The probable explanation of the differences of opinion expressed in the literature is that suggested by Möhlau and Koch: that the products reported have been mixtures of the products of condensation in different proportions. In this case derivatives would exhibit different properties according as the reaction with vinylresorcinol (V) or that with ethylidenediresorcinol (III) (dihydroxymethylxanthene (IV)) had predominated.

Summary

1. The primary product of the catalytic condensation of acetylene and resorcinol has been secured in crystalline form and identified as vinylresorcinol (V).

2. The dimethyl ether of vinylresorcinol (VII) has been shown to give the dimethyl ether of β-resorcylic acid on oxidation.

3. Reduction of vinylresorcinol has been effected, the product being ethylresorcinol (VIII).

4. The primary product of the condensation of aldehyde and resorcinol has been shown to be vinylresorcinol (V).

5. The work of previous investigators who showed the presence of dihydroxymethylxanthene (IV) in the products has been corroborated.

6. No evidence whatsoever in support of Causse's acetal formula (I) has been found, but a number of facts which argue against it have been observed.

NOTRE DAME, INDIANA

[CONTRIBUTION FROM THE LABORATORIES OF PHYSICAL CHEMISTRY AND AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE QUANTITATIVE STUDY OF THE PHOTOCHEMICAL ACTIVATION OF STEROLS IN THE CURE OF RICKETS. II

BY STANISLAW-KAZIMIERZ KON, FARRINGTON DANIELS AND HARRY STEENBOCK

RECEIVED JULY 18, 1928 PUBLISHED SEPTEMBER 5, 1928

In an earlier paper from these Laboratories,[1] there were described experiments in which cholesterol was exposed to monochromatic light to determine the minimum number of quanta necessary to secure its antirachitic activation. This work was confined to the use of the 265 m. μ line of the mercury arc. It was followed by an extensive research by Dr. Waldemar Vanselow in which all the important lines of the quartz mercury lamp were studied under a wide variety of conditions.[2] As recent researches from several laboratories have indicated that it is ergosterol rather than cholesterol itself which produces the vitamin D upon irradiation, it seemed desirable to repeat and amplify the experiments, using ergosterol instead of cholesterol.

[1] Fosbinder, Daniels and Steenbock, THIS JOURNAL, **50**, 923 (1928).

[2] Unpublished data.

Fortunately, in the meantime important improvements have been made in our photochemical technique, so that it is now possible to measure directly the optical absorption as well as the curative effect on animals. In the earlier work an attempt was made to determine the percentage absorption of ultraviolet light by means of a spectrophotometer but the results were too unsatisfactory to publish. By the new technique the percentage absorption was measured directly with a thermopile and galvanometer.

Ergosterol as the Pro-Vitamin

Nearly all of the experimental evidence now available points to the conclusion that ergosterol is the mother substance of vitamin D. Windaus working in collaboration with Rosenheim,[3] Hess[4] and Pohl[5] found that ergosterol can be rendered active in much smaller amounts than cholesterol and that it shows exactly the same absorption bands as those considered to be characteristic of the activatable contaminant of ordinary cholesterol. It was found also by these investigators that cholesterol can be freed from the contaminant by various chemical treatments such as bromination and subsequent reduction of the dibromide, boiling with animal charcoal, treatment with permanganate in acetone and over-irradiation. These results have been commonly interpreted as showing that ergosterol is the mother substance of vitamin D and that it is at present the only substance known to yield an antirachitic substance on irradiation.

A different point of view is held, however, by Bills, Honeywell and MacNair[6] and by Jendrassik and Kenényffi.[7] The former observers reported that highly purified cholesterol is still activatable and concluded that the residual activatability was due to something other than ergosterol. The latter assume an equilibrium between pure cholesterol and pro-vitamin D. Bills, Honeywell and MacNair used cholesterol twice purified by bromination in the laboratory of Windaus as well as cholesterol brominated three times in their laboratory. Cholesterol purified by boiling for three periods of eight hours each with Merck's blood charcoal was used also. All of these preparations after irradiation in the solid state for fifteen minutes gave positive results in feeding tests, but a level of 0.3% of the rachitogenic diet was necessary rather than the 0.01% level used in the case of the ordinary cholesterol. Spectroscopic examination showed that a concentrated solution of the purified cholesterol exhibited three absorption bands identified with ergosterol and two additional ones at 315 and 304 mμ. The ergosterol bands were 150 times weaker after purification but the curative effect was only 30 times weaker.

[3] Rosenheim and Webster, *Biochem. J.*, **21**, 389 (1927).

[4] Windaus and Hess, *Nachr. Ges. Wiss. Göttingen Math.-Phys. Klasse*, **175** (1926).

[5] Pohl, *ibid.*, 185 (1926).

[6] Bills, Honeywell and MacNair, *J. Biol. Chem.*, **76**, 251 (1928).

[7] Jendrassik and Kenényffi, *Biochem. Z.*, **189**, 180 (1927).

This suggestion that ergosterol is not the only pro-vitamin was so important that it was necessary to put the matter to independent test before continuing with the quantum efficiency tests of the next section on ergosterol. The experiments of Bills and collaborators were repeated using twice brominated cholesterol and cholesterol purified with "Norite" charcoal and with Merck's animal charcoal. Both curative tests and preventive tests were used with several different groups of rats, and although complete cures were not obtained (save in the case of cholesterol purified with Norite of which even 0.06% proved to be active), still there was always a marked lessening of the rachitic condition, confirming the findings of Bills, Honeywell and MacNair.

The results are capable of interpretation in a different way, however. The ergosterol lines were still present in the purified cholesterol of these investigators and the difference between the absorption measurements and the rat tests (150 versus 30) might have been due to the presence of new decomposition compounds absorbing generally in the short ultraviolet, so that the results could still be explained on the assumption that the ergosterol had not been completely removed in the purification. In a recent letter to *Nature*, Heilbron, Morton and Sexton[8] venture the opinion that the new bands of absorption at 315 and 304 present in the specially purified cholesterol of Bills and co-workers are due to cholesterilene formed in the course of oxidation. We would completely share their opinion inasmuch as a spectrogram taken through 20 cm. of a 10% solution of our inactive cholesterol showed such a heavy absorption below 290 mμ that the presence of various oxidation products absorbing ultraviolet light, but not activatable, cannot be questioned. It must be admitted, as Bills and co-workers point out, that the stability of ergosterol is quite remarkable in the presence of cholesterol and it would be extremely interesting to find the cause of this phenomenon. It is possible that the formation of mixed crystals is effective in this respect.

It was decided to attempt a more drastic purification of cholesterol. Twenty-five grams of commercial cholesterol was dissolved in 437 cc. of boiling acetone and after the addition of 1.25 g. of powdered potassium permanganate, the solution was refluxed for one hour. The manganese dioxide was then filtered off, 1.25 g. of fresh potassium permanganate was added and the solution was refluxed for one hour again and this treatment was repeated five times. The last filtrate was diluted cautiously with water and crystallized at 0°. The crystals were carefully washed with alcohol, taken up in boiling alcohol, filtered through hardened filter paper, the solution was brought to boiling, diluted with water and crystallized; 11.5 g. of snow-white crystals of cholesterol was obtained with a melting point of 147° (uncorr.).

[8] Heilbron, Morton and Sexton, *Nature*, **121**, 452 (1928).

This material was irradiated, fed and tested as in the preceding experiments, but it was completely inactive, giving no cure and the degree of protection was so small as to be within the limit of experimental error. In the light of these experiments it seems evident that the activatable impurity left in cholesterol after bromination and after boiling with charcoal is ergosterol.

The interesting formation of antirachitic cholesterol derivatives under the action of Floridin[9] described by Bills[10] and MacDonald[11] was also studied. These preparations were made according to the method of Bills and studied in curative and protective experiments. In the protective experiment the animals were evidently stunted. There was almost no growth, though the food intake was sufficient to allow growth at a moderate rate. The development of their bones and especially of the femora and humeri seemed to be out of any proportion to the body weight—in fact the bones would be almost normal for rats of the same age, but twice the weight. The amount of ash was perfectly normal and there was no histological evidence of rickets. The results of the curative test were striking, as they presented much greater variations than is usually encountered when a single preparation is tested on standardized rats. Nearly all of them showed, however, a positive line test. In agreement with Bills and MacDonald it seems certain that the substance formed is different from vitamin D.

Having been convinced that pure cholesterol cannot be rendered antirachitic by ultraviolet irradiation and that ergosterol is at present the only substance known to be specifically activated by radiant energy, a study of the photochemical activation of ergosterol was next undertaken.

Determination of the Quantum Efficiencies

The experimental procedure was similar to that described in the earlier work,[1] but it was markedly improved by the introduction of two new features and the results reported here are more accurate.

Procedure.—The apparatus consisted of a Hilger quartz monochromator and the intensity was measured as before by means of a Coblenz vacuum thermopile and a Leeds and Northrup galvanometer. The commercial quartz mercury vapor lamp was replaced by a water-cooled capillary arc constructed in this Laboratory. A detailed description of this lamp will be published independently. The intensity of isolated lines could be increased ten times by this means over those produced by our previous lamp. A second marked improvement over the original apparatus was obtained by the use of a double absorption cell placed inside of the spectrometer tube immediately in front of the thermopile. It was arranged to slide back and forth on a rack so that either the first cell containing the solvent or the second cell containing the solution of ergosterol could be placed in the path of the light. Each compartment of the cell was 19 mm. long, 1.5

[9] A Fuller's earth from Northern Florida possessing marked catalytic properties.

[10] Bills, *J. Biol. Chem.*, **67**, 753 (1926).

[11] Bills and MacDonald, *ibid.*, **68**, 821 (1926).

mm. wide and 17 mm. high. This arrangement eliminated, completely, errors due to reflection from the windows and absorption by the solvent.

The degree of activation obtained in the different experiments was determined as before by the line test and the amount of calcium deposition was described as negative, positive or very positive. The smallest amount of calcium deposition accepted as a positive test was that corresponding to a condition of the bone described in this Laboratory as indication of a line, provided that the next lower test in the same series showed distinctly less and the next higher distinctly more calcification. If two subsequent members of a series showed the same lowest degree of calcification the one requiring the more energy was taken.

It should be emphasized that although this method is at present the only one which is sufficiently sensitive to detect the exceedingly minute quantities of vitamin D formed, and is far more sensitive than any chemical test, it imposes, nevertheless, limitations on the accuracy of the test. Biological variations are to be expected in all experimental animals, but the rat colony used in these experiments had been so rigorously controlled that the rats may be considered to be as highly standardized as now appears possible.

The ergosterol was prepared by a method similar to that described by Windaus and Grosskopf.[12] The ergosterol was further purified by acetylation, recrystallization of the acetate from glacial acetic acid, saponification and repeated recrystallization from absolute alcohol. The final product melted at 163° (uncorr.). It was beautifully white and crystalline. The ergosterol was either irradiated in the dry state back of the slit as in the earlier work, or it was dissolved in optically pure alcohol. It was then placed in one of the compartments of the double cell in front of the thermopile. Two concentrations of the alcoholic ergosterol solution were used: 1/25% and 1/1250%. The results of the experiments carried out on different lines are tabulated below.

Discussion

It will be seen that the threshold value of activation lies for all the lines studied in the neighborhood of 700-1000 ergs (or 10×10^{13}–14×10^{13} quanta). The lower value corresponds to an amount of vitamin D equal to 6×10^{-8} g.

TABLE I

SOLID ERGOSTEROL

Subs., irradiated, mg.	Line, mμ	Time, secs.	Defl.	Abs., %	Abs. energy, ergs	Abs. quanta	Anti-rachitic action
25	265	3	11	100	234	3.2×10^{13}	Negative
25	265	6	10	100	425	5.9×10^{13}	Negative
25	265	10	10.5	100	744	10.3×10^{13}	Positive
25	265	10	16	100	1134	15.8×10^{13}	Very positive
25	265	20	16	100	2268	31.6×10^{13}	Very positive
25	265	40	16	100	4536	63.2×10^{13}	Very positive
25	265	60	16	100	6804	94.8×10^{13}	Very positive
25	265	90	16	100	10206	142.2×10^{13}	Very positive
25	280	6	6.5	100	276	3.9×10^{13}	Negative
25	280	22	4.5	100	701	10.0×10^{13}	Positive
25	280	35	5	100	1240	17.7×10^{13}	Positive
25	280	30	7.5	100	1594	22.8×10^{13}	Very positive
25	280	60	7.5	100	3188	45.6×10^{13}	Very positive
25	280	120	7.5	100	6376	91.2×10^{13}	Very positive

[12] Windaus and Grosskopf, *Z. physiol. Chem.*, **124**, 8 (1923).

TABLE II

ALCOHOLIC SOLUTIONS OF ERGOSTEROL

Subs. irradiated	Line, mμ	Time, secs.	Defl. solvent	Defl. soln.	Abs., %	Abs., energy, ergs	Abs. quanta	Antirachitic action
1/25%	256	15	6.5	0	100	691	9.0×10^{13}	Positive
1/25%	256	10	10	0	100	709	9.2×10^{13}	Positive
1/25%	256	22	6.5	0	100	1013	13.2×10^{13}	Positive
1/25%	256	30	6.5	0	100	1382	18.0×10^{13}	Very positive
1/25%	256	30	10	0	100	2126	27.7×10^{13}	Very positive
1/25%	256	60	10	0	100	4252	55.4×10^{13}	Very positive
1/25%	256	120	10	0	100	8504	110.8×10^{13}	Very positive
1/1250%	265	30	8.5	3.5	59	1063	14.5×10^{13}	Positive
1/1250%	265	120	8.5	3.5	59	4252	58.0×10^{13}	Very positive
1/1250%	265	600	8.5	3.5	59	21260	290.0×10^{13}	Very positive
1/25%	265	30	8.5	0	100	1807	25.1×10^{13}	Positive
1/25%	265	120	8.5	0	100	5228	100.4×10^{13}	Very positive
1/25%	265	475	8.5	0	100	28610	397.7×10^{13}	Very positive
1/1250%	280	30	5	2.5	50	531	7.6×10^{13}	Negative
1/1250%	280	120	4.5	2	56	2124	30.4×10^{13}	Positive
1/1250%	280	600	5	2.5	50	10620	157.0×10^{13}	Very positive
1/25%	280	30	5	0	100	1062	15.1×10^{13}	Positive
1/25%	280	120	5	0	100	4248	60.4×10^{13}	Very positive
1/25%	280	600	5	0	100	21240	302.0×10^{13}	Very positive
1/25%	293	20	4	0	100	567	8.5×10^{13}	Negative
1/25%	293	30	5	0	100	1063	15.8×10^{13}	Positive
1/25%	293	60	3.5	0	100	1488	23.7×10^{13}	Positive
1/25%	293	40	6.5	0	100	1842	27.5×10^{13}	Very positive
1/25%	293	65	5	0	100	2303	34.4×10^{13}	Very positive
1/25%	293	120	3.5	0	100	2976	44.4×10^{13}	Positive
1/25%	293	300	3.5	0	100	7440	111.1×10^{13}	Very positive

TABLE III

ERGOSTEROL ACETATE, ALCOHOLIC SOLUTION

Subs. irradiated	Line, mμ	Time, secs.	Defl. solv.	Defl. soln.	Abs., %	Abs. energy, ergs	Abs. quanta	Antirachitic action
1/25%	265	10	6.5	0	100	461	6.4×10^{13}	Negative
1/25%	265	20	6.5	0	100	922	12.8×10^{13}	Negative
1/25%	265	30	6.5	0	100	1382	19.2×10^{13}	Very positive
1/25%	265	45	6	0	100	1913	26.6×10^{13}	Positive
1/25%	265	60	7	0	100	2976	41.4×10^{13}	Very positive
1/25%	265	90	6	0	100	3827	53.2×10^{13}	Very positive
1/25%	265	120	7	0	100	5952	82.7×10^{13}	Very positive
1/25%	265	240	6	0	100	10200	141.8×10^{13}	Very positive

The calculation is similar to that given in the previous paper,[1] and is based on the Einstein law of photochemistry and also on the assumption that vitamin D is the only product formed from ergosterol upon irradiation with monochromatic light. The slight difference in favor of the 256 mμ line when compared with the 293 mμ line is probably not sufficiently marked to be beyond the limits of experimental error. The formation of vitamin

TABLE IV

EXPERIMENTS WITH A TIME LAG

Alcoholic Solution of Ergosterol

Subs. irradiated	Line, mμ	Time, secs.	Defl. solv.	Defl. soln.	Abs., %	Abs. energy, ergs	Abs. quanta	Antirachitic action
1/25%	265	6	7	0	100	298	4.1×10^{13}	Negative
1/25%	265	12	7.5	0	100	638	8.9×10^{13}	Negative
1/25%	265	15	7	0	100	744	10.3×10^{13}	Positive
1/25%	265	18	9	0	100	1148	15.9×10^{13}	Positive
1/25%	265	24	8	0	100	1361	19.3×10^{13}	Positive
1/25%	265	30	7.5	0	100	1594	22.1×10^{13}	Very positive
1/25%	265	50	5	0	100	1770	24.6×10^{13}	Very positive
1/25%	265	36	7	0	100	1786	24.8×10^{13}	Very positive

TABLE V

EXPERIMENTS WITH A NARROWER SLIT

Subs. irradiated	Line, mμ	Time, secs.	Defl. solv.	Defl. soln.	Abs., %	Abs., energy, ergs	Abs. quanta	Antirachitic action
1/25%	265	30	2.5	0	100	531	7.4×10^{13}	Negative
1/25%	265	50	2	0	100	709	9.8×10^{13}	Negative
1/25%	265	100	2	0	100	1418	19.6×10^{13}	Positive

D from ergosterol is thus shown to be independent of the wave length over practically the whole range of selective absorption and to be only a function of the incident energy. The efficiency is also independent of the state in which ergosterol is irradiated for solid ergosterol, a concentrated solution absorbing all the incident rays and a dilute solution absorbing only about 60% of the radiation require all the same number of ergs (or quanta) for activation. When working with crystals it is necessary to use a large excess, otherwise some light passes through the interstices and a lower quantum efficiency is found. For example, 5 mg. of ergosterol placed in the small box used for the purpose (area 70 mm.^{2}) required more than 2500 ergs for activation—calculated on the basis of complete absorption.

In order to determine whether further purification of the light would result in a higher quantum efficiency, a series of irradiations was carried out with one of the slits (between the prism and the double cell) cut down to $^1/_{11}$ of its original width. This change diminished the intensity of the light 3.5 times for the 265 mμ line, but did not alter the quantum efficiency as seen in Table V. A series of irradiations was carried out with ergosterol acetate in $^1/_{25}$% alcoholic solution (prepared in the course of purification of the ergosterol, m. p. 177°, uncorr.) The quantum efficiency is the same as for ergosterol. This fact confirms the statements of other investigators[13] and shows that the hydroxyl group apparently plays no role in the process of activation.

It was thought that some information concerning the mechanism of ac-

[13] Rosenheim and Webster, *Lancet, II*, 622 (1927); Windaus and Holtz, *Nachr. Ges. Wiss. Göttingen Math.-Phys. Klasse*, Heft. 2 (1927).

tivation could be gained by exposing ergosterol to intermittent radiation. It was, namely, possible that in the case of one of the stages of the process being a slow reaction the quantum efficiency might be altered by such means. The intermittent irradiation was carried out with a $^1/_{25}\%$ alcoholic solution of ergosterol, wave length 265 mμ. The light was thrown on the cell for the duration of one second at five-second intervals. A photographic camera shutter was used for the purpose. The results are tabulated in Table IV. No change in the quantum efficiency could be detected.

The quantum efficiency of antirachitic activation of ergosterol reported in the present work is smaller than that given in the earlier paper. As already stated the present values are to be considered more accurate and this fact ought not to convey the impression that the activatability of cholesterol is due to a substance having a higher quantum efficiency than ergosterol. A large number of experiments was carried out using various preparations of cholesterol, but lack of space prevents the publication of all of the tables. It is sufficient to say that in no case did we find a higher quantum efficiency than for ergosterol, but for one preparation which had been repeatedly recrystallized and was kept in an ice chest the quantum efficiency was found to be almost the same as for ergosterol, namely 1400 ergs for the 265 line (irradiated in the solid state). Experiments with the original V-1-24 preparation (exposed to air and kept since 1925 at room temperature) showed a lower quantum efficiency—1500 ergs were negative and 5000 distinctly positive. The presence of traces of oxidation products and impurities might easily explain the smaller quantum efficiency of ordinary cholesterol. The chemically purified cholesterols described in the first part of this paper were also exposed in the solid state to monochromatic radiation with the idea that differences in the residual amount of ergosterol present might be detected. These experiments failed in so far as the highest amount of energy used, namely, 200,000 ergs, was insufficient to activate any of them.

Ergosterol was irradiated in a $^1/_{25}\%$ solution in acetone. Acetone shows selective absorption in the same region as ergosterol. The results were negative and energy up to 28,000 ergs was ineffective.

Acetone was used as a solvent also with pure cholesterol with the idea in mind that the energy absorbed by the acetone might possibly be transferred to cholesterol with the formation of vitamin D. A 1% solution of chemically purified cholesterol was used. The results were entirely negative up to 90,000 ergs.

Interesting experiments have been published by Windaus[14] on the sensitization of ergosterol to visible radiation. The products were, however, entirely inactive antirachitically.

[14] Windaus and Brunken, *Ann.*, **460**, 225 (1928); Windaus and Borgeaud, *Ann.*, **460**, 235 (1928).

One of us (S.-K. K.) is indebted to the International Health Board of the Rockefeller Foundation for a fellowship during the tenure of which the work here reported was carried out.

Summary

1. Repeated boiling with permanganate in acetone solution removes completely the pro-vitamin from cholesterol. Cholesterol purified in this way and irradiated is inactive antirachitically even in large doses.

2. The photochemical formation of vitamin D from ergosterol under the action of monochromatic light has been studied quantitatively for different lines.

3. The quantity of radiant energy necessary to form an amount of vitamin D sufficient to cause a demonstrable deposition of calcium in the bones of a rachitic rat has been found to be constant over a wide range of radiations, 700–1000 ergs being necessary for the 256, 265, 280 and 293 mμ lines.

4. The quantum efficiency was independent of the state in which ergosterol was irradiated, the results being the same for irradiation of the solid or of solutions in alcohol of varying concentration.

5. The quantum efficiency was the same for ergosterol acetate as for ergosterol. The hydroxyl group plays no role in the process of activation.

MADISON, WISCONSIN

NEW BOOKS

Crystallographic Tables for the Determination of Minerals. By VICTOR GOLDSCHMIDT, Heidelberg, and SAMUEL G. GORDON, Philadelphia. Academy of Natural Sciences, Philadelphia, 1928. 70 pp. 17 × 26 cm. Price, $1.50.

In accordance with one of the fundamental principles of crystallography, the angles of every crystallized compound are constant and, except in the cubic system, characteristic of that compound. Angle measurement should accordingly constitute a useful method for the identification of unknown substances. Owing, however, to the existence of freedom of choice as to orientation and as to the unit forms, this method has never come into general use. Previous attempts to arrange crystals in determinative tables have either covered too few compounds or have been too complicated for anyone but the author and his immediate associates to use. In the present publication an effort is made to improve upon existing tabulations (without, however, giving any credit to earlier workers), over a thousand minerals being arranged according to crystal system, with data as to crystallographic constants, composition, specific gravity, hardness and miscellaneous properties.

When any new mineralogic work appears, it is the reviewer's custom, for judging how carefully it has been prepared, to look up at once what