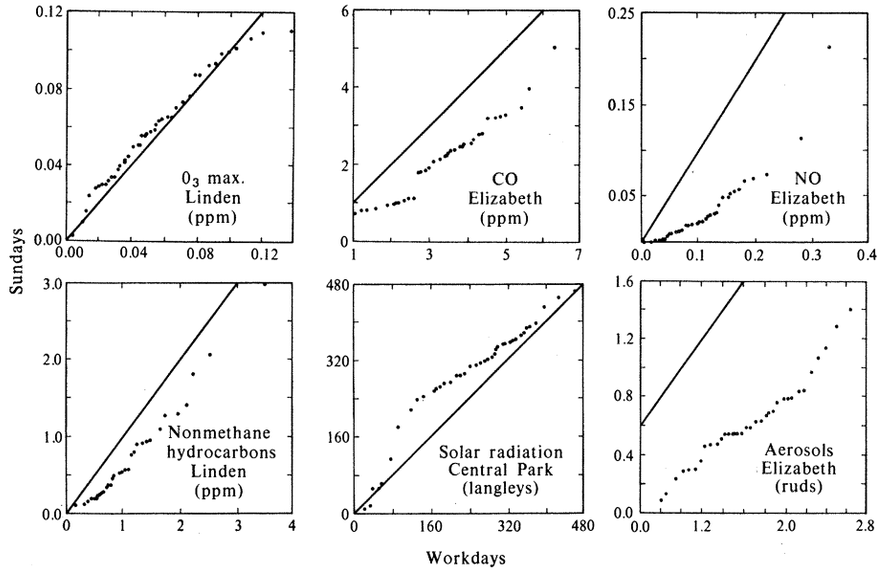

Fig. 1. Quantile-quantile plots for Sundays versus workdays.

are plotted against the corresponding quantiles of the other set of data (in this case the workday values). If the distributions are nearly the same, then the points of the plot lie nearly along the straight line $Y = X$.

Such *Q-Q* plots have been made for each of the 92 sets of air quality and meteorological data described above. For each of the variables the following patterns are consistent from site to site. The Sunday quantiles of NO, NO_2, CO, nonmethane hydrocarbons, aerosols, and total hydrocarbons are markedly lower than the workday quantiles, since on the *Q-Q* plots all these points are well below the line $Y = X$. The quantiles for aldehydes and CH_4 are slightly lower on Sundays than on workdays, whereas SO_2 shows no consistent pattern. The O_3 maxima are only slightly higher on Sundays, whereas O_3 averages are markedly higher. For all the meteorological variables the workday and Sunday quantiles are similar except solar radiation, mixing height, and vertical sigma which have noticeably higher Sunday quantiles.

Examples of patterns occurring at a particular site are presented in Fig. 1. Measurements from the Elizabeth, New Jersey, monitoring station (New Jersey Department of Environmental Protection) of NO, CO, and aerosols are shown, as are the closest data on O_3 and nonmethane hydrocarbons (Linden, New Jersey, Esso Research and Engineering Company, 2 km west of the Elizabeth site) and solar radiation (Central Park, New York City, National Weather Service, 21 km to the northeast). On all plots the line $Y = X$ has been drawn to facilitate comparison.

The *Q-Q* plots also convey several important facts that can only be seen by studying the entire distribution of each of the variables: the very highest O_3 maxima occur on workdays in this data set, but *all other* O_3 maxima and *all* average quantiles are higher on Sundays; for CO, NO, aerosols, and nonmethane hydrocarbons, the differences between Sunday and workday quantiles tend to increase with increasing concentration; for solar radiation the lowest and highest quantiles are nearly the same on Sundays and workdays, whereas the middle quantiles are considerably higher on Sundays. This increase in solar radiation would tend to increase vertical turbulence, which is consistent with the increased vertical sigma and mixing height. Since aerosols are a major absorber and scatterer of solar radiation in the troposphere (*5*), the increase in solar radiation on Sundays may well be due to the decrease in the Sunday aerosol concentrations (motor vehicles and industrial operations are major sources of aerosols).

We note in conclusion that the reduction in the concentrations of primary pollutants from 5 a.m. to 1 p.m. on Sundays may be regarded as a regional experiment to shed light on the effectiveness of reducing the O_3 concentration by a morning reduction of primary pollutants. Since the O_3 concentrations show little change in this experiment, it would appear that serious questions are raised about this reduction procedure.

W. S. CLEVELAND, T. E. GRAEDEL
B. KLEINER, J. L. WARNER*
Bell Laboratories,
Murray Hill, New Jersey 07974

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3. The measurement techniques used for the air quality data described here are as follows: NO, Griess-Saltzman colorimetry; NO_2, Griess-Saltzman colorimetry; SO_2, West-Gaeke colorimetry; aldehydes, modified Schiff's test [G. R. Lyles, F. B. Dowling, V. J. Blanchard, *J. Air Pollut. Control Assoc.* **15**, 106 (1965)] that measures formaldehyde plus ~ 10 percent acetaldehyde; CO, nondispersive infrared absorption; total hydrocarbons, flame ionization detection; CH_4, gas chromatography; nonmethane hydrocarbons, gas chromatography; aerosols, soiling index opacity by filter tape sampler; O_3, chemiluminescence.
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6. We are greatly indebted to R. Gnanadesikan and K. B. McAfee, Jr., of Bell Laboratories and R. E. Pasceri and C. Fleisher of the New Jersey Department of Environmental Protection for their valuable advice and assistance in the research described in this report.

* The authors of this report are listed in alphabetical order.

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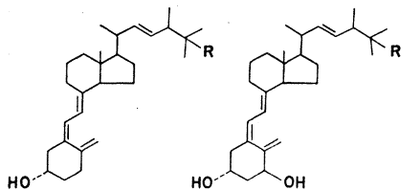
1α-Hydroxyvitamin D_2: A Potent Synthetic Analog of Vitamin D_2

Abstract. *A hydroxy analog of vitamin D_2, 1α-hydroxyvitamin D_2, has been synthesized and tested for biological activity. This vitamin derivative is active in stimulating intestinal calcium transport and bone calcium mobilization in the rat and exhibits antirachitic activity. Its biopotency is comparable to that of the corresponding vitamin D_3 analog, 1α-hydroxyvitamin D_3.*

Expression of biological activity of vitamin D_3 requires prior metabolic conversion of the vitamin to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] via the intermediate 25-hydroxy derivative (*1*). An entirely analogous transformation of vitamin D_2 (**1**, Fig. 1) to 25-hydroxyvitamin D_2 (25-OH-D_2) (**2**, Fig. 1) could be shown some years ago (*2*); and recent work (*3*) has established the further metabolism of **2**, by chick kidney preparations, to



1: R = H 3: R = OH
2: R = OH 4: R = H

Fig. 1. Structures of vitamin D_2 (**1**), 25-OH-D_2 (**2**), 1,25-$(OH)_2$-D_2 (**3**), and 1α-OH-D_2 (**4**).

1,25-dihydroxyvitamin D_2 [1,25-$(OH)_2$-D_2] (**3**, Fig. 1). We have also shown previously that the 25-deoxy derivative of 1α,25-$(OH)_2$-D_3, 1α-hydroxyvitamin D_3 (1α-OH-D_3), available by synthesis from cholesterol (*4*), represents a very potent analog of the natural vitamin D_3 metabolite, exhibiting activity in both normal and anephric animals. The preparation and biological activity of 1α-OH-D_3 has received much attention in the interim (*5–7*), and we therefore investigated the biological properties of the corresponding vitamin D_2 analog, 1α-hydroxyvitamin D_2 (1α-OH-D_2) (**4**, Fig. 1).

Analog **4** could be prepared from isoergosterone (ergosta-4,6,22-trien-3-one), which in turn was available from ergosterol by the procedures of Shepherd *et al.* (*8*). Selenium dioxide oxidation (*9*) of isoergosterone gave ergosta-2,4,6,22-tetraen-3-one, and treatment of the tetraenone with hydrogen peroxide in base (*10*) then yielded the crystalline (m.p., 143° to 145°C) 1α,2α-epoxide (1α,2α-epoxyergosta-4,6,22-trien-3-one) (*11*). Lithium reduction of the epoxide in a solution of ammonia and tetrahydrofuran (*5, 12*) gave 1α-hydroxy-7,8-dihydroergosterol (molecular weight, 412; m.p., 180° to 182°C). After acetylation to the corresponding diacetate (molecular weight, 486) and the usual allylic bromination and dehydrobromination sequence (*13*), 1α-hydroxyergosteryl diacetate (molecular weight, 484; λ_{max} 295, 283, 272 nm, where λ is the absorption wavelength) was obtained. Ultraviolet irradiation (*14*) of an ether solution of this diacetate furnished the previtamin derivative, which was isomerized to the vitamin skeleton and then hydrolyzed to 1α-OH-D_2 (**4**, Fig. 1). The new vitamin D_2 analog is characterized by its ultraviolet spectrum (λ_{max}, 265 nm; λ_{min}, 228 nm) and the mass spectra and nuclear magnetic resonance (NMR) spectra (Fig. 2).

For bioassay, male weanling rats (Holtzman) were fed a vitamin D–deficient low calcium diet for 3 weeks. Groups of five rats then received the appropriate dose of 1α-OH-D_2 (in 0.05 ml of ethanol) or were given ethanol alone (controls) by injection into the jugular vein. Intestinal calcium transport and bone mineral mobilization assays were performed (*15*).

The time course study (Fig. 3) illustrates the intestinal and bone activity of 1α-OH-D_2 in the vitamin D–deficient rat. Stimulation of intestinal calcium transport is noticeable within 3 hours after administration of the vitamin analog and reaches a maximum within about 12 hours. Stimulation of bone calcium mobilization, as measured by serum calcium concentration, follows a very similar pattern. The intestinal and bone response to 0.25 μg of 1α-OH-D_2 is essentially the same as that elicited by a similar dose of the corresponding vitamin D_3 analog, 1α-OH-D_3, both in terms of magnitude and duration over a 24-hour assay period (*4*). For example, a dose of 625 pmole (0.25 μg) of 1α-OH-D_3 gave an intestinal calcium transport ratio of 2.9 ± 0.2 (as compared to 1.5 ± 0.2 for controls) and serum calcium concentration

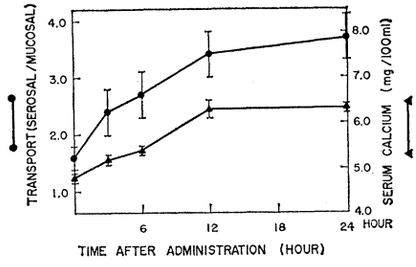


Fig. 3. Intestinal calcium transport (●—●) and bone calcium mobilization (▲--▲) response of vitamin D–deficient rats on a low calcium diet to a 0.25-μg dose of 1α-OH-D_2. Five animals were used for each measurement; the vertical bars represent the standard deviation from the mean.

of 6.7 ± 0.1 mg percent (as compared to 4.3 ± 0.1 for controls), 14 hours after administration (*4*).

Although we have as yet not tested 1α-OH-D_2 in anephric rats, it would appear almost certain that this analog, like 1α-OH-D_3, does not require a functional kidney (the site of 1α-hydroxylation of the vitamin) for activity. The antirachitic potency of 1α-OH-D_2 is three times that of vitamin D_2 in the stimulation of bone calcification.

The activity of 1α-OH-D_2 may depend on prior hydroxylation at C-25 to form the natural metabolite, 1,25-$(OH)_2$-D_2. The question regarding the functional significance of a hydroxyl at C-25 has not yet been resolved, but there is some evidence that an extended side chain (and, therefore, possibly a C-25 hydroxyl) is required for full activity. For example, the 1α-hydroxy analog possessing a two-carbon side chain (1α-hydroxypregnacalciferol) is devoid of all activity (*16*).

From a practical point of view, that is, ease of preparation or potential clinical application, analog **4** appears to offer no advantages over the previously prepared vitamin D_3 derivative,

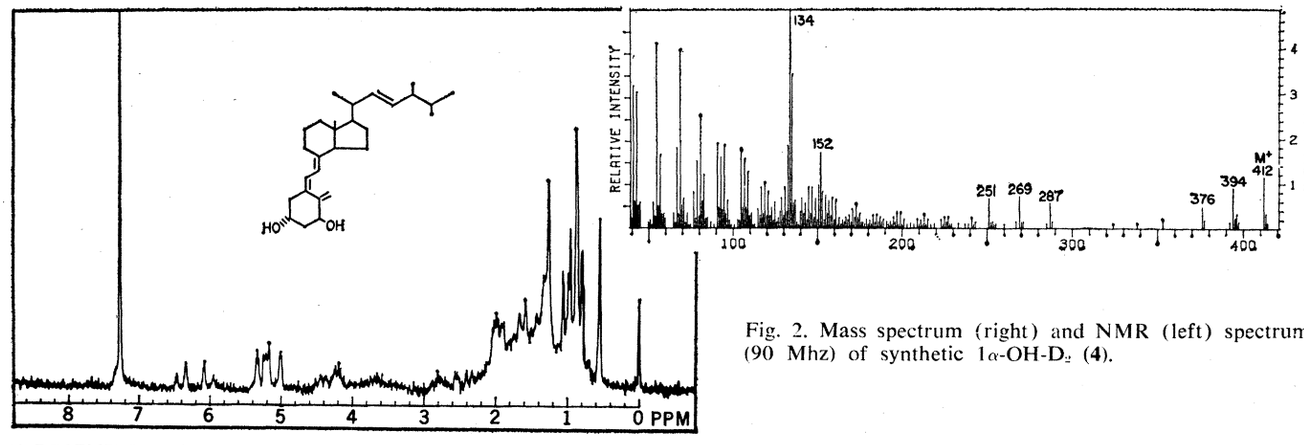


Fig. 2. Mass spectrum (right) and NMR (left) spectrum (90 Mhz) of synthetic 1α-OH-D_2 (**4**).

1α-OH-D_3. The new vitamin D_2 derivative should prove very useful, however, for detailed studies of the causes for the well-known, but poorly understood, discrimination of chicken and other bird species against vitamin D_2.

H.-Y. PETER LAM
H. K. SCHNOES, H. F. DELUCA
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison 53706

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Decrease in Free Cystine Content of Cultured Cystinotic Fibroblasts by Ascorbic Acid

Abstract. *The 100-fold increase in free cystine content characteristic of cultured skin fibroblasts from patients with nephropathic cystinosis was decreased more than 50 percent by addition of* L-*ascorbic acid to the culture medium at concentrations of 0.29 to 2.9 millimolar. Fresh ascorbic acid must be added to the culture medium daily to produce a progressive decrease of the free cystine content of the cells over a 3-day period. Upon removal of ascorbic acid from the medium, the free cystine content returns to its initial value.*

Nephropathic cystinosis is a metabolic disease that is inherited in an autosomal recessive manner and is characterized biochemically by a high intracellular content of free (nonprotein) cystine. As a result, cystine crystals deposit in the conjunctiva, bone marrow, lymph nodes, peripheral leukocytes, and many internal organs (*1*). Affected children have impairment of both renal tubular and glomerular functions, which leads to end-stage renal disease with uremia within the first decade of life.

The cystine is thought to accumulate in lysosomes (*2*), but the primary defect that leads to cystine storage remains unknown. This disease has recently been diagnosed in a pregnant woman, on the basis of an increased content of free cystine in cultured amniotic fluid cells (*3*). The pregnancy was terminated, and we studied the cystinotic fetal organs and found that most organs contained 50 to 100 times more free cystine than similar organs from normal controls (*3*). Only the adrenal gland of the cystinotic fetus had a free cystine content in the normal range. Since the fetal adrenal gland is very rich in ascorbic acid (*4*), we tested the effect of ascorbic acid on cultured skin fibroblasts from patients with nephropathic cystinosis. Such fibroblasts contain 100 times more free cystine than fibroblasts from controls (*5*).

When growth medium containing 0.57 m*M* L-ascorbic acid was added to cultured cystinotic skin fibroblasts every 24 hours, the cystine content declined steadily for 3 days (Fig. 1A), reaching less than 50 percent of the initial value. If the daily medium changes contained freshly prepared ascorbic acid, the cystine concentration of these cells remained at this low level. When ascorbic acid was omitted from the medium, the cystine content of the cells returned to the initial value within 3 days. In cultured fibroblasts from five patients with nephropathic cystinosis and one patient with the intermediate (late onset) type of cystinosis (*1*) this decline averaged 58.6 percent (range 48 to 78 percent) (Table 1). It was not possible to lower the free cystine content of cystinotic fibroblasts faster or to a greater extent by adding fresh ascorbic acid more frequently (every 12 hours) or by using higher concentrations. A similar decline in the free cystine content of these cells was observed after the addition of ascorbic acid in concentrations ranging from 0.29 to 2.9 m*M*; with 0.11 m*M*, however, the free cystine content declined only 25 percent.

Dithiothreitol [DTT, Cleland's reagent (*6*)], a powerful reducing agent, removes cystine from cystinotic fibroblasts, presumably by reducing cystine to cysteine (*7*). When DTT (1 m*M*) was added every 2 hours to cystinotic fibroblasts in a cystine-free medium, the intracellular cystine pool was depleted by more than 90 percent in 8 hours (Fig. 1B). If medium containing 133 μ*M* cystine was then added, the cells regained their initial intracellular free cystine content in 24 to 48 hours. In the presence of 0.57 m*M* ascorbic acid, the rate of reaccumulation decreased about 50 percent, and the eventual free cystine content of the cells was less than half that in the control cells (Fig. 1B).

The growth rate and morphological appearance by phase microscopy of both control and cystinotic fibroblasts were unchanged in the presence or absence of 0.57 m*M* ascorbic acid. This concentration of ascorbic acid caused no significant change in the *p*H of the growth medium (± 0.01 *p*H unit). Raising the concentration of ascorbic acid to 2.9 m*M* inhibited the growth of both control (*8*) and cystinotic fibroblasts. Of the intracellular amino acids other than cystine, only the concentrations of proline, hydroxyproline, lysine, and hydroxylysine were changed in 0.57 m*M* ascorbic acid (*9*). The protein content per cell was unaltered by concentrations of ascorbic acid ranging from 0.29 to 1.16 m*M*.

The mechanism by which ascorbic acid lowers the free cystine content of