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The Unimolecular Rates of Hydrolysis of 0.01 Molar Methyl- and Benzylfructofuranosides and -Pyranosides and of Sucrose in 0.00965 Molar Hydrochloric Acid at 20 to 60°

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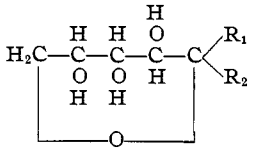
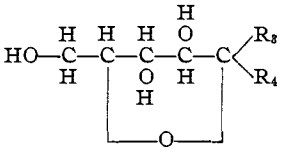
Quantitative comparisons, restricted to derivatives of the aldohexoses, led to the generalization that pyranosides are at least one hundred and fifty times as resistant to hydrolysis with aqueous acid as the corresponding furanosides.[1a] This ratio, however, is now known to be approximately ten for methylglycosides of the aldopentose, *d*-arabinose,[2] and also for methyl and benzylfructosides.[1a] The fructosides and probably the methyl 1-sorbosides[3] are, in addition, so unstable that the pyranoside forms hydrolyze at rates comparable to those of the furanosides derived from aldo sugars. (Sorbose is, like fructose, a ketohexose.) Thus, in the aldopentose and ketohexose series the rates of hydrolysis at 20° may be so similar that differences in these rates cannot be used to distinguish between the two types of ringed structure. The activation energies of these rates, nevertheless, might differ sufficiently[4] to characterize these furanosides and pyranosides. We have determined, therefore, the activation energies of hydrolysis of the fructosides whose structures are sketched[5] and which under our conditions gave quantitative yields of fructose and the alcohol. Such an investigation, carried out on substances closely related in chemical constitution, might develop, also, a valuable means of studying the kinetics of hydrolytic reactions.



Fructopyranosides

(1) α-Methyl $R_2 = OCH_3$
(2) β-Methyl $R_1 = OCH_3$
(3) β-Benzyl $R_1 = O-CH_2C_6H_5$



Fructofuranosides

(4) α-Methyl $R_3 = OCH_3$
(5) α-Benzyl $R_3 = O-CH_2-C_6H_5$
(6) Sucrose R_4 = α-glucopyranosido

One R of each of the pairs (R_1 R_2) (R_3 R_4) is always CH_2OH.

Materials.—The fructosides were recrystallized to constant rotation and kept in a desiccator over anhydrone. Their specific rotations in 1 to 2% aqueous solution (sodium light) were checked five months later at the close of the research and still deviated only slightly from the accepted values, given in parentheses. Found: pyranosides: α-methyl 43.5° (44–46.5°);[6] β-methyl −172.1° (−172.0°);[7] β-benzyl −130.6° (−130.0°);[1a] furanosides: α-methyl 92.3° (93.0°);[1b] α-benzyl 44.2 (45.7).[1b] Their aqueous solutions were non-reducing and neutral to brom thymol blue. The sucrose was U. S. Bureau of Standards sample 17, lot 3650,[8] while the fructose and glucose used in calibrations were recrystallized specimens with the correct rotations.

The stock solutions of "Reagent" hydrochloric acid were standardized before and after the research with potassium acid phthalate (U. S. Bureau of Standards sample 84) and remained unchanged at 1.93 and 0.0193 *N*. Stock solutions of reagent sodium hydroxide were 1.94 and 0.0194 *N*, the latter increasing to a final value of 0.0200 *N*.

Apparatus.—The hydrolyses were carried out in two glass cells. In one, the area of contact between stirrer and solution was five times that in the other.[9] Glass caps, ground to fit closely without grease, restricted the loss of water from either cell to less than 10 mg. in eight hours at 100°. This loss was a negligible fraction of the minimum residual volume of 10 cc. present in the experiments. A thermostat kept the temperature in the cells constant to 0.02°, the readings being made to 0.01° on glass mercury thermometers. These were calibrated in the same range with a platinum resistance thermometer reading on the international temperature scale. All glass apparatus was cleaned with chromic acid followed by thorough washing, finally with distilled water.

Methods.—About 0.001 mole of fructoside, weighed to 0.1 mg. in dry air, was dissolved in 40 cc. of distilled water in a calibrated 100-cc. volumetric flask. Exactly 50 cc. of 0.0193 *N* hydrochloric acid was added before the flask was filled to the mark with distilled water and the contents thoroughly mixed. The reaction cell was rinsed twice with 10-cc. portions of this solution. The remainder was then introduced and the cell with its contents shaken in boiling water, or a bath at 0°, until their temperature was within 1° of that of the thermostat. In this way, thermal equilibrium between solution and thermostat was obtained quickly and it was possible to withdraw the first

(1) For literature references see (a) Purves and Hudson, THIS JOURNAL, **59**, 1170 (1937); (b) *ibid.*, **59**, 49 (1937).

(2) Montgomery and Hudson, *ibid.*, **59**, 992 (1937).

(3) Schlubach and Graefe, *Ann.*, **532**, 211 (1937). This article reviewed the literature.

(4) Moelwyn-Hughes, *J. Gen. Physiol.*, **13**, 317 (1930).

(5) The position of the oxygen bridge linking was established in each case by the methylation method[1a] but the orientation of the α- and β-glycosides toward and away from the ring, respectively, is arbitrary in the sketch.

(6) Schlubach and Schröter, *Ber.*, **61**, 1216 (1928). We are greatly indebted to Dr. F. B. Cramer for a supply of this glycoside.

(7) Hudson and Brauns, THIS JOURNAL, **38**, 1216 (1916).

(8) Cane sugar purchased in a grocery hydrolyzed at the same rate.

(9) Forthcoming publication by the first named author.

sample for analysis less than fifteen minutes after the addition of the acid.

These samples, removed by carefully cleaned and dried pipets, varied from 6 cc. in the initial to 2 cc. in the final stages of hydrolysis. Each sample was immediately, but not quite completely, neutralized by discharge, with agitation, into a weighed amount of 0.00965 *N* sodium hydroxide at 5° contained in a tared (to 1 mg.) thin-walled Pyrex tube. The time of half addition was noted to within five seconds. The tube with the neutralized solution was promptly sealed with a well fitting, ungreased glass cap and weighed to within 1 mg. When the concentration of reducing sugar in the neutralized solution exceeded 0.04%, the solution was diluted to within this value with distilled water and the tube reweighed before being kept with the others at 0°. The whole manipulation, including any dilution necessary, occupied less than five minutes and trial showed that the reducing power of the solutions remained unchanged for at least six hours. In practice, the solutions were analyzed usually within one hour.

After at least seven samples had been removed at nearly equal intervals of time and the hydrolysis was 60 to 90% complete, the value of 100% was attained by shaking the cell and residual solution in boiling water for two to five minutes. It was then cooled quickly to 25° and a portion was withdrawn for analysis. The determinations made on the solutions obtained after the second and third heatings checked with each other and with theory within 1% in every case and were accepted as the final values in the calculations. In control experiments, a 0.01 molar solution of fructose underwent a decrease in reducing power of 0.034% per minute in 0.00965 *N* hydrochloric acid at 100° (measured from 0–34%, rate constant 3.4×10^{-4} min.$^{-1}$). A correction for this decrease in the final values of the hydrolyses was not made, as it was within the limit of error in all cases. Glucose in the same environment preserved its original reduction for a week.

Estimation of Degree of Hydrolysis.—The concentration of reducing sugar in the neutralized, partly hydrolyzed solutions was determined with the Shaffer–Hartmann–Somogyi alkaline copper reagent,[10] which was unaffected by unchanged glycoside. The reagent contained in one liter 5 g. of copper sulfate pentahydrate, 40 g. of anhydrous sodium carbonate, 7.5 g. of tartaric acid, and 0.7 g. of potassium iodate. When 5 cc. was heated with 5 cc. of fructose solution (reducing sugar 2 mg.) for fifteen minutes at 100°, 99% of the precipitated cuprous oxide was formed in the first ten minutes. One cc. of a 5% potassium iodide–9.2% potassium oxalate solution was added before the acidification and immediate titration of the liberated iodine with 0.002 *N* sodium thiosulfate.

Careful attention to all precautions mentioned[10] and the rigorous standardization of manipulative detail rendered the re-oxidation of cuprous oxide during the heating a constant quantity equivalent to 1.5 cc. of 0.002 *N* thiosulfate or to 0.08 mg. of fructose. The re-oxidation at room temperature in twenty hours was equivalent to 0.55 cc. of 0.002 *N* thiosulfate when the test-tubes remained capped and were not disturbed. The duplicate analyses and blanks were always carried out simultaneously. They were reproducible to one drop (0.07 cc.) of the thiosulfate solution equivalent to less than 0.005 mg. of reducing sugar. Over one thousand estimations were made with this precision.

The mg. fructose–cc. thiosulfate calibration plots (Fig. 1) were straight lines instead of the usual very slightly inflected ones, perhaps because the weight rather than the volume method was used in making dilutions. The slope of the lines sometimes varied slightly when a new batch of alkaline copper reagent was employed, as was the case every two or three weeks.

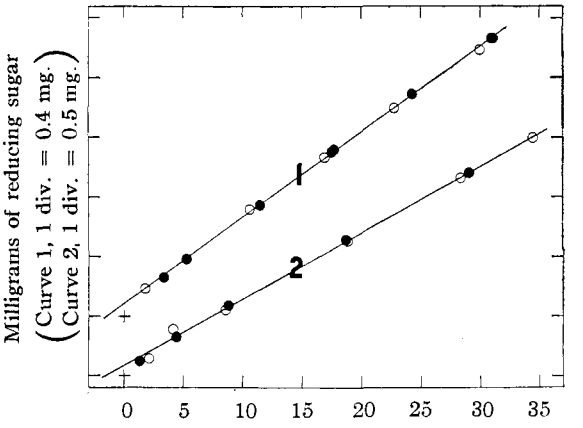


Fig. 1.—Plots of typical data which gave the concentration of fructoside in hydrolyzed solutions.

In Fig. 1, line 1, the circles represent the data for fructose and the filled circles those for fructose in 0.01 *M* α-methylfructopyranoside. Both sets of points lie on the same line, which was coincident with those obtained in similar experiments with all the other fructosides studied except sucrose. The reducing power of fructose, therefore, was not affected by the presence of 0.01 molar concentrations of fructoside[11] nor by the methyl or benzyl alcohol formed in the hydrolyses. Figure 1, line 2, represents similar data for partly and completely inverted solutions of sucrose. Each cross marks the origin of the line just above it.

The concentration of non-reducing fructoside in a partly hydrolyzed solution was calculated from the difference between the observed reducing power (in cc. of 0.002 *N* thiosulfate) and the reducing power when hydrolysis was complete.

Results

When the logarithms of the experimental values for the concentration of unchanged fructoside were plotted against time, straight lines were obtained. The initial portions to 60% complete hydrolysis of some typical plots are reproduced in Fig. 2. Circles and flagged circles representing duplicate experiments (which differed only in a

(10) (a) Shaffer and Hartmann, *J. Biol. Chem.*, **45**, 377 (1920–1921); (b) Shaffer and Somogyi, *ibid.*, **100**, 695 (1933).

(11) *Cf.* Richtmyer and Hudson, THIS JOURNAL, **58**, 2540 (1936).

fivefold change in the area of contact between the solution and stirrer) fell on the same straight lines with deviations usually less at the lowest temperatures (A curves) where errors due to timing and to evaporation during sampling were less. Usually the time axis has been shifted to separate the two sets of points. The first point of each set (nearest the left ordinate) represents data when hydrolysis was less than 2% complete and filled circles on line 6A represent solutions not stirred in the interval since the preceding point.

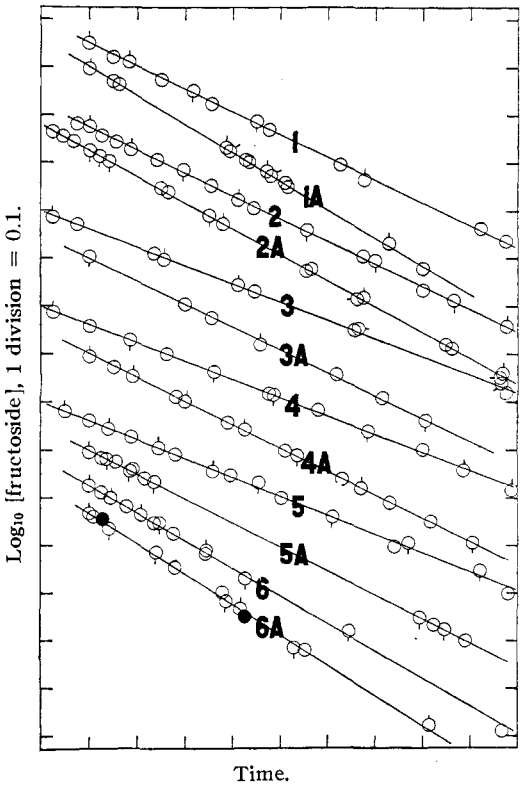


Fig. 2.—Typical plots of data which gave the fraction of fructoside hydrolyzed per minute. The numbered unlettered lines represent data at the highest temperature and the A lines at the lowest temperature listed in Table I for the compound with the same number. One division on the abscissas represents in minutes for line 1, 20; 1A, 400; 2, 10; 2A, 1000; 3, 5; 3A, 400; 4, 5; 4A, 100; 5, 10; 5A, 200; 6, 20 and 6A, 1000.

No extraordinary deviations from any of these straight lines occurred either for a five-fold change in the area of contact between stirrer and solution or when stirring was stopped or during the initial 4% of hydrolysis or when hydrolysis was 90% complete. The reactions, therefore, were homogeneous and followed an apparently unimolecular course with no change in the activity of the catalyst and no detectable side or reverse reactions. Investigations[1a] with 0.25 molar acid at 20° were thereby confirmed. The dependence of the rate upon the concentration of acid was determined only for sucrose at 30° (Table I) where the reaction was found first order with respect to the concentration of hydrochloric acid, although its concentration did not change in the reaction.

Sucrose in methyl alcohol containing hydrochloric acid gives initially an anomalous increase in the optical rotation followed by the usual decrease.[1a] This effect has not been observed in aqueous solutions nor is any anomaly apparent in the present measurements.

Average values of the fraction of fructoside hydrolyzed per minute (rate constants, k, Table I) were derived from the slopes of the best straight lines through plots of data like those in Fig. 2 and were not corrected for changes with temperature in the activity of the acid. This correction would be minute for the 0.00965 N hydrochloric acid used.[12] The deviations in k noted in Table I represent the spread between duplicate experiments but may not express the errors of measurement.

TABLE I

THE RATES AND ACTIVATION ENERGIES OF THE HYDROLYSIS OF APPROXIMATELY 0.01 MOLAR FRUCTOSIDES IN 0.00965 N HYDROCHLORIC ACID

		°C.	k in min.$^{-1}$ × 10^4	$\log k = \log a - b/T$ log a	b	E in kcal.
			Pyranosides			
1	α-Methyl	30.0	3.43 ± 0.07			
		40.0	15.3 ± .2	16.884	6170	28.2
		50.0	62.3 ± .8			
2	β-Methyl	30.0	1.25 ± .01			
		40.0	6.49 ± .1			
		50.1	28.1 ± .3	17.636	6530	29.9
		60.0	105 ± 2			
3	β-Benzyl	30.1	2.75 ± 0.05			
		40.0	12.3 ± .2	16.718	6150	28.1
		59 8	176 ± 2			
			Furanosides			
4	α-Methyl	30.0	11.3 ± 0.3			
		39.9	51.2 ± .7	16.516	5900	27.0
		50.0	176 ± 7			
5	α-Benzyl	20.2	5.84 ± 0.06			
		30.0	23.3 ± .3	15.522	5500	25.2
		40.0	90.3 ± 2			
6	Sucrose	30.0	1.44 ± 0.02			
		40.0	5.33 ± .05	14.695	5620	25.7
		50.0	21.0 ± .2			
		59.7	66.7 ± 1			
		30.0[a]	14.2[a]			

[a] Sucrose in 0.0965 N HCl.

The logarithms of k plotted against the reciprocal of the absolute temperature, $T(273.2 + °C.)$ fell on another set of straight lines. The lines

(12) Harned and Ehlers, THIS JOURNAL, **55**, 2179 (1933).

were drawn by giving weights of 1.0 to 1.2 to points from the highest to the lowest temperature, respectively, to allow for the increase in experimental accuracy at the lowest temperatures. In the case of α-methylfructofuranoside average values of $\log_{10} k$ at 39.9 and 50° deviated from the straight line (Fig. 3, line 4) by 2% and corresponded to determinations of copper reducing power when the cuprous oxide stood overnight before titration.

The activation energies, E (Table I), of the hydrolyses were derived from the negative of the slopes, b, of these lines. E (in calories) $= b$ ($\log_e$ 10) × gas constant, $R = 4.58\ b$. The constants $\log a$ and b of the equations $\log_{10} k = \log a - b/T$ for these lines are given also in Table I.

Discussion

Although the acid inversion of sucrose has been studied frequently,[4,13] the concentrations of sugar and of hydrochloric acid were much greater than the approximately 0.01 molar solutions used here and a quantitative comparison of our rate constants with previous data was not attempted. The activation energy of 25.7 ± 0.05 kcal. found in the present work on sucrose is in good agreement with 25.83[4] and 25.8[13a] kcal. obtained by others. The present method of following the hydrolysis, therefore, is felt to be at least as accurate and convenient as the best of its predecessors. This was not unexpected because the copper reduction technique, in contrast to polarimetric,[13a,b] dilatometric[13c] and calorimetric[13d] methods, need include no allowance for the mutarotation of the liberated reducing sugars.

TABLE II

DIFFERENCES IN THE CONSTANTS LOG a AND IN b AND E ASSOCIATED WITH KNOWN CHANGES IN THE STRUCTURE OF THE FRUCTOSIDES

Fructo Series	Structural difference	Δ log a	Δb	E, kcal.	Δb/Δ log a = T	T when log k = 0
β-Pyrano-	Methyl (2)			(29.9)		(370)
side	—	0.92	380	1.75	410°	
	Benzyl (3)			(28.1)*		(368)*
α-Furano-	Methyl (4)			(27.0)		(358)
side	—	.99	400	1.8	405	
	Benzyl (5)			(25.2)		(354)
Methyl-	β (2)					
pyrano-	—	.75	360	1.65	480	
side	α (1)			(28.2)*		(366)*
α-Methyl	Pyranoside (1)					
	—	.37	270	1.2	730	
	Furanoside (4)					

(13) These papers also review other work: (a) Lamble and Lewis, *J. Chem. Soc.*, 233 (1915); (b) Scatchard, THIS JOURNAL, **48**, 2259 (1926); (c) Hitchcock and Dougan, *J. Phys. Chem.*, **39**, 1177 (1935); (d) Sturtevant, THIS JOURNAL, **59**, 1532 (1937).

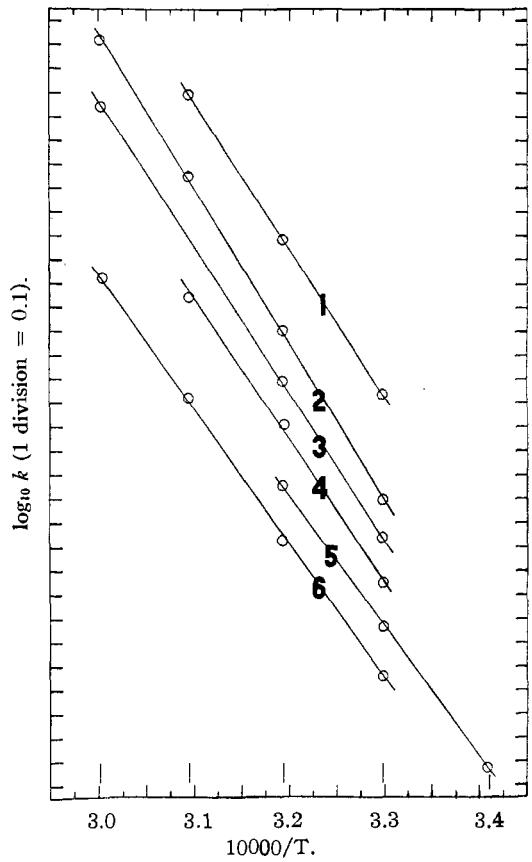


Fig. 3.—Plots which gave the activation energies, E, in Table I. The vertical lines in the plot along the abscissas are at values of $1/T$ at 60, 50, 40, 30 and 20° from left to right, respectively. The numbered lines represent the data for the compound with the same number in Table I.

It follows from Tables I and II that the activation energies as well as the rates of hydrolysis of fructopyranosides and fructofuranosides are too similar in magnitude to be diagnostic of the ringed structure. It is interesting to note that the substitution of methyl for benzyl glycosides in the β-pyranoside or α-furanoside series caused increases in b (and E) which were identical within the experimental error. This identity and the approximate equality of the Δ log a's suggests that the log a's and b's (which determine the rates of hydrolysis and their variations with temperature) may represent a sum of terms each characteristic of a portion of the chemical structure[14a] or of an equilibrium involved in the hydrolysis.[14b,c]

The changes in E noted in Table II may be due

(14) *Cf.* (a) Hudson's rules of isorotation, THIS JOURNAL, **31**, 66 (1909); (b) Steinhardt, *Kgl. Danske Videnskb. Selskab, Math. fys. Medd.* (in English), **44**, 11 (1937); (c) Svirbely and Schramm, THIS JOURNAL, **60**, 330 (1938).

not only to changes in the strength of the bond hydrolyzed but also to variations in the number of squared terms, n, contributing energy to the reaction. In the case of vibrational energy n is twice the number of degrees of freedom. In gases, the number of squared terms contributing energy to the reaction may be shared among all the particles concerned.[15] We will assume that these conclusions are valid for the above hydrolyses in dilute solution[16] when the same number of molecules react in unit time at the same temperature. This occurs when $\log k = \log a - b/T = \log k' = \log a' - b'/T$ or when $T = \Delta b/\Delta \log a$. Then as $E_{obsd.} = E - [(n/2) - 1]\ RT$, $\Delta E = R(\log_e 10)\,\Delta b = RT\ \Delta n/2$, and at $T = \Delta b/\Delta \log a$, $-\Delta n = 2\ (\Delta \log a)\ \log_e 10 = 4.61\,\Delta \log a$ or $-\Delta n = 4.24$ (4.56), 3.46 and 1.7 for the respective variations in the fructoside structure listed in Table II.

It is not surprising, in view of the complexity of the activation process, that the changes in the calculated numbers of degrees of freedom for these reactions in solution are not integers. The increase in n when benzyl replaces methyl as the aglycone is consistent, also, with the greater number of active degrees of freedom of the benzyl group at these temperatures. The confirmation and interpretation of these mathematical relationships as well as those which evaluate the log a factors for reactions between molecules and ions must await data on a more extended series of structurally related glycosides.

It has been found that log a increases with b for most reactions. This is true at a given temperature because reactions with rate constants, k, greater than 0.1 (half life, $0.693/k = 7$ min.) are too fast and those with $k < 10^{-4}$ (half life 6930 min.) are too slow to measure conveniently. At a given temperature, therefore, we have $-1 > (\log a - b/T) > -4$ and, thus, for those reactions which are measurable at a given temperature, large increases in b must be accompanied by an increase in log a. The fact that this may not be true for small changes in log a or b within the range of the above difference is shown by comparing the log a and b factors for reactions (5) and (6), Table I.

Another frequently used rule is that the temperature at which rates of reactions equal a given value increases with E. For example, when $\log_{10} k = 0$; $\log a - b/T = 0$ and $T = b/\log a$ so that b is expected to increase faster than log a. A consideration of reactions (3) and (1) Table II (starred values) indicates that this regularity may not hold when the changes are small.

This research was carried out with the financial support of the Chemical Foundation Cellulose Fund.

Summary

1. Shaffer–Hartmann–Somogyi estimations of copper reducing power were used to follow the hydrolysis of 0.01 molar solutions of several fructosides in 0.00965 molar hydrochloric acid. The method was convenient and accurate.

2. Unimolecular rate constants, k, were determined from 20 to 60° and were given by the equation $\log k = \log a - b/T$. Activation energies, $E = 4.58b$, were for α-methyl, β-methyl and β-benzylfructopyranoside 28.2, 29.9 and 28.1 kcal.; for α-methyl and α-benzyl fructofuranoside 27.0 and 25.2 kcal., and for sucrose 25.7 kcal., respectively.

3. The constants log a and b or E increased when (1) the methyl group replaced the benzyl group, (2) the α- replaced the β-isomers and (3) pyranosides replaced furanosides, but the differences were too small to provide a reliable method of distinguishing one particular ring structure from another as in the aldohexose series.

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(15) Fowler, "Statistical Mechanics," The Macmillan Co., New York, N. Y., 1936, p. 716.

(16) Some justification for this view even when reaction occurs in solution is given by experiments on the reduction of dichromic acid upon absorption of light by quinine in aqueous sulfuric acid. Light in the near ultraviolet is absorbed largely by the quinoline structure of quinine [Heidt and Forbes, THIS JOURNAL, **55**, 2701 (1933)] while reaction probably occurs at the secondary hydroxyl group. Furthermore, quantum yields changed when groups attached to the quinoline structure were altered although these groups were not oxidized by dichromate in this reaction. Both of these facts imply a transfer of energy involving several degrees of freedom within the quinine molecule before reaction occurs.