[CONTRIBUTION FROM THE GEORGE HERBERT JONES CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO]

# PROVITAMIN D ACTIVITY AND STRUCTURE—ADDITION OF GRIGNARD REAGENTS TO 7-KETOCHOLESTERYL ACETATE*

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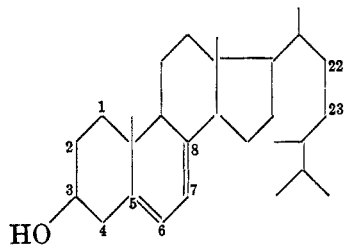
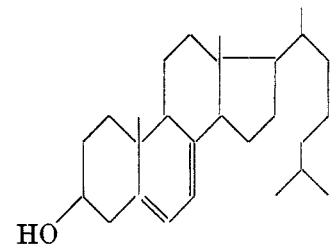
*Received November 23, 1936*

In recent years considerable chemical and biological evidence has been accumulated to show that antirachitic provitamin activity is not restricted to ergosterol, but is exhibited by several structurally related compounds, notably 22-dihydroergosterol (I)[1] and 7-dehydrocholesterol (II).[2]




I




II

The high activatability of these substances indicates that provitamin activity is associated with the pair of conjugated double bonds at $C_{5-6}$ and $C_{7-8}$, and is probably independent of minor changes in the structure of the side-chain.

During the past thirteen months we have investigated the preparation of 7-dehydrocholesterol and its substitution products from cholesterol. As the first step in the preparation of derivatives of this type we have studied the products of the addition of various Grignard reagents to 7-ketocholesteryl acetate. Although the work is still incomplete, the recent publication of Bann, Heilbron, and Spring on 7-methylenecholesterol[3] suggests that duplication of effort may be avoided by disclosure of our observations to date.

* The authors are indebted to Eli Lilly and Co. for a generous grant which made this work possible, and to Mr. C. R. Eckler of the Lilly Research Laboratory for the biological assays reported in this paper.

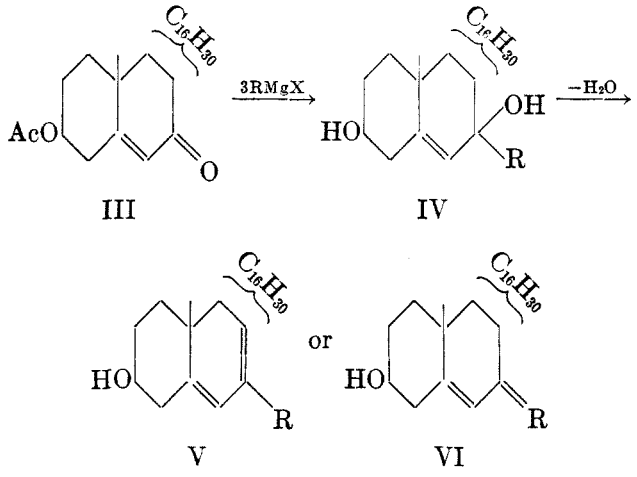
[1] WINDAUS AND LANGER, *Ann.*, **508**, 105 (1933).

[2] WINDAUS, LETTRÉ, AND SCHENCK, *ibid.*, **520**, 98 (1935).

[3] BANN, HEILBRON, AND SPRING, *J. Chem. Soc.*, **1936**, 1274.

## THE ACTION OF GRIGNARD REAGENTS ON 7-KETOCHOLESTERYL ACETATE

The addition of three moles of a Grignard reagent to 7-ketocholesteryl acetate (III) may give rise to the following products.




The tertiary alcohol (IV) should, on partial dehydration, give rise to one or both of two isomeric dienols (V, VI). The product of annular dehydration (V) should exhibit intense absorption of light between 2600 and 3000 Å, and should be susceptible to isomerization by ultra-violet light.

Our study included the reaction of 7-ketocholesteryl acetate with the following Grignard reagents: methylmagnesium iodide, and ethyl-, isobutyl-, phenyl-, allyl-, cyclopentyl-, cyclohexyl-, and *tert.*-butylmagnesium bromides. Only the first four of these reactions have been studied exhaustively; the study of the others is still in progress. The interaction of 7-ketocholesteryl acetate and methylmagnesium iodide yielded both 7-hydroxy-7-methylcholesterol and 7-methylenecholesterol, but the latter was the sole product if the Grignard addition compound was refluxed for a long time or if it was hydrolyzed with dilute hydrocholoric acid. The only isolable product of the reaction between the ketone and ethylmagnesium bromide was 7-ethylidenecholesterol. Reaction of the ketone with isobutylmagnesium bromide yielded an uncrystallizable oil, from which 7-isobutylidenecholesteryl benzoate was isolated on esterification. Saponification of the benzoate yielded 7-isobutylidenecholesterol. The sole product obtainable from the reaction of the ketone with phenylmagnesium bromide was 7-hydroxy-7-phenylcholesterol. Attempts to dehydrate the latter have been unsuccessful up to the present time.

## ABSORPTION SPECTRA

The absorption spectra (Fig. 1) of 7-alkylidene derivatives of cholesterol show a single intense band at 2400 Å, as a consequence of the conjugation at $C_5$ and $C_7$. The striking difference from the spectra of ergosterol derivatives, and the similarity to the typical absorption curves of $\alpha$, $\beta$ unsaturated ketones is worthy of note. The lack of absorption exhibited

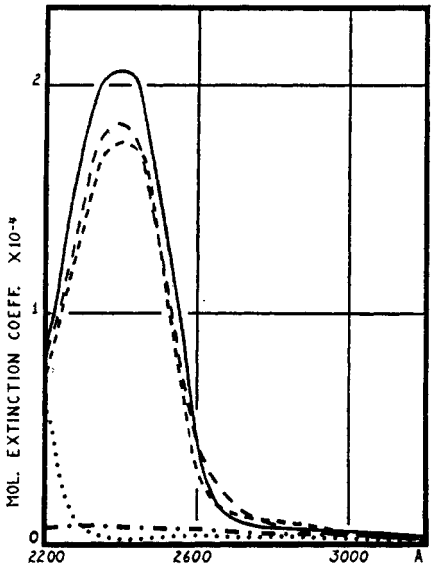



FIG. 1

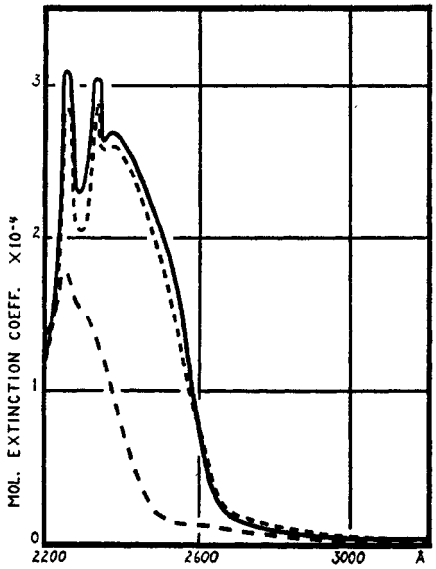



FIG. 2

FIG. 1.—ABSORPTION SPECTRA
———— 7-methylenecholesterol
— — — — 7-isobutylidenecholesterol
---------- 7-ethylidenecholesterol
—·—·—·— 7-hydroxy-7-methylcholesterol
..... 7-hydroxy-7-phenylcholesterol

FIG. 2.—ABSORPTION SPECTRA
———— 7-ethylidenecholesteryl benzoate
---------- 7-isobutylidenecholesteryl benzoate
— — — — 7-hydroxy-7-phenylcholesteryl benzoate

by 7-hydroxy-7-phenyl-, and 7-hydroxy-7-methylcholesterol is not unexpected. The absorption curves of some of the benzoates are shown in Fig. 2.

## POSITION OF THE SECOND DOUBLE BOND

The position of the double bond in the side-chain of the 7-alkylidene derivatives was determined as follows: 7-ethylidenecholesteryl acetate

was oxidized by means of chromic acid in glacial acetic acid. The product was identified as 7-ketocholesteryl acetate by its melting-point and by the melting-point of a mixture with an authenticated specimen. The similarity in the absorption spectra of the three alkylidene derivatives was interpreted as sufficient evidence for their identity with respect to the position of the second double bond.

## ANTIRACHITIC ACTIVATABILITY

The 7-alkylidene derivatives, irradiated with ultra-violet light, were inactive on rats in daily doses of 0.1 milligram. We have, however, succeeded in obtaining a substance of low, but unquestionable antirachitic provitamin activity from the crude product of the reaction between 7-ketocholesteryl acetate and isobutylmagnesium bromide. Heating to 200°, or slow distillation at low pressure, of the crude reaction product yielded an oil which, when irradiated, was active on rats in daily doses of 0.01 milligram. None of the crude products arising from the other Grignard reactions could be thus activated. One might seek an explanation of the activatability of this crude product in the possibility that in the course of the Grignard reaction some direct reduction of the ketone may have taken place. This side-reaction would lead to the formation of 7-hydroxycholesterol, which on partial dehydration would yield the highly activatable 7-dehydrocholesterol. However, the distillation, at low pressure, of 7-hydroxycholesterol, prepared by the method of Windaus, Lettré, and Schenck,[2] yielded an oil which was completely inactive after irradiation.

Another tentative hypothesis to explain the unusual behavior of the isobutyl derivative is now under experimental investigation in this laboratory, as is the active principle in this preparation. We hope to publish the results of our findings shortly.

## EXPERIMENTAL

*Grignard reaction.*—The following general procedure was adopted. 7-Ketocholesteryl acetate (6.60 g.—0.015 mole), dissolved in 30 cc. of anhydrous benzene, was dropped slowly into the solution of the Grignard reagent prepared in the usual manner from 0.1 mole of the halide. After addition, the solution was refluxed for from four to six hours, and then decomposed by means of iced ammonium sulfate solution. The reaction product was extracted with ether, the ether solution dried with anhydrous sodium sulfate, and the solvent removed.

*7-Hydroxy-7-methylcholesterol.*—The oily Grignard reaction product was dissolved in 25 cc. of hot methanol, the solution filtered, and allowed to cool slowly. A yield of 2.25 g. of 7-hydroxy-7-methylcholesterol was obtained; prisms from methanol, containing solvent; needles from benzene; m. p. 164–165°.

*Anal.* Calc'd for $C_{28}H_{48}O_2$: C, 80.8; H, 11.52.
Found: C, 81.0; H, 11.49.

*7-Methylenecholesterol.*—Concentration and cooling of the filtrate of the above yielded 1.10 g. of 7-methylenecholesterol; further concentration of the mother-liquor yielded an addition quantity of 0.95 g. The product crystallizes from methanol in the form of tufts of needles, containing solvent, which could not be removed by drying at 40° under extremely low pressure; m. p. 81–82°.

*7-Hydroxy-7-methylcholesteryl monobenzoate.*—The diol (0.5 g.) was dissolved in 2 cc. of pyridine, and 1.0 g. of benzoyl chloride added. The mixture was allowed to stand for 24 hours. The gummy reaction product was washed several times with water, then digested with boiling methanol, and the insoluble residue dissolved in hot acetone. The benzoate was obtained in needles; m. p. 172–173°.

*Anal.* Calc'd for $C_{35}H_{52}O_3$: C, 80.7; H, 10.00.
Found: C, 80.6; H, 10.11.

*7-Methylenecholesteryl benzoate.*—Treatment of the alcohol with pyridine and benzoyl chloride yielded the benzoate; slender prisms from acetone; m. p. 139–140°.

*Anal.* Calc'd for $C_{35}H_{50}O_2$: C, 83.7; H, 9.96.
Found: 83.6; H, 9.79.

*7-Ethylidenecholesterol.*—The Grignard reaction product was dissolved in hot methanol, filtered, and cooled. No crystalline product could be isolated, but the lack of absorption exhibited by the crude material indicated that it probably consisted almost entirely of the diol. A few drops of hydrochloric acid was added to the methanol solution, and the latter was refluxed for one hour. Upon cooling of the solution, 7-ethylidenecholesterol separated in large, flat, elongated prisms; m. p. 66–68°.

*Anal.* Calc'd for $C_{29}H_{48}O$: C, 84.5; H, 11.67.
Found: C, 84.3; H, 11.45.

*7-Ethylidenecholesteryl benzoate.*—Treatment with benzoyl chloride in pyridine in the usual manner yielded the benzoate; needles from acetone; m. p. 109–110°.

*Anal.* Calc'd for $C_{36}H_{52}O_2$: C, 83.7; H, 9.92.
Found: C, 83.6; H, 9.71.

*7-Ethylidenecholesteryl acetate.*—7-Ethylidenecholesterol (1 g.) was refluxed for one hour with 5 cc. of acetic anhydride, and the reaction mixture poured into cold water. The solid which precipitated was filtered off and crystallized from hot acetone; glistening plates, m. p. 110–111°.

*Anal.* Calc'd for $C_{31}H_{50}O_2$: C, 81.9; H, 11.01.
Found: C, 81.7; H, 10.95.

*7-Isobutylidenecholesteryl benzoate.*—The Grignard reaction yielded a colorless gum which could not be crystallized. It was dissolved in 10 cc. of pyridine, and 5 g. of benzoyl chloride was added in small portions with cooling. After standing at room temperature for 48 hours, the product was washed several times with water, then digested with boiling methanol, and the residue dissolved in hot acetone. On cooling, 7-isobutylidenecholesteryl benzoate was obtained; needles from acetone; m. p. 164–165°.

*Anal.* Calc'd for $C_{35}H_{56}O_2$: C, 83.8; H, 10.37.
Found: C, 83.8; H, 10.36.

*7-Isobutylidenecholesterol.*—Three hundred fifty milligrams of the above benzoate was refluxed with 8 cc. of 0.5 *N* alcoholic potassium hydroxide for 15 minutes. The solution was then cooled, water was added, and the precipitated white solid was filtered off and dissolved in a small quantity of methanol. On cooling, 7-isobutyli-

denecholesterol came down in flat prisms (m. p. 120–121°), containing solvent, which could not be removed by long drying *in vacuo*.

*7-Hydroxy-7-phenylcholesterol.*—The crude reaction product was dissolved in hot benzene and filtered. The product was then precipitated by means of petroleum ether, filtered off, and recrystallized from a mixture of benzene and petroleum ether. 7-Hydroxy-7-phenylcholesterol came down in the form of extremely fine needles; m. p. 151–152°.

*Anal.* Calc'd for $C_{33}H_{50}O_2$: C, 82.8; H, 10.46.
Found: C, 82.6; H, 10.48.

*7-Hydroxy-7-phenylcholesteryl monobenzoate.*—The usual reaction with benzoyl chloride in pyridine yielded the benzoate, needles from acetone; m. p. 201–202°.

*Anal.* Calc'd for $C_{40}H_{54}O_3$: C, 82.4; H, 9.27.
Found: C, 82.3; H, 9.20.

*Oxidation of 7-ethylidenecholesteryl acetate.*—The acetate (0.5 g.) was dissolved in 50 cc. of glacial acetic acid; 0.4 g. of chromic acid, dissolved in a mixture of 5 cc. of water and 5 cc. of acetic acid, was added in small portions, while the solution was maintained at a temperature of between 40 and 50°. A few cubic centimeters of alcohol was added after the solution had stood for two hours, and the solvent was evaporated *in vacuo*. The crystalline residue was dissolved in hot dilute acetic acid. The product which came down on cooling melted at 154–156°, and showed no depression of melting point when mixed with an authenticated specimen of 7-ketocholesteryl acetate; m. p. 157°.

## SUMMARY

The reaction of 7-ketocholesteryl acetate with various Grignard reagents has been studied.

Of the two isomeric dienols which might result from the dehydration of the tertiary alcohol formed in the Grignard reaction, in each case the only substance obtained was the product of side-chain dehydration. None of the 7-alkyl-7-dehydro derivatives were found.

A substance of antirachitic provitamin activity was obtained by heating the crude product of the reaction between 7-ketocholesteryl acetate and isobutylmagnesium bromide.