) using a hexane/ethyl acetate (82:18) solvent system. The oily diester **18** was collected at Rv 78 mL (40 mg, 76%): ^{1}H NMR (500

MHz, $CDCl_3$) δ 0.531 (3H, s, 18-H_3), 0.847 (3H, d, J = 6.4 Hz, 21-H_3), 1.224 (6H, s, 26- and 27-H_3), 2.35 (1H, m), 5.45 and 5.57 (1H and 1H, each m, 1β- and 3α-H), 7.45 (4H, t, J = 7.0 Hz, Ar-H), 7.57 (2H, m, Ar-H), 8.07 (4H, m, Ar-H);^{13}C NMR (125 MHz, $CDCl_3$) δ 11.0 (CH_3), 18.6 (CH_3), 21.0 (CH_2), 22.9 (C), 22.9 (CH_2), 23.9 (CH_2), 25.4 (CH), 26.9 (CH_2), 28.6 (CH_2), 29.2 (CH_3), 29.4 (CH_3), 34.1 (CH_2), 36.0 (CH), 36.3 (CH_2), 36.4 (CH_2), 36.6 (CH), 36.7 (CH_2), 40.8 (CH_2), 43.1 (C), 44.4 (CH_2), 49.1 (CH), 53.9 (CH), 69.9 (CH), 70.6 (CH), 71.1 (C), 128.4 (CH), 129.5 (CH), 129.6 (CH), 130.5 (C), 130.8 (C), 132.9 (CH), 132.9 (CH), 134.3 (C), 135.2 (C), 166.0 (C); HRMS (ESI): calcd for $C_{41}H_{52}O_5Na$ $(M + Na)^+$: 647.3707, found: 647.3722.

Biological studies

In vitro studies (VDR binding, HL-60 differentiation and 24-hydroxylase transcription assays) as well as tests *in vivo* (bone calcium mobilization and intestinal calcium transport) were performed as previously described.[19] All animals were managed in accordance with University of Wisconsin standards and protocols for animal care and use. Our experiments were approved by the College of Agricultural and Life Sciences Institutional Animal Care and Use Committee.

Crystalographic studies

Crystal data for the suprasterol analogue 17. $C_{27}H_{46}O_4$, M = 434.64, T = 100 K, orthorhombic, space group $P2_12_12_1$, Z = 4, a = 6.8943(2), b = 11.6898(4), c = 32.2785(11) Å, αβγ = 90°, V = 2601.42(15) Å^3, Dx = 1.110 g·cm^{-3}, 7954 unique data ($2\theta_{max}$ = 30.550), 6631 with $F_0^2 > 2\sigma(F_0^2)$, R = 0.0463, Rw = 0.0990, S = 1.040. In addition to one molecule of the suprasterol 17, there was also one molecule of water in the asymmetric unit.

Structure determination of suprasterol analog 17. The structure of the vitamin **17** was determined in a single-crystal X-ray diffraction measurement on a Kappa Apex II Ultra CCD diffractometer with TXS rotating molybdenum anode and multilayer optics (λ = 0.71073 Å, 50.0 kV, 24.0 mA).[20] Temperature of measurement was 100 K. The 2450 frames were measured with scan width of 0.5° and counting time of 20 s. Indexing, integration and scaling were performed with the original Bruker Apex II software.[21] The multi-scan absorption correction was applied in the scaling procedure.

The structures were solved by direct methods[22] approach using the SHELXS-97 program and refined with the SHELXL-97.[23] The refinement was based on F^2 for all reflections except

those with negative intensities. Weighted R factors wR and all goodness-of-fit S values were based on F^2, whereas conventional R factors were based on the amplitudes, with F set to zero for negative F^2. The $F_0^2 > 2\sigma(F_0^2)$ criterion applied only for R factors calculation was not relevant to the choice of reflections for the refinement. The R factor based on F^2 is about three times as large as the one based on F. All hydrogen atoms were located in idealized geometrical positions. Scattering factors were taken from Tables 4.2.6.8 and 6.1.1.4 from the International Crystallographic Tables Vol. C.[24]

Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC-1423643.

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Notes and references

[1] Rappoport, Z. The chemistry of dienes and polyenes. In *The chemistry of functional groups,* Volume 1 and 2. Rappoport, Z. (Editor) March 1997 and August 2000, An Interscience Publication, John Wiley & Sons, Ltd.

[2] (a) Holick, M.F. Vitamin D: A millenium perspective. *J. Cell Biochem.* **2003**, *88*, 296-307. (b) Chen T.C.; Lu Z.; Holick, M.F. Photobiology of vitamin D. In *Vitamin D: Physiology, molecular biology and clinical applications*, by Holick, M.F.; Humana Press, 2010. (c) Holick, M.F. Photobiology of vitamin D. In *Vitamin D*, Third Edition, by Feldman, D.; Pike, J.W.; Adams, J.S.; Elsevier Academic Press, 2011.

[3] Jacobs, H.J.C. ; Havinga, E. Photochemistry of vitamin D and its isomers and of simple trienes. *Adv. Photochem.* **1979**, *11*, 305-373.

[4] Windaus, A.; Gaede, J.; Koser, J.; Stein, G. Über einige krystallisierte Bestrahlungsprodukte aus Ergosterin und Dehydro-ergosterin. *Justus Liebigs Ann. Chem*. **1930**, *483*, 17-30.

[5] Westerhof, P.; Buisman, J.A.K. Investigations on sterols. VIII. Some hitherto unknown irradiation products of ergosterol. *Recl. Trav. Chim. Pays-Bas* **1956**, *75*, 1243-1251.

[6] Dauben, W.G.; Baumann, P. Photochemical transformations. IX. Total structure of suprasterol II. *Tetrahedron Lett*. **1961**, 565-572.

[7] Havinga, E. Vitamin D, example and challenge. *Experientia* **1973**, *29*, 1181-1316

[8] Bakker, S.A.; Lugtenburg, J.; Havinga, E. Studies on vitamin D and related compounds. XXII. New reactions and products in vitamin D_3 photochemistry. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 1459-1464.

[9] Jacobs, H.J.C.; Gielen, J.W.J.; Havinga, E. Effects of wavelength and conformation on the photochemistry of vitamin D and related conjugated trienes. *Tetrahedron Lett.* **1981**, *22*, 4013-4016.

[10] Dauben, W.G.; Kellogg, M.S. Mechanism of the photochemical transformation of a 1,3,5-hexatriene to a bicyclo[3.1.0]hex-2-ene. *J. Am. Chem. Soc.* **1972**, *94*, 8951-8953.

[11] Padwa, A.; Brodsky, L.; Clough, S. Orbital symmetry and steric control in the photorearrangement of 1,3,5-hexatrienes to bicyclo[3.1.0]hex-2-enes. *J. Am. Chem. Soc.* **1972**, *94*, 6767-6775.

[12] Okamura, W.H.; Do, S.; Kim, H.; Jeganathan, S.; Vu, T.; Zhu, G.-D.; Norman, A.W. Conformationally restricted mimics of vitamin D rotamers. *Steroids* **2001**, *66*, 239-247.

[13] Kulesza, U.; Sigüeiro, R.; Mouriño, A.; Plum, L.A.; DeLuca, H.F.; Sicinski, R.R. Synthesis of 9-alkylated calcitriol and two 1α,25-dihydroxy-9-methylene-10.19-dihydrovitamin D_3 analogues with a non-natural triene system by thermal sigmatropic rearrangements. *J. Org. Chem.* **2013**, *78*, 1444-1450.

[14] Kulesza, U.; Mouriño, A.; Plum, L.A.; DeLuca, H.F.; Sicinski, R.R. Synthesis of novel 19-norvitamin D_3 analogs with unnatural triene system. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 23-26.

[15] Woodward, R.B.; Hoffmann, R. *The conservation of orbital symmetry*; Verlag Chemie: Weinheim, Germany, **1970**.

[16] (a) Salem, L. Theory of photochemical reactions. *Science* **1976**, *191*, 822-830. (b) Salem, L. The sudden polarization effect and its possible role in vision. *Acc. Chem. Res.* **1979**, *12*, 87-92.

[17] Jacobs, H.J.C. Photochemistry of conjugated trienes: Vitamin D revisited. *Pure Appl. Chem.* **1995**, *67*, 63-70.

[18] (a) Okamura, W.H.; Midland, M.M.; Hammond, M.W.; Abd. Rahman, N.; Dormanen, M.C.; Nemere, I.; Norman, A.W. Chemistry and conformation of vitamin D molecules. *J. Steroid Biochem. Mol. Biol*. **1995**, *53*, 603-613. (b) Eguchi, T.; Ikekawa, N. Conformational analysis of 1α,25-dihydroxyvitamin D_3 by nuclear magnetic resonance. *Bioorg. Chem.* **1990**, *18*, 19-29. (c) Wing, R.M.; Okamura, W.H.; Rego, A.; Pirio, M.R.; Norman, A.W. Studies on vitamin D and its analogs. VII. Solution conformations of vitamin D_3 and 1α,25-dihydroxyvitamin D_3 by high-resolution proton magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **1975**, *97*, 4980-4985.

[19] (a) Chiellini, G.; Grzywacz, P.; Plum, L.A.; Barycki, R.; Clagett-Dame, M.; DeLuca, H.F. Synthesis and biological properties of 2-methylene-19-nor-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactones-weak agonists. *Bioorg. Med. Chem.* **2008**, *16*, 8563-8573. (b) Glebocka, A.; Sicinski, R.R.; Plum, L.A.; Clagett-Dame, M.; DeLuca, H.F. New 2-alkylidene 1α,25-dihydroxy-19-norvitamin D_3 analogues of high intestinal activity: Synthesis and biological evaluation of 2-(3′-alkoxypropylidene) and 2-(3′-hydroxypropylidene) derivatives. *J. Med. Chem.* **2006**, *49*, 2909-2920.

[20] APEX2, 2010.3-0; Bruker AXS Inc.: Madison, WI, USA, **2010**.

[21] (a) SAINT, 7.68A; Bruker AXS Inc.: Madison, WI, USA, **2010**. (b) Sheldrick, G. M. SADABS, University of Gottingen: Germany, **1996**.

[22] Sheldrick, G.M. Phase annealing in SHELX-90: direct methods for larger structures. *Acta Crystallogr., Sect. A* **1990**, *46*, 467-473.

[23] Sheldrick, G.M. SHELXL93. *Program for the Refinement of Crystal Structures.,* Univ. of Göttingen, Germany.

[24] *International Tables for Crystallography*; Wilson, A.J.C. Ed., Kluwer: Dordrecht, 1992; Vol. C.