There is therefore by this study added one more bit of evidence for the induced dissymmetry of the carbonyl group, and for the non-homogeneous nature of its absorption band.

Work is being continued along the same lines in this Laboratory on other cyclic ketones, and on similar compounds involving doubly-bound oxygen.

Summary

Tables and curves are given for the ultraviolet absorption and rotatory dispersion of 3-methylcyclohexanone in cyclohexane solution. These afford further evidence for the non-homogeneous nature of the carbonyl absorption band, and for the induced dissymmetry of the carbonyl group.

WELLESLEY, MASS. RECEIVED SEPTEMBER 15, 1936

[FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

The Oxidation–Reduction Potentials of Derivatives of Thioindigo. I. Thioindigo Tetrasulfonate

BY PAUL W. PREISLER AND LOUIS H. HEMPELMANN

Until the mathematical formulation of the potential changes of the two-step oxidation–reduction process, involving one electron per step, by Michaelis[1] and by Elema,[2] resulting from their work upon the natural pigment pyocyanine, the potentials of all organic oxidation–reduction systems previously measured, which required two electrons per molecule for reduction, were considered to be one-step processes involving a pair of electrons.

Sullivan, Cohen and Clark,[3] who originally investigated systems of indigo sulfonate–leuco indigo sulfonate, considered these to be of the one-step two-electron class. Preisler and Shaffer[4] found that in the *p*H range of 10 to 12, red intermediate colors were formed, between the blue colors of the oxidants of indigo sulfonates and the yellow colors of the fully reduced reductants, when the oxidants were reduced by gradual addition of sodium hydrosulfite. A determination of the oxidation–reduction potentials in alkaline buffers revealed that these systems exhibited the characteristics of a two-step process.

An investigation of the potentials of simple and substituted sulfonates of the closely related thioindigo[5] is being conducted, to ascertain whether these exhibit the two-step oxidation–reduction process, to obtain further information on the nature of intermediary reduction products, and to study the effects of substitution on the potentials.

Since the initiation of this work, Remick[6] has reported on the potentials of thioindigo disulfonate and its reduction product and has concluded that his results with this compound show "... that the dye underwent a dimolecular reduction, involving but one electron per molecule of dye. The experimental data conform with the following mathematical formulation and establish this as the first system observed to undergo a thermodynamically reversible, dimolecular, oxidation–reduction reaction. No evidence of a second step was found." His mathematical formulation is developed on the assumption that this type of chemical reaction occurs.

This unusual interpretation of the thioindigo–leuco thioindigo system prompts the presentation at this time of results of work on the potentials of thioindigo tetrasulfonate, a representative derivative, which have been found to follow the same general mathematical formulations to which other previously studied organic systems seem to conform.

The potentials of the thioindigo tetrasulfonate–leuco thioindigo tetrasulfonate mixtures in acidic buffers of *p*H below 3 give typical curves approaching in shape those of a one-step two-valent process and in buffers of *p*H above 10.5 give curves showing transition into the shapes characteristic of the two-step process. In the *p*H range below 3, the E'_0–*p*H curve of the system oxidant–semiquinone lies in a region of more negative potential, and the E'_0–*p*H curve of the system semiquinone–reductant in a region of more positive potential, than that of the resultant oxidant–reductant curve; in the *p*H range above 10.5 the relative

---

(1) L. Michaelis, *J. Biol. Chem.*, **96**, 703 (1932).

(2) B. Elema, *Rec. trav. chim. Pays-Bas*, **50**, 807, 1004 (1931); *J. Biol. Chem.*, **100**, 149 (1933).

(3) M. X. Sullivan, B. Cohen and W. M. Clark, *U. S. Public Health Reports*, **38**, 1669 (1923).

(4) P. W. Preisler and P. A. Shaffer, abstracts of papers presented at the American Chemical Society meeting, Chicago, Ill., 1933 (manuscript in preparation).

(5) P. Friedländer, *Ber.*, **39**, 1060 (1906): *Ann.*, **351**, 410 (1907).

(6) A. E. Remick, THIS JOURNAL, **58**, 733 (1936).

positions are reversed. The much deeper red colors of the partly reduced solutions in buffers above *p*H 10.5 offer additional evidence of semiquinone formation.

Experimental

Thioindigo tetrasulfonate may be prepared as a well-crystallized tetrapotassium salt by the following method. Suspend 10 g. of powdered thioindigo[7] (Rowe: Colour Index No. 1207) in 100 cc. of metallic-salt-free 30% excess sulfur trioxide, fuming sulfuric acid, in a long-necked flask lightly stoppered with a glass bulb. Heat for four hours or longer in a suitable bath at 145–150° under a hood since sulfur trioxide fumes are evolved copiously. Cool, pour the mixture into 1 liter of water and filter. Add small portions of 50% filtered potassium carbonate solution until but little carbon dioxide is evolved on addition and the mixture is still slightly acid to congo red. Filter off the light red precipitate and wash with a filtered 5% KAc–0.3% HAc solution to remove sulfate. Dissolve the precipitate in 1 liter of water, heat to boiling, add 100 cc. of a filtered 50% KAc–3% HAc solution and allow to cool slowly. Filter off the crystalline red salt and wash at least ten times with filtered 5% KAc–0.3% HAc solution and then at least ten times with 95% alcohol followed by some absolute alcohol; yield about 80%.

Analyses of material prepared by this method were made for sulfur and potassium: sulfur by fusion with sodium dioxide, potassium perchlorate and benzoic acid, in a Burgess–Parr bomb, precipitating as barium sulfate, igniting and weighing; for potassium by ashing to potassium sulfate and weighing. Samples were dried for five hours at 200° or at 135° and 5 mm. The moisture of different preparations varied from 6 to 9%.

Potassium: found, 20.03, 19.97, 20.01, average 20.00%; calcd., 20.34%. Sulfur: found, 24.66, 24.68, average 24.67%; calcd., 25.02%. Sulfur–potassium atomic ratio, 1.505, corresponding to 6.02 S to 4.00 K, calcd. 6 to 4. The slightly low values for potassium and sulfur are perhaps due to incomplete drying. Some of the moisture in the original sample is probably in the combined state, since the sample becomes much darker red on heating. The position of the KSO_3-groups was not determined but by analogy to indigo they are considered to be in the 5,5′,7,7′-positions.

The potentials of two platinum electrodes, immersed in the buffered solution of the thioindigo tetrasulfonate and its reduction products, were measured by a potentiometer system against a saturated calomel cell connected to the solution by a 5% agar-saturated potassium chloride bridge. Electrodes usually agreed to within 0.0002 volt. The calomel cell was standardized against a hydrogen electrode in 0.05 *M* acid potassium phthalate. The *p*H values of the buffers were determined by a hydrogen electrode.

The mixture of oxidant and its reduction products was made by titrating the deoxygenated, buffered, oxidant solution with a reducing agent similarly prepared. The volume at the beginning of the titration was 55 cc. and about 10 to 15 cc. was added. The E_0' values and slopes were determined graphically; the table summarizes the results.

POTENTIALS OF MIXTURES OF EQUIVALENT AMOUNTS OF OXIDANTS AND REDUCTANTS IN BUFFERS AT VARIOUS *p*H

*p*H	E_0' ox-red volt	$(E_{1/4} - E_{3/4})/2$	E_0' ox-semi	E_0' semi-red	Type buffer
0.04	0.408	(Between			HCl
.78	.362	0.014–0.015)			HCl
1.09	.345				HCl
1.48	.323				Citrate
2.05	.290				Citrate
2.97	.236				Citrate
3.56	.200				Citrate
4.00	.177				Citrate
4.46	.162				Citrate
4.73	.147				Citrate
5.42	.116				Acetate
6.53	.084				Phosphate
7.48	.047				Veronal
8.52	.018	0.015	−0.037	+0.073	Veronal
9.75	−.019	.018	− .037	− .001	Carbonate
10.29	−.034	.024	− .040	− .028	Carbonate
10.93	−.054	.030	− .034	− .074	Phosphate
11.18	−.059	.036	− .029	− .089	Phosphate

The buffers were 0.05 to 0.10 *M* in buffering ion and the reactants about 0.0005 *M;* no correction was made for the *p*H changes accompanying the reduction. An equivalent amount of sodium hydroxide was added to the titanium trichloride titrating solutions to compensate for the excess hydrochloric acid (7.493 *N*) in the commercial titanium trichloride (1.29 *M*) preparations. Titanium trichloride was used as reducing agent in buffers from *p*H 0.04 through 4.73; sodium hydrosulfite in the more alkaline buffers.

Commercial tank nitrogen, deoxygenated by passing over heated copper, was used to deoxygenate and stir the solutions. Temperature variations were less than 0.1° and the bath was maintained at 30.0°.

The number of equivalents involved in the complete reduction of indigo tetrasulfonate was determined by comparing the quantity of standard titanium trichloride solution required to reduce a known quantity with that required by a standard ferric chloride solution: 0.404 g. of thioindigo tetrasulfonate containing 5.6% moisture, was made up to 100.00 cc. Of this solution, 5.00 cc. was added to 50.00 cc. of hydrochloric acid, the final *p*H of this mixture being 0.78. At this *p*H, the potential mixture curve most closely resembled a two-electron shape curve, as given by $E_h = E_0' - (RT/nF) \log_e [(\text{Red})/(\text{Ox})]$, the E_0' values calculated from experimental measurements, taken at 1-cc. intervals, were within 0.001 volt of the theoretical E_0' for all mixtures containing from 6.3 to 94.9% of reductant. This solution required for complete reduction to a pale yellow solution, 15.8 cc. of a titanium trichloride solution in similar buffer. Five cc. of a 0.0101 *N* ferric chloride solution added to 50.00 cc. of about 1 *N* hydrochloric acid with titrated titanium trichloride of the same strength as used in the indigo tetrasulfonate determination required 15.6 cc., both end-points being determined graphically. Calculation shows that within the limits of

---

(7) Obtained through the courtesy of the Society of Chemical Industry in Basle.

experimental error, two equivalents of reducing agent per mole of indigo tetrasulfonate were used. Similar relationships hold at all other *p*H values studied. The data of Knecht and Hibbert[8] on the use of titanium trichloride for determination of thioindigo led to the same conclusion.

The initial concentration of the oxidant was changed by adding 35 cc. of the above solution of hydrochloric acid of proper strength to make final *p*H of 0.78 and diluted to 55.00 cc. and then titrated with a titanium trichloride solution of seven times the concentration of the previously used solution. The E_0' of this titration was within 0.001 volt of the E_0' of the above titration. The maximum calculated *p*H change would correspond to about 0.002 volt. Concentration apparently has but little effect on potential in this *p*H region. All other titrations were made at about 0.0005 *M* initial oxidant concentration. The effect of concentration changes in the region of apparent semiquinone formation was not studied because of certain decompositions of the materials in the alkaline buffers, which would make the results questionable. The occurrence of semiquinones, rather than meriquinones, is not absolutely established but the results fit the general theoretical considerations and are analogous to those with indigo sulfonates where semiquinone formation seems the most likely explanation.

Discussion

The relationships existing between the two component oxidation-reduction systems and their resultant can be more easily recognized by a study of the graph showing the corresponding curves of E_0' against *p*H.

The change in slope of the resultant oxidant-reductant E_0'-*p*H curve at *p*H 4.5 indicates an ionization in the reductant; a color change to deeper yellow accompanies the ion formation. The experimental points from *p*H 8.5 to 11.2 set the slopes of the two primary E_0'-*p*H curves in this region. The *pK* of the semiquinone may be either lower or higher than this first *pK* at 4.5 of the reductant: if lower, the slopes of the semiquinone-reductant E_0'-*p*H curve (shown by the long-dash line), as it extends into more acid regions from *p*H 8.5, will change from 0.06 to 0.12 at the *pK* of the reductant and then to 0.06 again at the *pK* of the semiquinone; and, if higher, it will change (shown by the short-dash line), from 0.06 to 0.00 at the *pK* of the semiquinone and from 0.00 to 0.06 at the *pK* of the reductant. The slope of the oxidant-semiquinone E_0'-*p*H curve is 0.00 at *p*H 8.5 or higher *p*H and will change to a slope of 0.06 at the *pK* of the semiquinone. The relative positions of the *pK* values will determine which order of slopes will prevail, the curves drawn are for illustration of the effect only and do not represent accurate location.

An exact estimation of the *pK* of the semiquinone cannot be made with the data at hand: the instability of the potentials and the momentary deepening of the red color on addition of reducing agent in buffers about *p*H 5 suggests that a different ionic species of the semiquinone with somewhat slower reaction rate is perhaps present in this region, although its relative position to the first *pK* of the reductant is indeterminate. This would suggest that the *pK* of semiquinone is higher than the *pK* of reductant and that the system is of the type indicated by the short-dash lines.

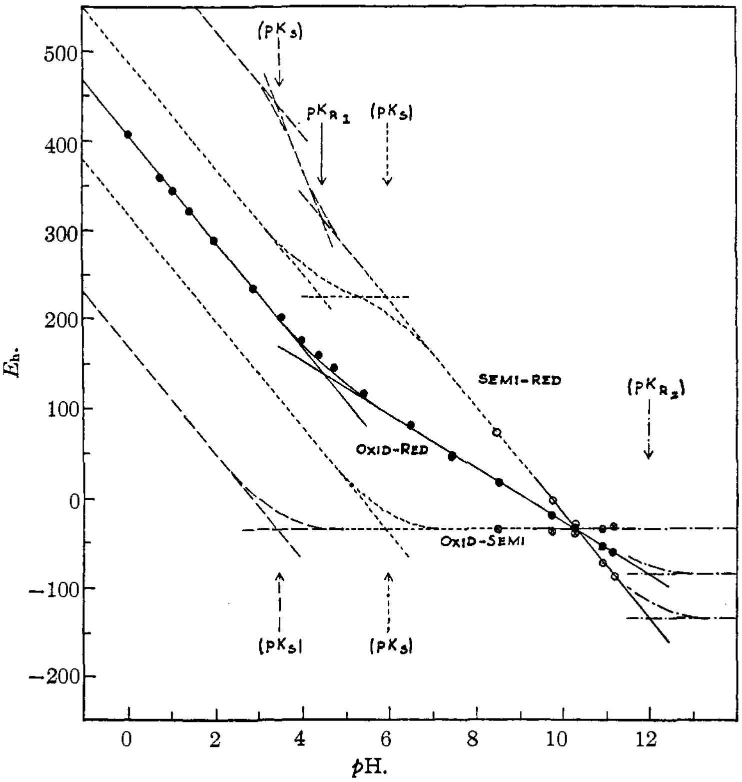

Fig. 1.

The decomposition of materials in buffers of *p*H greater than 11.5 prevents the estimation of the second *pK* of the reductant. At higher *p*H than this *pK*, all E_0'-*p*H slopes would be 0.00 (shown by short-dash long-dash line).

The above interpretation seems consistent with the results obtained and follows the general theory

---

[8] E. Knecht and E. Hibbert, *Ber.*, **40**, 3821 (1907).

which apparently holds for other readily reversible electromotively active organic oxidation–reduction systems. Preliminary experiments with certain other substituted thioindigo sulfonates indicate that these also exhibit semiquinone formation.

The formulation by Remick for the thioindigo disulfonate–leuco thioindigo disulfonate system was developed by considering that the reduction of two molecules of oxidant involving two electrons, one for each molecule, resulted in the formation of one molecule of reductant. This interpretation cannot be applied to the data presented for the tetrasulfonate, which show that one molecule of oxidant requires two reducing equivalents for conversion into one molecule of reductant. The introduction of the additional two sulfonic acid groups probably would not change the fundamental character of the reduction process of the thioindigo nucleus. The oxidation–reduction process for the several homologous indigo sulfonates has been found to be of the same type.

A comparison of the constants of indigo tetrasulfonate (E_0 = 0.365 volt and pK_{R1} = 6.9) and thioindigo tetrasulfonate (E_0 = 0.409 volt and pK_{R1} = 4.5) reveals that the substitution of S for N into the two heterocyclic organic nuclei would increase the E_0 value and the acidic dissociation of the reductant.

The instability of the oxidant or its reduction products and the semiquinone formation make thioindigo tetrasulfonate unsuitable for use as an oxidation–reduction potential indicator, except in acid *p*H regions below about *p*H 2.5.

Summary

The tetrapotassium salt of thioindigo tetrasulfonate has been prepared after direct sulfonation of thioindigo with fuming sulfuric acid. The oxidation–reduction potentials of the system thioindigo tetrasulfonate–leuco thioindigo tetrasulfonate have been determined for the *p*H range 0.0 to 11.5. The orange-red oxidant is converted into the yellow reductant by a reaction involving two reduction equivalents per molecule. The constants of the system established are E_0 = 0.409 volt and the first *pK* of the reductant is 4.5. Formation of a deeper red semiquinone was noted in the *p*H region from 8.5 to 11.5. Except in acid solution of *p*H less than 2.5, the material is not particularly suitable as an oxidation–reduction indicator.

ST. LOUIS, MO. RECEIVED AUGUST 31, 1936

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE SCHOOL OF PHARMACY OF THE UNIVERSITY OF MARYLAND]

The Preparation of Some Organic Mercurials from Diazonium Borofluorides

BY MELVIN F. W. DUNKER,[1] EDGAR B. STARKEY AND GLENN L. JENKINS

Of the many methods of making aromatic mercury compounds, only two have made use of the diazonium salts. McClure and Lowy[2] noticed that on reducing electrolytically a diazonium solution using mercury electrodes, a small amount of mercurial was formed. They were also successful in preparing the corresponding mercury derivatives by stirring the diazonium solution with finely divided mercury produced mechanically. Nesmejanow[3] has succeeded in preparing the mercury derivatives of nuclei containing no group or groups such as alkyl, halogen, hydroxyl, etc., by treating the diazonium chloride–mercuric chloride double salt with precipitated copper powder; but when the nuclei contained groups such as carboxyl, nitro, sulfonic acid, etc.,[4] temperatures ranging from −10 to −70° were necessary.

Using more stable diazonium compounds such as the diazonium borofluorides of Balz and Schiemann,[5] and by producing very finely divided mercury by reduction with stannous chloride directly in the reaction mixture, it was found that some aromatic mercurials could be prepared in better yields. The replacement of the diazonium group with mercury was carried out at room temperature. In the course of the preparation of the necessary diazonium borofluorides, better yields were obtained by diazotizing in borofluoric acid.

The probable equations are illustrated by the following

---

[1] Abstracted in part from a thesis submitted by Melvin F. W. Dunker to the Graduate School of the University of Maryland for the degree of Master of Science.

[2] McClure and Lowy, THIS JOURNAL, **53**, 319 (1931).

[3] Nesmejanow, *Ber.*, **62**, 1010 (1929).

[4] Nesmejanow and co-workers, *ibid.*, **67**, 130 (1934).

[5] Balz and Schiemann, *ibid.*, **60**, 1186 (1927).