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ACKNOWLEDGMENTS

The authors acknowledge financial support from the University of Manitoba Research Board and the Medical Research Council of Canada for the award of a summer studentship to Catherine Savage. The authors are grateful to Dr. A. D. Friesen of the Winnipeg Rh Institute Inc., Winnipeg, Manitoba, Canada, for the measurement of protein concentrations and for helpful discussions.

Degradation of Crystalline Ergocalciferol [Vitamin D_2, (3β,5*Z*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol]

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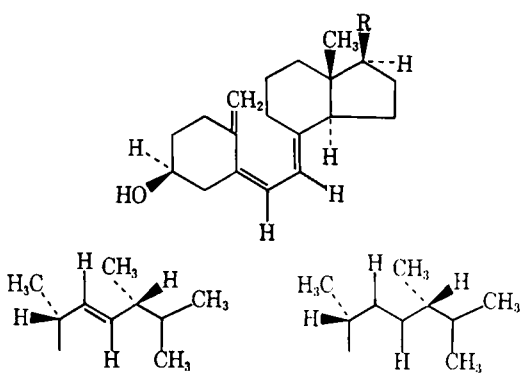
Received June 15, 1981, from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.* Accepted for publication October 17, 1983.

Abstract □ The products of the degradation of crystalline ergocalciferol were investigated. These studies showed that numerous acidic and neutral oxidation products were formed resulting in the complete destruction of the triene functionality. Separation of the neutral products by preparative TLC led to material identified as the Windaus ketone IIa, 2,3,3a,4,5,6,7,7aβ-octahydro-7aα-methyl-1*R*-(1α,1*R*,4*R*,5-trimethyl-2*E*-hexenyl)-4*H*-inden-4-one.

Keyphrases □ Ergocalciferol—crystal degradation products, liquid chromatography, identification of a Windaus ketone □ Liquid chromatography—determination of the crystal degradation products of ergocalciferol

Solid-gas reactions are often catalyzed by heat and/or light; thus, these reactions are of importance when studying the mechanism of drug degradation (1-6). This paper reports studies on a specific class of solid-gas reactions: solid-oxygen reactions. A review (1) reports our preliminary studies on the complex heat- and light-catalyzed solid-oxygen reactions of vitamin D_2, ergocalciferol (Ia). This paper presents these studies in greater detail.



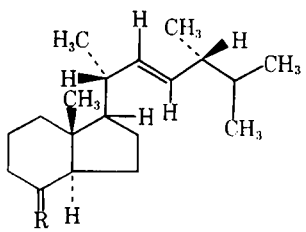
Ia, R = Ib, R =

The oxidative degradation of ergocalciferol has been known for over 40 years (7-11); however, the structures of the solid-state degradation products have not been elucidated. Thus, it is the aim of this study to isolate and unequivocally identify these products. Initially, the decomposition of ergocalciferol in room fluorescent light and air was investigated, resulting in the identification of the Windaus ketone (IIa).

EXPERIMENTAL SECTION

Reagents—Ergocalciferol[1] purchased in sealed ampules was used in all experiments. All reagents employed were of either reagent, spectral, or ACS grade. Methanol used in recrystallization of compounds was purified by reflux with magnesium and iodine followed by distillation over molecular sieves.

Apparatus—All melting points were obtained on a hot stage[2] and are uncorrected. IR spectra[3] were determined neat or as KBr pellets. NMR spectra were obtained using a 60-MHz instrument[4] with either $CDCl_3$ or acetone-d_6 as the solvent and 1% tetramethylsilane as the internal standard. Low-[5] and



Ia, R = 0
Ib, R = NNHCONH$_2$

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Kofler Hot Stage.
[3] Beckman IR-33; Beckman Instruments, Irvine, Calif.
[4] Varian Anaspect EM 360; Varian Associates, Palo Alto, Calif.
[5] Determined by Dr. I. Jardine and associates using a DuPont 21-492B mass spectrometer.

0022-3549/84/0900-1322$01.00/0

high-resolution[6] mass spectra were determined using mass spectrometers operated in both the electron-impact and chemical-ionization modes.

Chromatography—Thin-layer silica gel chromatography plates containing fluorescent indicator[7] were used. Mixtures were applied to the preparative layer silica gel chromatography plates[8] with an applicator[9].

Synthesis of 2,3,3a,4,5,6,7,7aβ-octahydro-7aα-methyl-1*R*-(1α,1*R*,4*R*,5-trimethyl-2*E*-hexenyl)-4*H*-inden-4-one (IIa) and Its Semicarbazone (IIb)—Compounds IIa and IIb were synthesized according to the procedure of Windaus and Grundman (12). Compound IIb was crystallized as a white solid, mp 222°C [lit. (12) mp 222°C]; IR (KBr): 3440, 3240, 3170 (NHC=O amide), 1680 (primary amide), 1570 (secondary amide), and 960 cm^{-1} (*trans* RHC=CHR); ^{1}H-NMR (acetone-d_6): δ 0.57 (s, 3, C-18 CH_3), 0.80 (d, 6, C-26,27 CH_3), 0.90 (d, 3, C-28 CH_3), 1.1 (d, 3, C-21 CH_3), 1.1–2.9 (m, 15), 5.2 (m, 2, 22-H, 23-H), 5.6 (d, 2, H_2N—C=O), and 8.2 ppm (s, 1, HNC=O); MS: *m/z* 333 (M^+, 58%).

Anal.—Calc. for $C_{20}H_{35}N_3O$: C, 72.03; H, 10.57; N, 12.60; O, 4.80. Found: C, 71.91; H, 10.68; N, 12.80[10].

Degradation of Ergocalciferol in the Solid State—The experiments, performed in triplicate by different investigators at different times of the year, led to similar results. Crystalline ergocalciferol from a newly opened refrigerated ampule was spread in a petri dish and left exposed to artificial light and room air (with average humidity, room temperature, and atmospheric pressure). The colorless needles gradually changed to an orange powder. The IR and NMR spectra of the degrading compound gradually changed for the first 6 months and then remained unchanged after 6 months.

A sample of ergocalciferol (0.81 g), which had been stored for 6 months at room temperature, chloroform (150 mL), and a 10% $NaHCO_3$ solution (150 mL) were stirred for 5 min at room temperature. The organic phase was removed at reduced pressure. Thin-layer chromatography of the residue [cyclohexane–ethyl acetate–chloroform–acetic acid (5:2:1:1)] indicated three fractions ($R_f \times 100$: 0, 31, 53):

1. Material *A* R_f (× 100) 0; 0.038 g; 46% of the recovered material was an amber oil that could not be crystallized. IR (neat): 3400 (broad), 1700 (C=O, broad), and 1620 cm^{-1} (C=C); MS (C.I.): *m/z* 309 (5.7%), 279 (11.0), 153 (11.0), 143 (28.0), and 91 (100.0).
2. Material *B* R_f (× 100) 31; 0.023 g; 28% of the recovered material was an amber oil that could not be crystallized. IR (neat): 3400 (ROH), 1710 (C=O, broad), and 1620 cm^{-1} (C=C); MS (C.I.): *m/z* 309 (40.5%), 307 (33.8), 294 (22.3), 293 (100.0), 292 (24.3), 291 (86.5), 279 (27.0), 275 (72.3), and 125 (21.6).
3. Material *C* R_f (× 100) 53; 0.021 g; 26% of the recovered material was an amber oil that could not be crystallized. IR (neat): 2490 (C—H), 1700 (C=O), and 1630 cm^{-1} (C=C); MS (C.I.): *m/z* 209 (11.3%), 278 (22.6), and 277 (100). The exact mass of the 276 peak was determined to be 276.247.

Material *C* and IIa co-chromatographed on silica gel plates using a three solvent system: cyclohexane–ethyl acetate chloroform–glacial acetic acid (5:2:1:1); chloroform–methanol (9:1); and ethanol.

Preparation of the Semicarbazone Derivative of Material *C*—A mixture of material *C* (0.04 g), semicarbazide hydrochloride (0.26 g, 0.002 mol), sodium acetate (0.41 g, 0.01 mol), and methanol (10 mL) was heated to a reflux temperature in an 80°C oil bath. Water (0.5 mL) was added dropwise over a 5 min period. The mixture was refluxed for 0.5 h, then cooled. Recrystallization of the material from methanol gave a white crystalline solid (0.005 g), mp 220°C, the IR spectrum (KBr) of which was identical to that of the aforementioned semicarbazone, IIb.

[6] Determined by the Purdue University Spectral Services Department using a Hitachi RMV-6A mass spectrometer.

[7] Silica Gel 60 F-254 plates, 0.25 mm; E. Merck A. G., Darmstadt, West Germany.

[8] Silica Gel GF Prep Pre-Coat plates, 1.0 mm; Camag, Inc., Milwaukee, Wis.

[9] Kontes Chromaflex Streaker; Kontes Glass Co., Vineland, N.J.

[10] Microanalyses were performed by Dr. C. S. Yeh and associates, Purdue University, West Lafayette, Ind.

RESULTS AND DISCUSSION

Degradation of ergocalciferol in ordinary fluorescent light at average room temperature and humidity results in the oxidation and fragmentation of the triene functionality. The ^{1}H-NMR spectrum of degraded ergocalciferol showed that the peaks assigned to the vinyl protons of the triene system at δ 4.7, 5.0, 5.94, and 6.14 ppm had disappeared while the peaks assigned to the vinyl protons of the C-22 to C-23 olefinic bond remained unchanged (13). In addition, carbonyl absorption appeared in the IR spectrum of the degraded material. In the NMR spectrum the peak attributed to the C-18 CH_3 absorption had decreased in intensity and shifted downfield. A broad, D_2O exchangeable peak, appeared between δ 3.3 and 5.38 ppm.

The degraded material *C* can be identified as IIa based on co-chromatography and the preparation of the semicarbazone derivative which had the same melting point and IR spectrum as the semicarbazone derivative of authentic IIa. Material *B* appeared to contain two components with structures similar to IIa. The high-resolution MS indicate that these components correspond to IIa with one or two hydrogen atoms replaced by hydroxyl groups. These two components have so far resisted complete characterization.

These results are consistent with literature reports which indicate that in air and light crystalline ergocalciferol decomposes, while storage under vacuum results in increased stability (7–9, 11). The extensive oxidation observed in our studies does not rule out other nonoxidative pathways of degradation, but it does indicate that oxidation is the major pathway of ergocalciferol degradation.

The nucleus of the ergocalciferol molecule undergoes extensive oxidation; however, the side chain appears to remain intact. This may be due to the lesser reactivity of the side-chain olefin and to the crystal packing which promotes oxidation of the ergocalciferol nucleus. Preliminary studies of cholecalciferol (Ib) degradation indicate this compound to be much more stable, confirming earlier reports (14). The greater stability exhibited by cholecalciferol may be due to different crystal packing. Recently, studies of hydrocortisone *tert*-butyl acetate have shown that different crystal forms have different reactivity towards oxygen, presumably because of different crystal packing (15). Crystals of cholecalciferol may also contain fewer defects or sites for potential nucleation, therefore being more stable.

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ACKNOWLEDGMENTS

This research was supported by NIH Grant GM21174.