

Isomerization experiments with different methods, which started from crystallized neo- β -carotene U gave, with some variations, the stereoisomers mentioned above. The compound U is comparatively resistant to moderate heating, *e. g.*, a solution of 3 mg. in 25 ml. of petroleum ether, when kept at 60° for one hour, contained about three-fourths of the final pigment in the form of unchanged compound U. In melt experiments the same stereoisomers were obtained as in the corresponding experiments with β -carotene and a further reversible zone was observed (neo- β -carotene D, 474.5, 441.5 m μ). The melt was kept at 135° for fifteen minutes and because of the lower temperature (as compared with the β -carotene melt) and the partial formation of the more intensely colored β -carotene, the total loss in extinction was only one-fourth to one-fifth of the initial value in this case. Of the total photometric value of the melt 40% was caused by unchanged U-isomer, 22% by β -carotene formed and 38% by seven minor stereoisomers. No slightly colored or colorless and fluorescent breakdown products were observed.

Summary

As a contribution to the stereochemistry of polyenes, the isomerization of β -carotene was

studied, under the influence of refluxing, iodine catalysis, hydrochloric acid catalysis, and by melting the crystals. The chromatograms showed a number of pigments, nine or ten of which were stereoisomers of β -carotene. Some of them are very labile. On addition of iodine they all yield a preponderant quantity of β -carotene and minor stereoisomers. One of the new isomers, "neo- β -carotene U," has been crystallized in a yield of 17% of the starting material. It is adsorbed above β -carotene and thus does not follow an earlier suggested rule concerning the location of polyene-hydrocarbon isomers on the Tswett column. It is tentatively assumed that the U isomer contains one *cis* double bond, and Gillam's pseudo- α -carotene two such bonds, out of the five stereochemically available double bonds. An observed labile stereoisomer which shows spectral maxima at 20 m μ . shorter wave lengths than β -carotene may possess four or five *cis* bonds in its molecule.

PASADENA, CALIFORNIA

RECEIVED APRIL 27, 1942

[FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK]

An Extension of the Acidity Scale

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After it had been recognized that the concentration of the hydrogen ion was only to a first approximation a useful concept for an acidity scale, *pH* was then defined as the logarithm of the reciprocal of the activity, instead of concentration, of the hydrogen ion. Because of the essential uncertainty involved in the definition of the activity of a single ionic species such as the hydrogen ion, one may avoid committing oneself to a definition in terms of strictly thermodynamical concepts and adopt the following working definition

$$pH = \frac{E - E_0}{2.3RT}$$

where *E* is the e. m. f. of a cell composed of a hydrogen electrode, the solution to be measured, a saturated potassium chloride bridge and a calomel cell. The constant *E*₀ is chosen either so that in a very dilute solution of hydrochloric acid (say 10⁻⁴ *M*) *pH* equals the logarithm of the reciprocal of the concentration of the acid, or so that the *pH* in an equimolecular mixture of acetic acid and sodium acetate of infinitely low ionic strength equals the *pK* of acetic acid, whether this *pK* be determined by a thermodynamical or by a non-thermo-

dynamical method, such as the conductivity method.¹ These two ways of fixing the value of *E*₀ are, for all practical purposes, compatible with each other.

This working definition is valid only on the assumption that the liquid junction potential of the above galvanic cell is irrelevant, either because it is negligibly small, or because it is constant for such cases of *pH* determinations as practically may occur. This assumption breaks down for solutions of large ionic strength and especially for solutions of very high acidity or alkalinity. Here, the liquid junction potentials can by no means be said to be irrelevant. If one tries to establish a *pH* scale for extremely acid solutions, another working definition of the acidity scale has to be chosen, and, possibly, such that in the region of lower acidities it coincides with the ordinary *pH* scale. One approach to this problem has been made by Hammett,² who, by means of color-

(1) One of the more recent presentations of this much discussed problem is given by MacInnes in "The Principles of Electrochemistry," Reinhold Publishing Corporation, New York, N. Y., 1939.

(2) L. P. Hammett, *Chem. Rev.*, **16**, 67 (1935); "Physical Organic Chemistry," McGraw-Hill Book Company, New York, N. Y., 1940.

metric studies of a suitably chosen set of acidity indicators in the form of dyestuffs, has established an acidity scale in terms of an "acidity function," which in the range of pH , say 12 to 1, coincides with the pH scale.

The writers encountered during their studies on bivalent reversible oxidation-reduction systems an opportunity of establishing an acidity scale on a different basis. The principle of the method is this.³

In a bivalent, reversible system one may distinguish three levels of oxidation-reduction: the reduced (R), the semi-oxidized (S), and the totally oxidized (T) form. The normal potential of the R,S system, or the normal potential of the lower step of oxidation, will be designated as E_1 ; that of the S,T system, or that of the higher step of oxidation, as E_2 ; and the normal potential of the R,T system, or the mean normal potential, as E_m . These three normal potentials are related to each other as follows

$$E_m = (E_1 + E_2)/2$$

All of these normal potentials depend on pH . Hence, also the difference $E_2 - E_1$ depends on pH . This difference will be referred to as the "spread" of the normal potentials. On plotting E_1 and E_2 against pH , the curves consist to the first approximation of rectilinear sections with slopes of $n \times$

0.06 volts per pH unit (at 30°), where n is an integer, including 0, and scarcely ever > 3 (see, for instance, Fig. 1). From this approximation one arrives at the more correct form of the curves by rounding out the discontinuities. For example, the potential of the true, rounded-out curve at the pH of the intersection of two straight lines (pK) differs from that of the point of intersection by 18 millivolts.⁴ This rule is of practical use for rounding out the preliminary discontinuities of the plots. Similarly, the E_m curve, as preliminarily plotted, consists of rectilinear sections with slopes of $n \times 0.03$ volts per pH unit, and here the correction for rounding out the edges is 9 millivolts at the pH of the intersection.

A further consequence of these rules is that also the spread, $E_2 - E_1$, depends on pH in a definite manner: in some pH intervals the spread may be independent of pH ; in other pH intervals the spread may depend linearly on pH , the slope being either 0.06 or an integer multiple thereof. The transition from a region where the spread is independent of pH to a region where it is 0.06 is, of course, not a discontinuous one, but has to be rounded out according to the same principle as mentioned before. A change of this slope is brought about by a change of acidic ionization of the dye in one of its levels of oxidation-reduction.

Within the range of well definable pH values, plots of the three normal potentials against pH have been previously established for numerous dyestuffs. On working in very acid solutions there are two factors interfering with an unambiguous extension of the plot. First of all, there is no way of measuring pH by the hydrogen electrode. In the second place, there is no way of obtaining absolute values of the oxidation-reduction potential. It is true, that by a method described in a previous paper,⁵ the liquid junction potential can be kept constant for all practical purposes during the time of the titration experiment, but the absolute value of the potential⁶ cannot be determined. What one can measure is only the change of the potential during the titration. So, neither E_1 nor E_2 nor E_m can be measured, yet the spread, $E_2 - E_1$, can be measured.

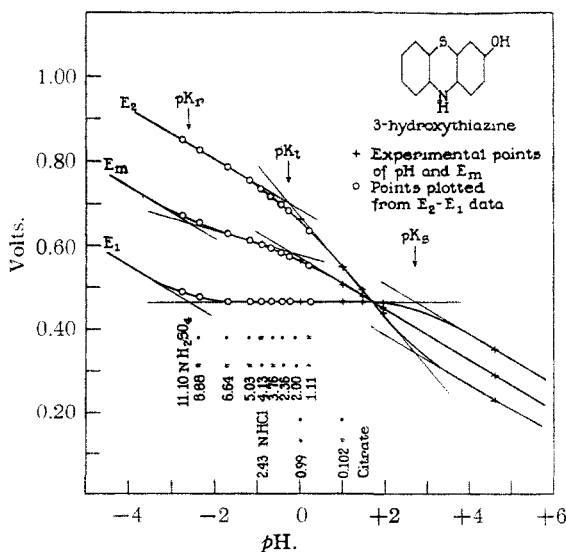


Fig. 1.

(3) The writers are fully aware of the fact that what follows is not quite easy to follow for anybody not thoroughly familiar with the theory of the equilibrium in a reversible two-step oxidation-reduction system. Detailed presentations of this subject are L. Michaelis, *Chem. Rev.*, **16**, 243 (1935); L. Michaelis and M. P. Schubert, *ibid.*, **22**, 437 (1938); L. Michaelis, *Ann. N. Y. Acad. of Sciences*, **XL**, 39 (1940).

(4) Furthermore, at that pH which equals $pK \pm 1$ the analogous correction is 2.4 mv., and at any pH still further apart from pK this correction is practically negligible.

(5) L. Michaelis, M. P. Schubert and S. Granick, *THIS JOURNAL*, **62**, 204 (1940).

(6) "Absolute," of course, only in so far as the potential of the normal hydrogen electrode is arbitrarily taken as zero.

Now the spread depends on the acidity, and this functional relationship can be used for an acidity scale which, in the ordinary acidity range, coincides with pH . The procedure is best explained by an example. A suitable dyestuff is chosen. 3-Hydroxythiazine⁷ is, by far, the best for reasons which will presently be understood. For this dyestuff it is easy to establish a plot of the normal potentials in the pH range from alkaline solution to acidities still measurable by the hydrogen electrode, say to pH 1 (the right-hand part of Fig. 1). Now the curves, which from pH 2 into the more acid region are all linear, can be extended into the region of higher acidity to the left, leaving all slopes unchanged, as long as one can be sure that no acidic ionization constant of the dye in any of its three forms, R, S or T interferes. Hereby one can establish a bridge from what may be called the known region of pH into the unknown region.

A series of potentiometric titrations in solutions of sulfuric acid furnishes the data for the spreads of the potentials at various concentrations of the acid (Table I). Thus, one can graphically correlate the spread with the concentration of the acid (Fig. 1). For instance, a titration experiment in 1.11 *N* sulfuric acid gave for the spread, $E_2 - E_1$, the value 174 millivolts. Now we mark that ordinate in the plot where the spread is 174 millivolts, and read on the abscissa that the pH here is +0.24. Hence, it is legitimate to ascribe to a 1.11 *N* sulfuric acid, the pH value +0.24. In a similar way, the pH values of sulfuric acid at other concentrations can be ascertained.

This procedure is legitimate for the extension of the pH scale to about 0. From here on, the oxidized form (t-hydroxythiazine) begins to change its state of acidic ionization by attaching a proton. Since the absorption spectra of the two forms of the oxidized dye are different, it is easy to find by a spectrophotometric method, such a concentration of sulfuric acid in which the ratio of the two forms is 1:1. The pH of this acid solution may be identified with the pK of the dye. The uncertainty involved in this simplified assumption will be discussed later on. To the left-hand side from pH 0, the E_2 curve must change its slope from

0.06 to 0.03. Now, one has to find the point of intersection in order to draw the straight line with slope 0.03 at the correct height above the abscissa. This is accomplished as follows. By a potentiometric oxidation-reduction experiment in such a sulfuric acid solution in which the ratio of the two acidic levels of the dye is 1:1, one finds a certain value of the spread for the normal potentials. In order to construct the two intersecting tangents and the correct value of the rounded-out curve at this point of intersection, one utilizes the rules stated above, saying that the normal potential of the T,S system at the point of intersection of the two tangents must be 18 millivolts lower than the point of intersection itself. In this way, the point of intersection is found to lie at pH -0.25. This, then, is also the exponent of acidic ionization, pK . Now, from the potentiometric oxidative titration experiments at higher acidities and the spreads derived from them, one can correlate the higher concentrations of sulfuric acid each with a definite pH value. This procedure can be extended to a pH about -2. Here, an acidic dissociation constant of the reduced form of the dye begins to interfere. By studying the ultraviolet absorption spectra of the reduced form, it was possible to obtain a semi-quantitative confirmation of the existence of this constant in the neighborhood of the acidity expected. It is safer to restrict the exploitation of this dye only to that region in which the pK of the reduced form of the dye does not yet interfere.

Now we select another dyestuff, 3-aminothiazine, utilizing the pH data obtained from oxythiazine. This is shown in Fig. 2. The points obtained within the ordinary pH range with aminothiazine are marked +. At higher acidities, up to -2, we can correlate the "spreads" with the pH of the acid solutions, as obtained from the first dye (Fig. 1). By optically determining the acidic ionization constant of the new dye in the same fashion as with the first, the plot can be extended up to pH -4. Here, again, a dissociation constant of the reduced form begins to interfere. For this reason, we proceed to another dye, 3,9-diaminothiazine (thionine) (Fig. 3) which permits us to extend the pH scale to about -6 in an analogous manner. This is as far as it was possible to go as yet.

On extending the pH scale by this method to the range of high acidity, there can be no objection to the procedure described, as long as the dye-

(7) As regards the nomenclature of the dyes, we follow our previous suggestion [S. Granick, L. Michaelis and M. P. Schubert, *THIS JOURNAL*, **62**, 1802 (1940)] of using the name of the leukodye which is always more convenient than that of the quinonoid form. When one of the three levels of oxidation-reduction is to be referred to in a special case, these three levels, then, are distinguished by the prefixes, r-, s- and t-.

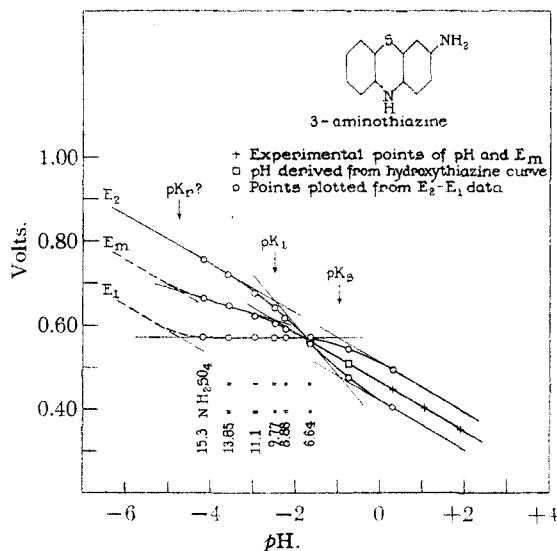


Fig. 2.

stuff in any of its three levels of oxidation-reduction (the R, S and T forms) does not change its state of acidic ionization. As soon as an acidic ionization constant of the dyestuff in one of its three levels of oxidation-reduction becomes noticeable, the following uncertainty begins to interfere. The optical method to be described presently yields that concentration of sulfuric acid where the ratio of the concentrations of the two forms of the dye, in their two levels of acidic ionization, is 1:1. What we want to know, however, is that concentration of the acid in which the ratio of the *activity* of the two forms is 1:1. Since the two forms of acidic ionization of necessity differ

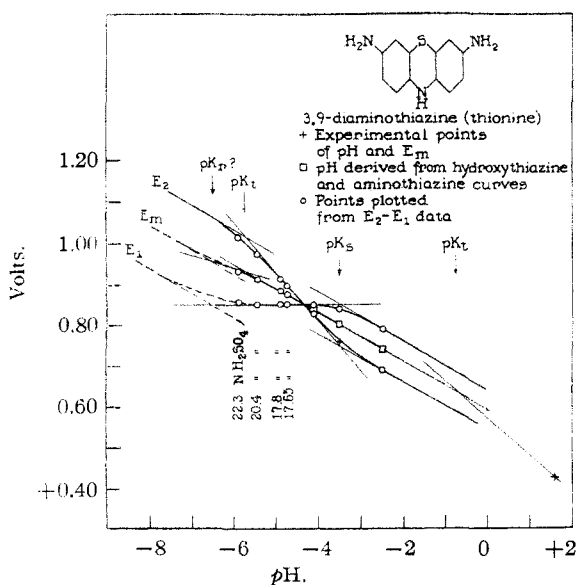


Fig. 3.

with respect to the number of free electric charges, the activity coefficients for the two forms will not be alike. On identifying the apparent ionization constant, as determined optically, with the thermodynamical ionization constant, one commits an error. This is the limitation of the method. Although the direction of the error may be recognizable from theoretical reasoning, there is no way of finding out a reasonable quantitative correction. For this reason, no correction will be applied at all in this paper, leaving any possible refinement for later work. Very likely, in sulfuric acid solutions the error in this procedure is small, and one should not over-emphasize it. The error may be estimated to rise up to a few tenths of a *pH* unit in the worst cases.

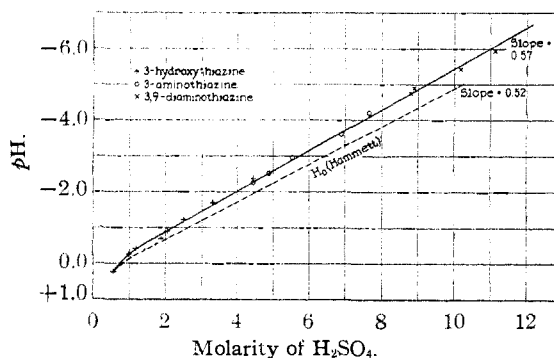


Fig. 4.

The results of the method may be understood, without further comment, from Figs. 1-4. The last of these figures permits of a comparison of this extended *pH* scale with Hammett's acidity function, H_0 . It is striking that in both scales there is a linear relationship between *pH* (or acidity function) and the concentration of sulfuric acid above 1 *M* sulfuric acid.⁸ Why this should be so is not yet explained. The slopes in the two methods are not quite the same: 0.52 for Hammett's function, 0.57 for ours. The writers are more impressed by the similarity than by the discrepancy between the two scales considering the different approaches used.

Optical Determination of the Ionization Constants.—Only two constants, those of the T forms of hydroxythiazine and aminothiazine, are essential for the plotting of the curves. The others that have been determined are not necessary but serve as a nice confirmation of the theory. For example, the *pK* value for the R form of oxythiazine in acid solution can be approximately es-

(8) N. F. Hall and W. Spengeman, *THIS JOURNAL*, **62**, 2487 (1940).

TABLE I
 3-HYDROXYTHIAZINE

| Solvent | pH directly measured with H ₂ electrode | E _m , volts | Index potential E _i , mv. | Spread E ₂ - E ₁ , mv. | pH values from spread |
|--|--|------------------------|--------------------------------------|--|-----------------------|
| Acetate buffer | +4.62 | +0.288 | 14.9, 15.0 | -120 | |
| Citrate buffer | +1.96 | + .449 | 19.4, 19.5 | - 22 | |
| Citrate buffer | +1.47 | + .477 | 26.0, 25.8 | + 24 | |
| 0.112 N HCl | +1.02 | + .506 | 43.6, 43.8 | + 81 | |
| 1.11 N H ₂ SO ₄ | +0.29 | + .543 | 87.0, 87.0 | +174 | +0.24 |
| 0.990 N HCl | +0.02 | + .555 | 99.0, 99.0 | +198 | - .02 |
| 2.00 N H ₂ SO ₄ ^a | +0.03 | + .550 | | +217 | - .25 |
| 2.36 N H ₂ SO ₄ ^a | .. | (+ .523) | | +233 | - .40 |
| 3.76 N H ₂ SO ₄ | | | | +252 | - .70 |
| 2.43 N HCl ^b | | | | +270 | - .90 |
| 4.13 N H ₂ SO ₄ ^a | | | | +270 | - .90 |
| 5.03 N H ₂ SO ₄ ^a | | | | +295 | -1.20 |
| 6.64 N H ₂ SO ₄ | | | | +324 | -1.68 |
| 8.88 N H ₂ SO ₄ | | | | +363 | -2.35 |

^a These experiments are new data. The others are taken from the previous papers by the authors. *pK* of T-TH⁺ at 2.00 N H₂SO₄.

 TABLE II
 3-AMINOTHIAZINE REDUCTION WITH Pd + H₂ AND TITRATION WITH POTASSIUM DICHROMATE IN SAME ACID

| Solvent | pH | E _m | E _i , mv. | E ₂ - E ₁ , mv. | pH values from spread of E ₂ - E ₁ |
|---|-------|----------------|----------------------|---------------------------------------|--|
| Acetate buffer | +4.62 | +0.293 | 15.4 15.6 | | |
| Citrate buffer | 1.91 | + .452 | 15.4 15.3 | | |
| Citrate buffer | 1.02 | + .506 | 15.9 15.8 | | |
| 1.11 N H ₂ SO ₄ | 0.29 | + .548 | 15.6 15.3 | - 99 | |
| 3.58 N H ₂ SO ₄ | | | 16.3 16.4 | - 67 | |
| 6.64 N H ₂ SO ₄ | | | 20.0 19.2 | - 19 | |
| 8.88 N H ₂ SO ₄ | | | 30.5 30.2 | + 42 | -2.25 |
| 9.77 N H ₂ SO ₄ ^a | | | 38.0 38.0 | + 67 | -2.50 |
| 11.1 N H ₂ SO ₄ | | | 54.5 54.0 | +106 | -2.95 |
| 13.85 N H ₂ SO ₄ ^a | | | | +148 | -3.60 |
| 15.3 | | | | +188 | -4.20 |

^a New data. *pK* of TH⁺-TH₂⁺⁺ at 10.0 N H₂SO₄.

 TABLE III
 REDUCTIVE TITRATIONS OF THIONINE WITH TiCl₃

| H ₂ SO ₄ N | E _i , mv. | E ₂ - E ₁ , mv. | pH values from spread E ₂ - E ₁ |
|----------------------------------|----------------------|---------------------------------------|---|
| 9.35 | 15.5 15.3 | -99 | |
| 12.9 | 16.0 16.0 | -78 | |
| 15.0 | 19.3 19.7 | -18.9 | |
| 17.65 | 31.4 32.8 | +48 | -4.75 |
| 17.80 | 38.0 38.5 | +68 | -4.90 |
| | 39.0 39.0 | | |
| 20.4 | 64.0 66.0 | +130 | -5.45 |
| 22.3 | 78.0 80.0 | +158 | -5.90 |

pK of TH⁺ - TH₂⁺ at 3.5 N H₂SO₄ (this *pK* value is only needed to get correct E_m, but is not necessary for the pH determination). *pK* of TH₂⁺⁺ - TH₃⁺⁺⁺ at 21.2 N H₂SO₄.

timated from the fact that the E₂ - E₁ values increase only slightly above 9 N sulfuric acid.

The *pK* values of the oxidized forms were determined by means of a Pulfrich photometer. The absorption spectra of the T and TH⁺ forms of hydroxythiazine overlap. By selecting several suitable filters where the ratios of the absorption of the two components are different, the determination of the ionization constant is made possible. The *pK* values of aminothiazine TH⁺ - TH₂⁺⁺ and of thionine TH₂⁺⁺ - TH₃⁺⁺⁺ are more readily determined, since there is scarcely any overlapping of the absorption spectra. The acid concentration is increased until the extinction is found to be just half the extinction of the dye with one less proton (the dyestuff concentration, of course, remaining constant).

The determination of the ionization constant of the reduced form of oxythiazine is made possible, because the R form has a sharp absorption band at 3100 Å. in contrast to the RH⁺ form which does not absorb at all in this region. By increasing the acidity in a series of steps, it was found that an absorption curve corresponding to one containing 50% of the concentration of the reduced form was obtained in a region between 8.5-12.0 N sulfuric acid, most probably in the neighborhood of 9.5 N sulfuric acid.

We wish to thank Dr. G. I. Lavin for taking the ultraviolet absorption plates of aminothiazine and Dr. E. G. Pickels for photometering these plates.

Summary

An extension of the pH scale for aqueous solutions of sulfuric acid up to 11 molar is accomplished by utilizing what is defined in the text as the spread of the normal potentials of certain dyestuffs which form large amounts of semiquinone radicals at suitable acidities. The results obtained from the use of three suitable dyes: hydroxythiazine, aminothiazine and thionine, can be compared with the Hammett acidity scale. In spite of the different approaches of Hammett's method and ours, we find a striking similarity between Hammett's results and our own.