2. The effective ion sizes of the quaternary bromide and picrate in chlorobenzene are about one ångström unit smaller than in a number of other solvents; this result indicates that specific interaction between solvent and solute can appear even in the case of strong electrolytes.

SCHENECTADY, N. Y. RECEIVED DECEMBER 8, 1939

[CONTRIBUTION NO. 164 FROM THE BIOLOGICAL RESEARCH LABORATORIES, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS]

Relative Efficiency of Active Wave Lengths of Ultraviolet in Activation of 7-Dehydrocholesterol

BY JOHN W. M. BUNKER, ROBERT S. HARRIS AND L. MALCOLM MOSHER

The sterols which have received major attention in respect to their photochemical conversion into antirachitics are ergosterol and cholesterol, associated respectively with lower plants and with animal materials. Crystalline ergosterol may be regarded as the precursor of vitamin D_2 (Calciferol), the principal form of antirachitic vitamin now available from commercial activation; crystalline cholesterol, on the other hand, does not itself constitute a significant provitamin, but its derivative, 7-dehydrocholesterol, when irradiated with appropriate ultraviolet energy, is converted into vitamin D_3,[1,2] a form which has been found only in animal tissues.

In the present study the quantum efficiencies of certain wave lengths of ultraviolet in the activation of 7-dehydrocholesterol in ether have been measured and compared.

Irradiation Procedure.—Details of the irradiation procedure already have been published.[3] A cylindrical quartz vessel of 80 cc. capacity was filled with a 0.1% solution of 7-dehydrocholesterol[4] in anhydrous ether, C. P., and placed in the optical axis of the beam emerging from a quartz monochromator.[5] Exposure times were calculated from data on preliminary tests so that an approximation of 33 U. S. P. XI units (10 Steenbock units) would be produced with each batch. Since one batch would give sufficient active material for assay on only ten rats and since the protocol for this series called for groups of forty or fifty rats each, the irradiation procedure was repeated four or five times for each wave length. This was done over a period of five days during which time repetitive irradiations were scattered at random through the whole series, thereby distributing any undetected variations in the conditions of the irradiation. At each wave length each unit batch was subjected to 2475×10^{12} quanta of monochromatic ultraviolet energy.

(1) Boer, Reerink, van Wijk and van Niekerk, *Proc. Acad. Sci., Amsterdam*, **39**, 622 (1936).

(2) Windaus and Bock, *Z. physiol. Chem.*, **245**, 168 (1936).

(3) Harris, Bunker and Mosher, THIS JOURNAL, **60**, 2579 (1938).

(4) Acknowedgment is made for the contribution of the 7-dehydrocholesterol by Winthrop Chemical Company.

(5) Bunker and Harris, *New Engl. J. Med.*, **216**, 165 (1937).

Feeding of Activated Materials.—All batches of 7-dehydrocholesterol solution irradiated at a given wave length were pooled with three ether washings of the quartz vessel and with olive oil in the proportion of one gram of oil for each rat to be fed. The ether was removed under carbon dioxide in a moderate vacuum and the oil residue thoroughly incorporated into Steenbock #2965 ration in the proportion of 29 g. of diet to each gram of oil solution. The eight milligrams of treated 7-dehydrocholesterol which this oil contained had an expected potency of about 3.3 U. S. P. units of vitamin D.

These mixtures were then fed to groups of rats previously rendered rachitic in twenty-one days on the Steenbock #2965 ration. Group size was selected as follows (based on probability predictions from previous similar experiments involving a total of approximately three hundred rachitic rats): 40 rats each for the testing of materials irradiated with 2483 Å., 2537 Å., 2652 Å., and 3025 Å.; 50 rats each for testing 2804 Å., 2894 Å., and 2967 Å.; and 18 rats for testing 3130 Å., which previous tests had shown to be probably inactive.

During the six-day feeding period each animal consumed its quota of 30 g. of ration. Empty feeding jars were replenished with additional amounts of the #2965 diet, unfortified, until the end of the seventh day.

Observations.—A total of 328 rats entered the test period and all but two survived. At the end of this period these animals were killed, the left leg of each was X-rayed, then line tested, and the right legs were taken for ashing.

A summary of essential data including line test observations is presented in Table I. The whole bone ash values (on a fat-free dry basis) were between 31.7 and 33.6% for all active wave lengths; 28.8% for the inactive 3130 Å. line; and 27.3% for the rachitic controls.

In a previous communication we expressed the opinion that recorded differences between group responses must be greater than the quantities which by purely statistical computation are barely significant, if they are to be accepted as real. This restriction of the use of statistical methods applies to the evaluation of data derived from the usual sized bioassay groups but the restriction is lifted when the number of animals tested concurrently is sufficiently large.

Interpretation of Observations.—In respect to bone ash determinations, the group values for the active wave lengths varied within a small range

inconsistently with respect to X-ray and line test evaluations which are mutually confirmatory. This strengthens the opinion that bone ash determinations, because of variations inherent in the method or for some other undetected reason, fail to afford criteria sufficiently precise for detection of small differences in degrees of antirachitic response in rats. The line test or the roentgenogram procedures are more delicate.

TABLE I

SUMMARY OF EXPERIMENTAL DATA

Wave length, Å.	Number of rats	Weights, g. First	Weights, g. Last	Healing response	Arithmetic mean response
2483	2	55.0	72.0	0.5	
	10	52.0	72.9	1.0	
	11	54.5	73.1	1.5	
	12	53.4	72.3	2.0	
	5	55.2	77.2	2.5	1.6000[a]
Total	40				
2537	1	50.0	64.0	0.0	
	2	55.5	76.0	0.5	
	6	52.2	69.5	1.0	
	14	53.5	72.7	1.5	
	10	52.5	68.0	2.0	
	7	52.4	78.7	2.5	1.6375
Total	40				
2652	2	56.5	74.5	0.5	
	7	53.1	76.4	1.0	
	11	53.1	72.5	1.5	
	17	53.1	73.9	2.0	
	3	52.0	70.7	2.5	1.6500
Total	40				
2804	4	53.5	72.3	0.5	
	7	51.9	73.9	1.0	
	15	52.4	71.5	1.5	
	18	52.7	71.3	2.0	
	5	43.2	68.4	2.5	
	1	52.0	66.0	3.0	1.6600
Total	50				
2894	2	54.0	76.0	0.0	
	2	53.0	75.7	0.5	
	5	53.2	74.8	1.0	
	14	52.1	70.5	1.5	
	13	53.2	74.5	2.0	
	12	52.4	73.7	2.5	
	1	56.0	67.0	3.0	
	1	58.0	..	Died	1.7551
Total	50				
2967	1	50.0	62.0	0.5	
	4	54.8	79.0	1.0	
	11	52.7	72.5	1.5	
	21	53.1	74.3	2.0	
	6	50.8	70.3	2.5	
	5	51.8	68.8	3.0	
	0	..	..	3.5	
	1	51.0	60.0	4.0	
	1	56.0	..	Died	1.9796
Total	50				
3025	4	52.5	78.8	0.0	
	1	50.0	72.0	.5	
	6	54.5	77.0	1.0	
	9	54.3	75.3	1.5	
	17	53.7	73.8	2.0	
	2	51.0	68.5	2.5	
	1	50.0	59.0	3.0	1.5500
Total	40				
3130	18	53.4	74.4	0.0	0.00

[a] The computation of means to the fourth decimal is for subsequent mathematical treatment of these values.

In respect to line test observations, inspection of the arithmetic mean responses indicates a peak of response for 2967 Å. over all other wave lengths, and a suggestion that 2894 Å. may be slightly more active while 3025 Å. is slightly less active than the remaining four wave lengths.

This interpretation by inspection was tested by statistical analysis in which the significance of the differences among arithmetic mean responses was determined. That a real difference of means existed was shown when the variance within groups was proven significantly less than the variance among groups.

In order to determine which differences among means are real, the "t" test was applied, as an appropriate method in view of the numbers of observations available.

Let "t" represent the ratio of the difference between two means to the best estimate of a standard error of that difference. Then

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

where $\overline{X}_1$, $\overline{X}_2$ = any two arithmetic mean response values, N_1, N_2 = the number of observations in the two groups, respectively, s = the best estimate of a standard deviation of a single group based on the data of the two groups considered, the value "s" being derived from the expression

$$s^2 = \frac{\Sigma(X_1 - \overline{X}_1)^2 + \Sigma(X_2 - \overline{X}_2)^2}{(N_1 - 1) + (N_2 - 1)}$$

where X_1, X_2 = each observation in the first or second group, respectively.

A "significant" value of "t" was taken to be 2.5, which gives approximately nineteen out of twenty chances that the differences found are real.

By the above test it appears that the response to 7-dehydrocholesterol activated by 2967 Å. is

TABLE II

SHOWING ESSENTIAL DATA USED IN THE "t" TEST

Wave lengths compared, Å.	Difference of arithmetic means	$s\sqrt{\frac{1}{N_1}+\frac{1}{N_2}}$	"t"	Interpretation
2483–2537	0.0375	0.13055	0.287	
2483–2652	.0500	.11930	.419	
2483–2804	.0600	.12035	.498	
2483–2894	.1551	.13355	1.161	
2483–**2967**	.3796	.12840	2.956	Significant
2483–3025	.0500	.14320	0.349	
2537–2652	.0125	.12555	.100	
2537–2804	.0225	.12530	.180	
2537–2894	.1176	.13810	.852	
2537–**2967**	.3421	.13315	2.569	Significant
2537–3025	.0875	.14850	0.589	
2652–2804	.0100	.11600	.086	
2652–2894	.1051	.12955	.811	
2652–**2967**	.3296	.12425	2.653	Significant
2652–3025	.1000	.13870	0.721	
2804–2894	.0951	.12630	.753	
2804–**2967**	.3196	.12195	2.621	Significant
2804–3025	.1100	.13590	0.809	
2894–**2967**	.2051	.14800	1.386	
2894–3025	.2245	.13290	1.689	
2967–3025	.4296	.14335	2.997	Significant

significantly greater than for any other wave length examined except for 2894 Å., which seems to have an effect intermediary between the effects of adjacent lines. There appears to be no significant difference between the activities of any other wave lengths tested.

The statistical analysis of results confirms the interpretation derived by inspection, and indicates that the lower efficiency of 3025 Å. may be regarded as not significant.

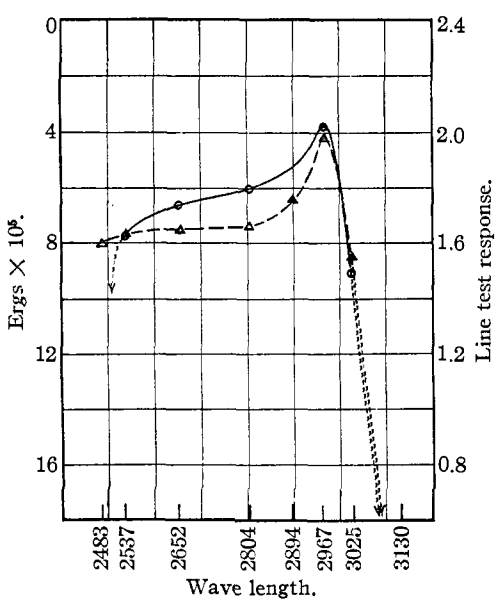

Fig. 1.—Relative antirachitic efficiencies of certain wave lengths of ultraviolet as determined by animal response: ○ = from direct irradiation of skin; Δ = from feeding of activated 7-dehydrocholesterol.

Discussion.—When mammals are exposed to sources of ultraviolet of suitable wave length and energy, rickets can thereby be cured or prevented. It is assumed that there is produced in living tissues, by such activation of the skin, vitamin D of one of the major types. Although vitamin D_2 is absorbable from the intestinal tract of mammals and exerts in them an antirachitic therapeutic effect, its precursor, ergosterol, is not absorbed nor has it been identified in mammalian tissues. On the other hand, 7-dehydrocholesterol has been identified as the provitamin of animal tissues[1,2] and may be presumed to be the precursor of the vitamin D produced by irradiation of mammalian skin.

It has been shown[5,6] that rats whose skins have been irradiated with dominant mercury lines in the region bounded by 2537 and 3025 Å. exhibit an antirachitic response. The degrees of response to the various wave lengths tested are not equal per unit of applied energy, the potency of line 2967 Å., as determined by us,[5] being significantly greater than that of the other wave lengths. It also has been reported[3] that the photochemical activation of ergosterol (in ether) results in substantially equal production of antirachitic per quantum of ultraviolet energy applied over a similar range of wave lengths. This may be taken as an indication of a difference between ergosterol and the provitamin of the skin.

The results of this research are displayed in Fig. 1, together with those of a previous investigation of the activation of the skin of living rats. It is to be noted that there is a qualitative similarity between the activation curve of 7-dehydrocholesterol and of rat skin, whereas the activation of ergosterol[3] is dissimilar. This further strengthens the hypothesis that 7-dehydrocholesterol is the predominant provitamin D of the skin.

Summary

1. The photochemical activation of crystalline 7-dehydrocholesterol in ether by monochromatic ultraviolet of 2483, 2537, 2652, 2804 and 3025 Å. is substantially uniform per quantum of energy applied.

2. On a quantum basis, the activation by 2967 Å. is significantly greater than for any other

(6) Knudson and Benford, *J. Biol. Chem.*, **124**, 287 (1938).

wave length examined, with the possible exception of 2894 Å.

3. The activation by 2894 Å. appears to be intermediate between that of 2967 Å. and the other wave lengths tested.

4. No demonstrable antirachitic properties were produced by irradiation of 7-dehydrocholesterol in ether by ultraviolet of 3130 Å.

5. The superior effectiveness of 2967 Å. in antirachitic activation of 7-dehydrocholesterol parallels the significant superiority of this wave length in inducing healing upon direct irradiation of depilated rachitic rats, which supports the hypothesis that 7-dehydrocholesterol is a significant precursor of vitamin D in the skin.

CAMBRIDGE, MASS. RECEIVED NOVEMBER 16, 1939

[CONTRIBUTION NO. 211 FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Identification of Organic Compounds. I. Chlorosulfonic Acid as a Reagent for the Identification of Aryl Halides

BY ERNEST H. HUNTRESS AND FREDERICK H. CARTEN[1,2]

Although the action of chlorosulfonic acid has been studied upon many compounds, either as a means of introducing the sulfonic acid group or the chlorosulfonyl group, no systematic attempt to employ it as a reagent for the identification of organic compounds has been reported. The present paper reports the study of chlorosulfonic acid as applied to the identification of a considerable number of halogenated aromatic hydrocarbons.

The object of this work was to find a set of standard conditions such that treatment of the sample with chlorosulfonic acid would yield readily the corresponding arylsulfonyl chloride which in turn could be converted to the corresponding sulfonamide. The process of introducing the $-SO_2Cl$ group is hereafter designated as "chlorosulfonylation."

Two standard procedures (I and II) for effecting chlorosulfonylation have been devised and tested upon a large number of compounds. The first carries out the chlorosulfonylation in chloroform solution; the second uses no solvent. Both procedures employ a relatively large excess of chlorosulfonic acid in order to convert the intermediate sulfonic acid (to be expected from one mole of chlorosulfonic acid) as completely as possible to the corresponding sulfonyl chloride. The yields of arylsulfonyl chloride thus obtained are generally high (60–90%) and the process is extremely simple to execute.

(1) This paper is constructed from part of a dissertation submitted by Frederick H. Carten to the Faculty of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1939.

(2) This work was assisted by a grant from the Warren Fund of the American Academy of Arts and Sciences for which grateful acknowledgment is hereby made.

Conversion of the arylsulfonyl chloride thus obtained to the corresponding arylsulfonamide was effected either by treatment with concentrated aqueous ammonium hydroxide, or by reaction with solid ammonium carbonate. The yields on this step were invariably nearly quantitative.

In a few cases the reaction with chlorosulfonic acid yielded abnormal products. Thus fluorobenzene, iodobenzene, *o*-dichlorobenzene and *o*-dibromobenzene at 50° with chlorosulfonic acid in the absence of any solvent, chloroform, yielded the corresponding sulfones. In a few other instances a small amount of the corresponding sulfones accompanied the usual sulfonyl chloride. From the point of view of identification, however, these sulfones serve just as well as the sulfonyl chlorides. Furthermore, in those cases where they accompany the sulfonyl chloride, they are readily separable from the final sulfonamide by their insolubility in alkali, the sulfonamides readily dissolving and being reprecipitable on acidification.

Another abnormal type of reaction occasionally observed was nuclear chlorination. This occurred notably with *p*-diiodobenzene and 1,2,4,5-tetrachlorobenzene but does not interfere with the identification of these compounds since the substitution products are readily obtained and have characteristic melting points.

Unsatisfactory results were obtained with the following compounds: *o*-chloroiodobenzene, *p*-chloroiodobenzene, *m*-chloroiodobenzene, *o*-bromoiodobenzene, *p*-bromoiodobenzene, *o*-iodotoluene, *m*-iodotoluene and *p*-iodotoluene. Treatment