UDC 547.92.07

9. **Eiji Ohki**: Steroid Studies. XV.[1] Synthesis of 4,4-Dimethylcholecalciferol and Its Conversion into 10-Isosteroid.

(*Institute of Applied Microbiology,* University of Tokyo*)

Cholecalciferol (vitamin D_3) (I) with antirachitic activity is the most active of known vitamin D's and is the only one of the D group to be isolated from animals (fish). Cholecalciferol is thought to be formed in the body by oxidation of cholesterol to 7-dehydrocholesterol (II) and synthesized from it by irradiation of ultraviolet rays.[2] In recent years, biosynthetic mechanism for the formation of cholesterol began to be clarified[3] and in 1957, 4,4-dimethylzymosterol (III) was discovered as the intermediate metabolite in the biosynthesis of cholesterol from lanosterol.[4,5] It seemed of biochemical significance, therefore, to prepare cholecalciferol possessing *gem*-dimethyl groups in 4,4-positions (IV) corresponding to (III) and to examine its vitamin D-like activity.[6]

The starting material to be used in this synthesis, 4,4-dimethyl-7-dehydrocholesterol (V) was prepared by the known method of Woodward, *et al.*,[7] or that of Bloch.[5]

A solution of (V) in ether containing 10% of benzene was irradiated with ultraviolet rays and the degree of its change was followed through ultraviolet spectra. When about 40% of (V) had undergone change, the reaction product was treated and the amorphous resinous matter so obtained was fractionally recrystallized as 2,4-dinitrobenzoate. 2,4-Dinitrobenzoate (VII) of dimethylcholecalciferol (IV) was isolated from this mixture and mild saponification of (VII) finally afforded the objective 4,4-dimethylcholecalciferol (IV), m.p. 71~73°, $[\alpha]_D$ +23.7°. (IV) showed ultraviolet spectrum similar to that of vitamin D's with absorption at 259 mμ and its infrared spectrum exhibited absorption at 896 cm^{-1} due to C-H out-of-plane deformation vibration of the vinylidene group. (IV) gave characteristic Liebermann-Burchard reaction and coloration to antimony trichloride. The yield was around 15% but this is better than the yield of ergocalciferol from ergosterol under the same conditions and purification is much easier.

A 2,4-dinitrobenzoate (VIII) of m.p. 122~126°, $[\alpha]_D$ +90°, was obtained as a by-product by fractional crystallization from the recrystallization mother liquor of (VII) but its saponification failed to produce any free alcohol as crystals. The ultraviolet spectrum and coloration to antimony trichloride of the free alcohol are quite similar to those of (IV). When (VIII) is allowed to stand over a long period of time or heated in benzene, thermal equilibrium is set up between (VIII) and (VII), and (VII) can be isolated by fractional recrystallization. It is assumed from these facts that (VIII) is a compound corresponding to the precalciferol type[8] known as the precursor of calciferol.

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* Mukoga-oka, Yayoi-cho, Bunkyo-ku, Tokyo (大木英二).

[1] Part XIV. K. Tsuda, E. Ohki, J. Suzuki: This Bulletin, **7**, 522(1959).

[2] cf. W.H. Sebrell, Jr., R.S. Harris: "The Vitamins — Chemistry, Physiology, Pathology," **II**, 132(1954). The Academic Press Inc., New York.

[3] cf. K. Bloch: Vitamins and Hormones, **15**, 119(1957).

[4] F. Gautschi, K. Bloch: J. Am. Chem. Soc., **79**, 684(1957); P.B. Schneider, R.B. Clayton, K. Bloch: J. Biol. Chem., **224**, 175(1957).

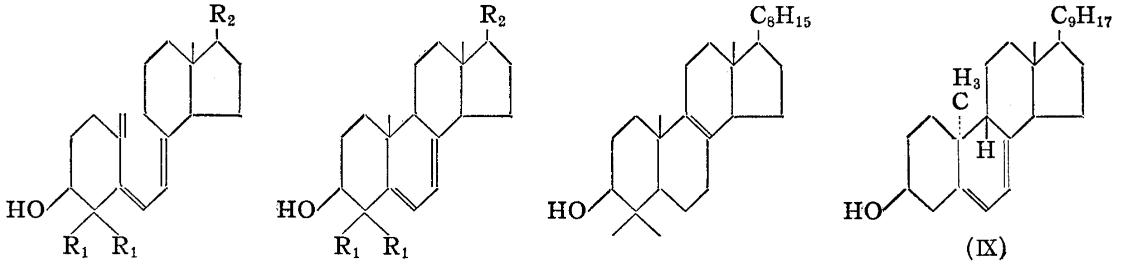
[5] F. Gautschi, K. Bloch: J. Biol. Chem., **233**, 1343(1958).

[6] Vitamin D_2 with ergosterol side-chain, 4,4-dimethylergocalciferol (VI), was synthesized in 1955 by G. Cooley, B. Ellis, and V. Petrow (J. Chem. Soc., **1955**, 2998) and these workers also reported of its antirachitic activity.

[7] R.B. Woodward, A.A. Patchett, D.H.R. Barton, D.A.J. Ives, R.B. Kelley: J. Am. Chem. Soc., **76**, 2852(1954); J. Chem. Soc., **1957**, 1131.

[8] L. Velluz, G. Amiard: Compt. rend., **228**, 692(1949). There are two theories as to this structure; one being that it is a *cis-trans* isomer of tachysterol (L. Velluz, G. Amiard, B. Goffinet: Compt. rend., **240**, 2156(1955)) and the other, that it is a *cis-trans* isomer of calciferol (H.H. Inhoffer, K. Brückner, R. Grundel, G. Quinkert: Ber., **87**, 1407(1954)).

The by-products of vitamin D synthesis reported to date include lumisterol[9] (IX), tachysterol, toxisterol, and suprasterol. For the purpose of preparing 10-isosteroid, to be reported in subsequent paper, photochemical reaction conditions for formation of lumisterol-type compounds (IX) from (X) were examined. The light in a shorter wave-length region was cut by the use of a solvent of ethanol-benzene mixture or ether, addition of xylene, salicylic acid, or phthalic acid, or by the use of a solution filter of potassium chromate, but this was found to slow down the reaction velocity and decrease the yield. Purification of the product as the 2,4-dinitrobenzoate proved the presence of (VII) and (VIII) alone. Therefore, it was believed that the *gem*-dimethyl group in the 4-position interfered in the cyclization of (V) into a lumisterol type by light.



(I) $R_1=H$ $R_2=C_8H_{17}$
(IV) $R_1=CH_3$ $R_2=C_8H_{17}$
(VI) $R_1=CH_3$ $R_2=C_9H_{17}$

(II) $R_1=H$ $R_2=C_8H_{17}$
(V) $R_1=CH_3$ $R_2=C_8H_{17}$

(III)

(IX)

Chart 1.

(IV) is labile to heat, same as calciferol, and heating of (IV) to 195~200° results in formation of an amorphous resin. Its purification by acetylation affords the acetate (X) of m.p. 71~73°, $[\alpha]_D$ +543°, in 60% yield. Saponification of (X) finally gives the free alcohol (XI), m.p. 72~74°, $[\alpha]_D$ +533°.

Ultraviolet absorption maxima of (X) and (XI) are present at 276 and 284.5 mμ, very similar to that of (V), and the compounds should possess homoannular diene-type chromophor. The abnormally large degree of optical rotation of (X) and (XI) indicates that they are compounds of the type of pyrocalciferol (XII) or isopyrocalciferol (XIII) formed by pyrolysis of ergocalciferol.[10]

Dehydrogenation of (X) with mercuric acetate affords a trienol (XIV), m.p. 131~133°, $[\alpha]_D$ −42.6°, with ultraviolet absorption maximum at 318.5 mμ.[11] On the other hand, dehydrogenation of the acetate (XV) of (V) affords a trienol (XVI) of m.p. 159~161°, $[\alpha]_D$ −72.5°, with ultraviolet absorption maximum at 323 mμ. (XIV) and (XVI) are not the same substance and it is assumed that they are steric isomers with different configuration relative to 10-position, being in a connection of dehydrolumisterol and dehydroergosterol.[12]

As for the hydroxyl at 3-position of (XI), result of pyrolysis reaction suggests 3β-configuration *a priori*. Oxidation of (XI) with chromium trioxide and pyridine or by the Oppenauer method affords a dienone (XVII), m.p. 89~91°, $[\alpha_D]$ +493°, and its reduction with lithium aluminum hydride or with sodium ethoxide gives a dienol (XVIII), m.p. 95~98°, $[\alpha]_D$ +606°, which is an epimer of (XI). Since the ultraviolet absoption of (XVIII) does

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9) cf. A. Windaus, K. Dithmar, F. Fernholz : Ann., **493**, 259(1932); A. Windaus, M. Peppe, W. Wunderlich : *Ibid.*, **533**, 118(1938).

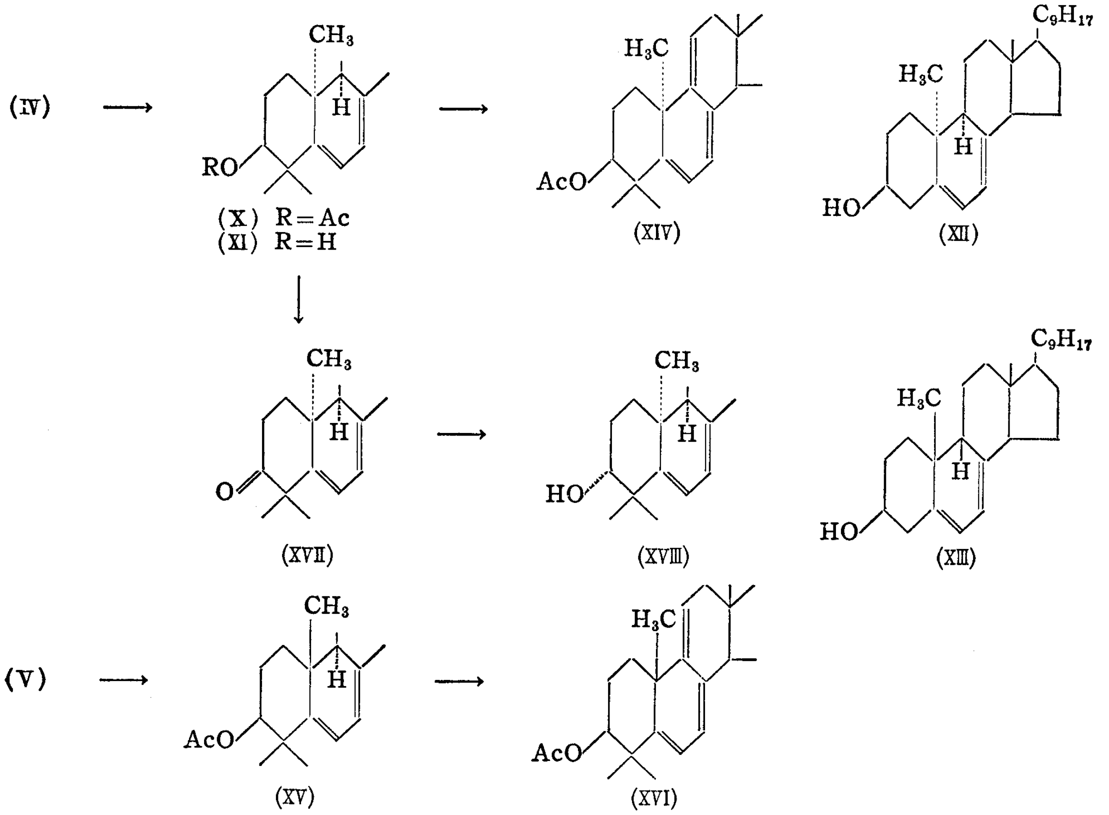
10) For conformation of (XII) and (XIII), see J. Castells, E. R. H. Jones, R. W. Williams : Proc. Chem. Soc., **1958**, 7.

11) The structure of (XIV) was assigned as formulated by the method of Windaus (cf. Footnote (12)) but 5,7,14-triene structure is also possible. cf. D. H. R. Barton, T. Bruun : J. Chem. Soc., **1951**, 2728.

12) A. Windaus, K. Dimroth : Ber., **70**, 376(1937).

not differ from that of (XI) which means that there is no chemical change in the diene system and (XVIII) possesses a hydroxyl of 3α-configuration.[13] Consequently, 3β-OH in (XI) should have an axial conformation and (XI) is considered to be a compound of the pyrocalciferol-type[10] (XII) with 10α-conformation.

It may be concluded from the foregoing facts that the predominance of 10α-isomer in the dienol obtained by pyrolytic cyclization of (IV) is due to the rigidly fixed indane skeleton (C/D ring juncture) of the steroid, as evidenced by molecular model, and in the bonding of B-ring to C-ring, a thermally more stable and more planer structure is formed from the pyrocalciferol type, utilizing equatorial bond at 9-position rather than from isopyrocalciferol type using the axial bond.[14]



Experimental

All melting points are uncorrected. Rotations were determined in $CHCl_3$ and ultraviolet spectra in ether unless otherwise specified. The infrared spectra of all compounds were recorded and are in agreement with the reported structures. Analytical samples were dried for 15 hr. at room temperature in high vacuum.

9,10-Seco-4,4-dimethylcholesta-5,7,10(19)-trien-3β-ol (**4,4-Dimethylcholecalciferol**) (**IV**)—A solution of 7.5 g. of 4,4-dimethylcholesta-5,7-dien-3β-ol (V), m.p. 138~140°, $[\alpha]_D^{10}$ −159.9°,[4] in 300 cc. of ether-benzene (10 : 1) was irradiated for 4 hr. with a Hg vapor lamp. The solution was evaporated *in vacuo* to leave a gum (8.2 g.) which was digested with EtOH (30 cc.). 2.8 g. of the unchanged material that precipitated was removed by filtration. Complete removal of the solvent gave a yellow resin (5.3 g.) which was treated in pyridine (30 cc.) at 0° with 5 g. of 2,4-dinitrobenzoyl chloride, stirring at room temperature for 2 hr. After dilution of this reaction mixture with water and extraction with ether, the extract was washed with dil. HCl, dil. $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated in vacuum to leave a crystalline mass. Recrystallization from acetone gave 2,4-dinitro-

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[13] cf. P. Busse : Z. physiol. Chem., **214**, 211(1933); **233**, 224(1935).

[14] cf. M. C. Dauson, T. G. Halsall, E. R. H. Jones, G. D. Meakins, P. C. Phillips : J. Chem. Soc., **1956**, 3172.

benzoate of the recovered starting material (V) (0.6 g.) as needles, m.p. 200～202°, $[\alpha]^{10}_{D}$ −14.9° (c=2.1). *Anal.* Calcd. for $C_{36}H_{50}O_6N_2$: C, 71.25; H, 8.31; N, 4.62. Found : C, 70.93; H, 8.33; N, 4.73.

After filtration of 2,4-dinitrobenzoate of (V), the filtrate was evaporated *in vacuo* and the crystalline residue was chromatographed on alumina (100 g.). The fractions eluted with benzine-benzene (4 : 1, 1 : 1) were recrystallized from EtOH-acetone, yielding 2,4-dinitrobenzoate of 4,4-dimethylcholecalciferol (VII) (2.1 g.) as pale yellow needles, m.p. 144～146°, $[\alpha]^{10}_{D}$ +115.5° (c=2.2), $[\alpha]^{10}_{D}$ +91.1° (c=2.1, benzene). *Anal.* Calcd. for $C_{36}H_{50}O_6N_2$: C, 71.25; H, 8.31; N, 4.62. Found : C, 71.42; H, 8.40; N, 4.52. The UV spectrum showed an absorption from 280 to 220 mμ, increasing steadily in intensity.

The foregoing compound (VII) (1.5 g) in benzene (3 cc.) was treated with 2.5 cc. of methanolic NaOH solution (prepared from 0.7 g. of Na and 23 cc. of MeOH, diluted with 1.5 cc. of water). The mixture was stirred for 30 min. at room temperature, then diluted with ether, and filtered. The filtrate was washed with water, dried, and the solvent removed. The residue was treated with charcoal in acetone and recrystallized from MeOH, yielding 0.92 g. of 4,4-dimethylcholecalciferol (IV) as prisms, m.p. 71～73°, $[\alpha]^{10}_{D}$ +23.7° (c=1), +23.3° (c=1.3). *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found : C, 84.23; H, 11.60. UV λ_{max} 259 mμ (ε 23300).

Coloration of (IV) turned gradually from yellow, green to greenish blue by Liebermann-Burchard test and from yellow to dark brown by $SbCl_3$ test on paper after heating at 110° for 5 min.

By-product (VIII) of Irradiation of (V)—The mother liquor of recrystallization of (VII) was concentrated and upon cooling, provided a crystalline mass which, after several wasteful recrystallizations, gave (VIII) as granular prisms, m.p. 122～126°; $[\alpha]^{15}_{D}$ +90.9° (c=2.9), $[\alpha]^{15}_{D}$ +73.9° (c=2.4, benzene). *Anal.* Calcd. for $C_{36}H_{50}O_6N_2$: C, 71.25; H, 8.31; N, 4.62. Found : C, 71.16; H, 8.29; N, 4.62. The ultraviolet spectrum showed the same absorption as that of (VII).

Saponification of (VIII) in the manner as described for (IV) afforded a free alcohol, $[\alpha]^{14}_{D}$ +21.1° (c=3.7); UV λ_{max} 259 mμ (ε 16000), but its crystallization was unsuccessful.

A solution of 0.5 g. of (VIII) in benzene (30 cc.) was boiled for 20 hr. in N_2 atmosphere and then evaporated *in vacuo* to leave a crystalline mass which after several recrystallization gave (VII), m.p. 144～146°, $[\alpha]^{14}_{D}$ +93.5° (c=1.4, benzene). Yield, 0.22 g.

4,4-Dimethyl-10-isocholesta-5,7-dien-3β-ol (XI) and its Acetate (X)—1.89 g. of (IV) in a sealed tube, filled with N_2, was heated for 7 hr. in an oil bath at 195～200°. The oily product was treated with pyridine-Ac_2O at room temperature. The acetate so obtained was digested with 1～2 cc. of EtOH and allowed to stand in a refrigerator, from which crystals of (X) were obtained and crystallized from EtOH to plates, m.p. 71～73°, $[\alpha]^{16}_{D}$ +543.7° (c=1.7). Yield, 1.197 g. *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found : C, 81.82; H, 10.96. UV λ_{max} mμ (ε) : 276 (11000), 284.5 (10800), 297 (infl.).

The free alcohol (XI) was obtained by saponification of (X) with 5% KOH-EtOH and recrystallization from MeOH containing a few drops of water to prisms, m.p. 72～74°, $[\alpha]^{10}_{D}$ +533.3° (c=0.6). *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found : C, 84.65; H, 11.74. UV λ_{max} mμ (ε) : 276 (10000), 285 (10000), 297 (infl.).

4,4-Dimethylcholesta-5,7,9(11)-trien-3β-ol and its Acetate (XVI)—To a solution of 0.4 g. of (XV), m.p. 142～145°, $[\alpha]^{15}_{D}$ −100.7° (c=3.7),7) in 5 cc. of $CHCl_3$, 0.92 g. of $(AcO)_2Hg$ dissolved in 20 cc. of AcOH was added and the mixture was stirred at room temperature over night. The precipitate of AcOHg was filtered and washed consecutively with water, MeOH, and ether. The combined filtrate and washing was washed with 5% H_2SO_4, dil. $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated to leave a semi-solid residue which was recrystallized several times from acetone to 0.12 g. of (XVI) as plates, m.p. 159～160°, $[\alpha]^{10}_{D}$ −72.5° (c=3.1). *Anal.* Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 81.72; H, 10.74. UV λ_{max} mμ (ε) : 310 (9250), 323.8 (11200), 339 (7500).

The free alcohol of (XVI) was obtained by saponification of (XVI) with 5% MeOH-KOH and recrystallization from MeOH; m.p. 128～130°, $[\alpha]^{10}_{D}$ −156.5° (c=1.7). *Anal.* Calcd. for $C_{29}H_{46}O \cdot \frac{1}{4}H_2O$: C, 83.89; H, 11.29. Found : C, 83.91; H, 11.31. UV λ_{max} mμ (ε) : 311 (8200), 323 (9900), 339 (6600).

4,4-Dimethyl-10-isocholesta-5,7,9(11)-trien-3β-ol Acetate (XIV)—To a solution of 0.2 g. of (X) in 2.5 cc. of $CHCl_3$, 0.46 g. of $(AcO)_2Hg$ dissolved in 10 cc. of AcOH was added and the mixture was stirred at room temperature for 1 week. After treatment as described above, an oily material obtained was digested with a few drops of MeOH and allowed to stand in a refrigerator. The crude crystals so obtained were recrystallized from MeOH, yielding 0.088 g. of (XIV) as prisms, m.p. 131～133°, $[\alpha]^{7}_{D}$ −42.6° (c=1.3). *Anal.* Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found : C, 81.60; H, 10.63. UV λ_{max} 318.5 mμ (ε 15900).

The free alcohol was obtained by saponification of (XIV) with 5% KOH-EtOH but its crystallization was not effected.

4,4-Dimethyl-10-isocholesta-5,7-dien-3-one (XVII)—(i) About 10 cc. was distilled from a solution of 0.242 g. of (XI) dissolved in 40 cc. of toluene and 6 cc. of cyclohexanone, in order to remove moisture. 0.3 g. of Al(iso-PrO)$_3$ dissolved in 5 cc. of toluene was then added to the refluxing solution and refluxing was continued for 4 hr. After volatile component was removed by steam distillation, the residue was extracted with ether and the extract was washed with water, dried over Na_2SO_4, and evaporated.

The amorphous residue was chromatographed on alumina (8 g.). Recrystallization of the fractions eluted with hexane and hexane-benzene (10 : 1) afforded 0.158 g. of (XVII) as prisms, m.p. 89\~91°, $[\alpha]_D^{11}$ +493.2° (c=0.6). *Anal.* Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found : C, 84.98; H, 11.21. UV λ_{max} mμ (ε) : 276.5 (9500), 285 (9500), 298 (infl.).

(ii) To a stirred solution of 0.5 g. of CrO_3 in 30 cc. of pyridine, 0.25 g. of (XI) dissolved in 10 cc. of pyridine was added at 0° and allowed to stand at room temperature over night. After dilution with ether and filtration of precipitate, the filtrate was diluted with water, and extracted with ether. The extract was washed with dil. HCl, dil. $NaHCO_3$, and water, then dried over Na_2SO_4, and evaporated *in vacuo* to leave a crystalline mass of (XVII) which was recrystallized from MeOH. Yield, 0.12 g.

4,4-Dimethyl-10-isocholesta-5,7-dien-3α-ol (XVIII) and its Acetate—(i) A mixture of 100 cc. of ether containing 0.82 g. of (XVII) and 0.5 g. of $LiAlH_4$ was allowed to reflux for 2 hr., after which excess of the hydride was decomposed with a few drops of water. Following addition of dil. HCl (20 cc.), the mixture was extracted with ether. The extract was washed with water, dried over Na_2SO_4, and evaporated to afford crystals of (XVIII). This was recrystallized from MeOH to granular prisms, m.p. 95\~98°, $[\alpha]_D^{10}$ +606.0° (c=0.8). *Anal.* Calcd. for $C_{29}H_{48}O \cdot \frac{1}{4}H_2O$: C, 83.48; H, 11.71. Found : C, 83.34; H, 11.64. UV λ_{max} mμ (ε) : 276.7 (12000), 286.4 (11000), 297 (infl.).

The acetate of (XVIII) was obtained in the usual manner by acetylation with pyridine-Ac_2O and recrystallization from MeOH-EtOH, as prisms, m.p. 97\~98°. *Anal.* Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found : C, 82.24; H, 11.05.

(ii) To a solution containing 0.3 g. of (XVII) in 50 cc. of dehyd. EtOH, 2 g. of Na was added in small portions under vigorous boiling. After all the Na had completely dissolved, the reaction mixture was diluted with water and extracted with ether. The extract was washed with water, dried over Na_2SO_4, and evaporated to leave a crystalline mass of (XVIII), which was recrystallized from MeOH.

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Summary

In order to examine the vitamin D activity, 4,4-dimethylcholecalciferol (IV), m.p. 71\~73°, $[\alpha]_D$ +23.7°, was prepared by irradiation of ultraviolet rays on 4,4-dimethyl-7-dehydrocholesterol (V). Pyrolysis reaction of (IV) was found to result in preferential cyclization to pyrocalciferol-type compound and 4,4-dimethyl-10-isocholesta-5,7-dien-3β-ol (XI), m.p. 71\~73°, $[\alpha]_D$ +543°, was obtained in a good yield.

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