isolated from the seeds of both *Erythroxylon coca* and *Erythroxylon novogranatense*. It is identical with ecgonidine methyl ester prepared from natural cocaine, and is subject to the provisions of the Harrison Narcotic Act.

WASHINGTON, D. C. RECEIVED MAY 2, 1941

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Magnetic Measurements on Semiquinone Radicals in the Dissolved State

BY LEONOR MICHAELIS

Semiquinones arising in solutions of reversible dyestuffs on partial reduction have been identified in previous papers[1] as free radicals by using the method of slow reduction of the quinonoid form and observing the change, in time, of the magnetic susceptibility. In those previous papers the measurements were carried out with Will's apparatus, a modification of Quincke's method, based on the movement of the meniscus of the solution in the magnetic field. This method is very sensitive and often fulfills its purpose most satisfactorily. Difficulties are encountered with it in solutions of high viscosity; deep coloration of the solution is not favorable either. In the present paper another method, a modification of Gouy's, is used. Like Will's method, it is a differential one, and is essentially similar to that used by Freed and Kasper[2] and by Pauling and Coryell,[3] adapted to the particular purpose.

The "Isthmus electromagnet" (General Electric Company) with pole pieces 3 cm. in diameter, develops, at 10 amp. and pole gap of 1.35 cm. a field strength of 10,600 gauss, and under the conditions used in the following experiments the field strength is proportional to the current intensity at least up to 10 amp. The vessel suspended between the poles is a double vessel (1-cm. diameter, each compartment 10 cm. long), similar to that devised by Freed and Kasper, the dividing wall being located between the centers of the pole pieces. The solution to be measured is placed in the upper compartment, and some suitable liquid in the lower one, in order to compensate approximately the pull exerted on the upper half. In this way a differential method is established exhibiting high sensitivity. The compensating liquid is adjusted to the particular purpose. Often a 3% agar gel was used, which is free from the risk of rising air bubbles. In other cases, when working with a highly concentrated solution of sulfuric acid as a solvent, a similar solution of the acid without the dye was used as compensator.

The balance was a semi-micro magnetically-damped balance equipped with a scale of 200 divisions at the pointer which is read through a microscope, each division being equivalent approximately to a hundredth of a milligram. With such equipment the balance generally fulfills the task of a microbalance in a most convenient manner. During the experiment the weight of the vessel is balanced by counterweights so that the equilibrium position lies somewhere within the microscopic scale. All changes of susceptibility were measured by observing only the deflection on this scale arising from switching on the current abruptly with full strength, previously adjusted by a suitable resistance. Although under ordinary conditions the balance is very nearly critically damped, the deflection, after closing the current, exceeds the true equilibrium position a little. It is only the maximum deflection which is observed, and the calibration of the balance is made with respect to it. The resting position, before switching on the current, can be fixed to within ± 1 line of deflection, sometimes better, sometimes not quite so sharply, over a period of time sufficient to make the reading of a deflection which requires about fifteen seconds. Ample time should be given after breaking the current to reach again an equilibrium position as constant as possible, before again closing the current. The deflections are usually reproducible to ± 1, or ± 2 lines, and the amperage is chosen so as to reach a deflection of 20 to 120 lines, if possible. The readings were always recalculated for 10 amperes, on the assumption, ascertained by many preliminary measurements, that the deflection is strictly proportional to the square of the amperage under all conditions occurring, which shows that the time necessary to build up the magnetic field is negligibly small compared with the fifteen seconds needed to attain the maximum deflection. The deflections can be calibrated in terms of pull in milligrams, and even directly in terms of volume susceptibility, as follows. The lower compartment of the vessel is permanently filled with the compensator, say a 3% agar gel. The upper compartment is filled in one experiment with air, in another with water. To get the deflection into the range of the microscopic scale, for the vessel used in most of the experiments, 1.3 to 1.8 amp. are needed for air and 8 to 10 amp. for water. All values are recalculated for 10 amp. The algebraic difference of these two deflections corresponds, at 10 amp., to a change in volume susceptibility of 0.740×10^{-6} cgsm., of which $+0.020 \times 10^{-6}$ is the volume susceptibility of air, and -0.720×10^{-6} that of water. Herefrom each line of deflection can be directly calibrated in terms of susceptibility. Using the average value of ten successive readings, the calibration is reproducible to 1% and better, even over a period of weeks. With the vessel and pole distance used in most of

(1) L. Michaelis, G. F. Boeker, R. K. Reber, *et al.*, THIS JOURNAL, **60**, 202, 214, 1678 (1938).

(2) Simon Freed and Charles Kasper, *Phys. Rev.*, **36**, 1003 (1930).

(3) L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci.*, **22**, 159 and 210 (1936); Chas. D. Coryell, Fred Stitt and Linus Pauling, THIS JOURNAL, **59**, 633 (1937).

the following experiments, each line of deflection is equivalent to 1.12×10^{-5} g. of pull, or to 2.08×10^{-10} cgsm. units of volume susceptibility, at 10 amp. For the vessel and pole distance used for the experiment shown in Fig. 1, this figure was 2.42×10^{-10}.

Whenever in the following experiments a change of susceptibility can be attributed to the appearance of a free radical, the concentration of the radical, $[s]$, in moles per liter, can be calculated starting from the assumption that at 25° (which temperature varied on different days by not more than ±2°) the molar susceptibility of a free radical containing one unpaired electron is 1240×10^{-6} cgsm. units.[4] Hence the volume susceptibility of an n molar solution of a radical is

$$\chi_{vol.} = n \times 1.24 \times 10^{-6}$$

which is equivalent, under the conditions stated above, to 5950 lines of deflection. Consequently, on comparing the volume susceptibility of a solution initially containing no free radical, and that of the same solution after a free radical has been generated, the molar concentration $[s]$ of the radical is

$$[s] = \frac{2.08 \times 10^{-10}}{1.24 \times 10^{-6}} \times D = 0.000168D$$

where D is the difference in lines of deflection before and after the development of the radical. For the experiment shown in Fig. 1, 2.42 must be taken in place of 2.08. No correction for any diamagnetic effect is necessary, whenever a change of susceptibility in time is observed without opening the reaction vessel, since such a correction under all occurring circumstances lies entirely within the limits of error. When the reduction is brought about not by the slow action of a reducing agent such as glucose, but by the addition of a quickly reducing, but magnetically indifferent, substance, a correction for the slight effect of the addition of the foreign substance may be applied. By a magnetically indifferent reducing agent we mean a diamagnetic substance such as ascorbic acid which on being oxidized is converted into a diamagnetic substance again, not to a paramagnetic one.

On measuring solutions magnetically stable in time, the accuracy of the result easily can be brought to ±1% by averaging a sufficient number of individual readings, which may oscillate about their average value by ±2 lines of deflection. On pursuing the change in time in those experiments with a slowly acting reducing agent, no such averaging is possible; instead the best fitting curve has to be drawn. Here the limit of error is of course larger.

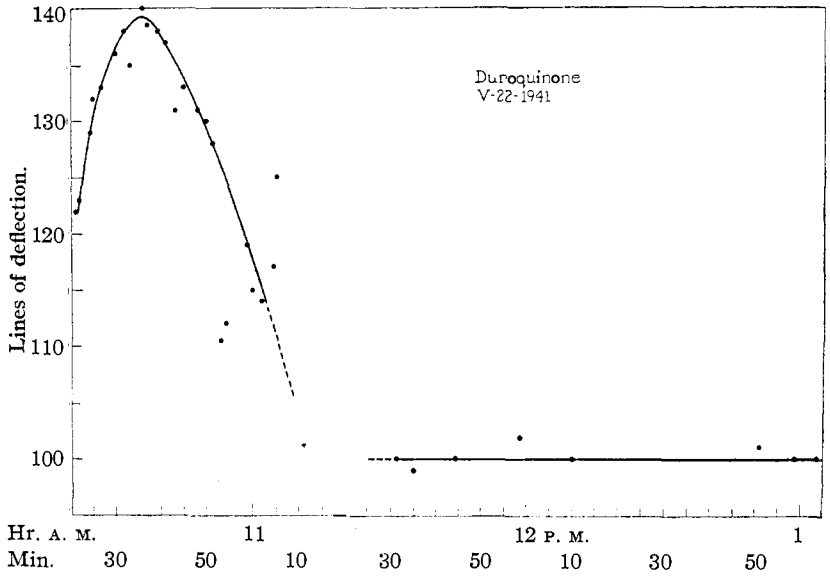

Fig. 1.—Solution of 0.232 g. of duroquinone; 1.10 cc. of 1.10 M sodium hydroxide, filled up with water to 10 cc., and 80 mg. glucose added. Ordinates: pull in lines of deflection (1 line corresponding to 2.42×10^{-10} unit of susceptibility, or to 0.000195 mole of a free radical per liter). Concentration of total duroquinone $[a] = 0.0142$; the maximum concentration of the radical $[s]$ is that corresponding to 39 lines of deflection; it is $= 0.00757$. Hence $[s/a]_{max.} = 0.53$.

As a first example an experiment with duroquinone is shown in Fig. 1. The solution was made up precisely as in experiment 9 of the previous paper[5] in which the maximum ratio of semiquinone to total dye was 0.48; the potentiometric data yielded 0.52. The present magnetic experiment gives 0.53. The agreement is very satisfactory, considering the fact that the total concentration of the dye is about a hundred times greater in the magnetic experiments than in the potentiometric one. It clearly indicates that neither the quinone nor the hydroquinone polymerizes on increasing the concentration, nor that

(4) E. C. Stoner, "Magnetism and Matter," London, 1934; W. Klemm, "Magnetochemie," Leipzig, 1936; J. H. Van Vleck, "Electric and Magnetic Susceptibilities," Oxford Univ. Press, 1932.

(5) Table II, THIS JOURNAL, 60, 1684 (1938).

the semiquinone radical undergoes any dimerization such as to form a diamagnetic compound analogous to benzoquinhydrone in the crystalline state.

Another example shows the development of the free radical on slow reduction of N,N′-dimethyl-γ,γ′-dipyridinium chloride, or methyl viologen.[6] In this case the reduction with glucose does not go to completion. The maximum concentration of the radical in the experiment shown in Fig. 2 corresponds to $[s] = 0.0081\ M$, which is only 42% of the total concentration of the substances used. Since the reduction by glucose does not go to completion even of the first step, this is just a qualitative experiment, showing the development of a radical.

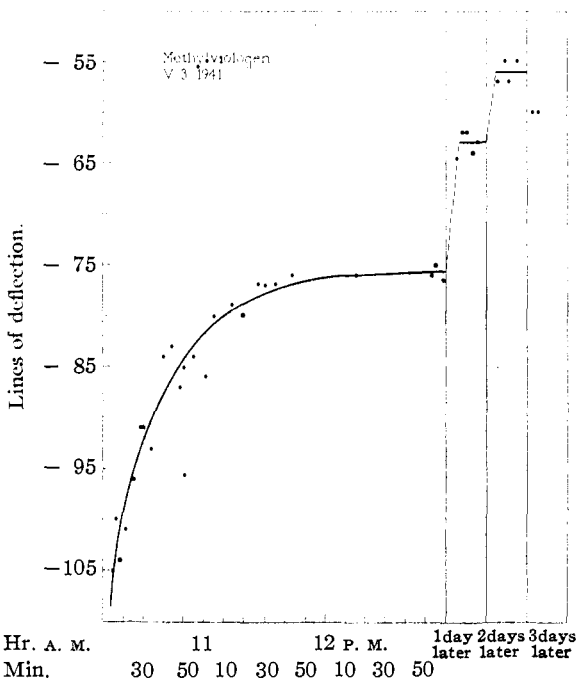

Fig. 2.—0.070 g. of methyl viologen, 1.5 cc. of 1.10 *M* sodium hydroxide, 60 mg. of glucose, filled up with water to 7.5 cc. Change of susceptibility in time. The maximum change is 50 lines of deflection, corresponding, for the vessel used, to a radical concentration of 0.0184 *M*; total concentration of the substance, 0.0422.

The following experiments show the free radical arising from partial reduction of thiazine dyestuffs in strongly acid solutions. No reducing agent is available such as would bring about the total two-step reduction at a rate slow enough to allow successive readings of the susceptibility. The following procedure was adopted. Choosing suitable dyestuffs and a suitable concentration of the acid, one can arrive at a condition such that the first and the second steps of reduction are distinctly separated and a considerable jump of the potential takes place when the first step of reduction is completed. In such a case ascorbic acid is a reducing agent which will bring about the first step of reduction but will not extend the reduction into the second step to any noticeable extent, even when using the ascorbic acid in excess. Observation of the color changes permits of trying for the best conditions under which this state of affairs prevails. The reduction by ascorbic acid is not instantaneous yet rather fast, and not slow enough to allow the observation of the change of susceptibility during the reduction. The dye is dissolved in sulfuric acid of suitable concentration and the magnetic pull is measured, using a similar solution of sulfuric acid without the dye as a compensatory liquid in the lower compartment of the vessel. After reading the deflection (average over 10 readings) a suitable amount of ascorbic acid is added and, when the reduction has proceeded as far as it can, that is to say, after ten to fifteen minutes, the readings are repeated. The difference in magnetic pull before and after addition of ascorbic acid reveals the concentration of the free radical produced by the reduction. A small correction is applied for the very slight change of magnetic pull brought about by the addition of ascorbic acid to the sulfuric acid. The correction due to the addition of ascorbic acid was found to be 5 lines of deflection, which must be subtracted from the total deflection. Two examples are shown, 3-aminothiazine and thionine. For aminothiazine the initial molar concentration of the dye was 0.0159, and the concentration of the free radical after reduction with ascorbic acid was 0.0119. For thionine, the initial molar concentration of the dye was 0.0178 *M* and that of the radical obtained from it, 0.0141 *M*. In both cases about 80% of the dye was converted into the free radical. In any case, these experiments show that even at such high concentrations of the dyes as exceed those used in the previous potentiometric experiments by more than a hundred times, no appreciable dimerization of the radical takes place.

Details of the Experiments.—(1).—0.045 g. of thionine (own preparation, $C_{12}H_{10}NSCl + H_2O$) is dissolved in 10 cc. of 22.6 *N* sulfuric acid; compensating liquid: the same acid without dye; magnetic pull at 10 amp. (average of 10 readings) −50.6 lines. Now 0.030 g. of ascorbic acid is added; pull, +33.7; difference, +84.1 lines; after

(6) (a) L. Michaelis and E. S. Hill, THIS JOURNAL, **55**, 1481 (1933); (b) L. Michaelis, *J. Gen. Physiol.*, **16**, 859 (1933).

TABLE I

OXONINE

	[a] Concn. of dye, *M*	Concn. of pyridine volume, %	Mol. concn. of NaOH	Mg. glucose added to 10 cc.	Max. difference of pull in lines of deflection	Max. concn. of semiquinone	$s/a_{max.}$	Plotted in Fig.
1	0.0200	20	0.0192	80	22	0.0037	0.185	3
2	.0200	20	.0176	80	17	.0029	.145	4
3	.0215	20	.0192	67	23	.00385	.192	5
4	.0189	20	.0176	40	18	.00302	.160	6
5	.0200	20	.0220	35	23	.00385	.197	7
						Average,	.18	
6	.0302	33	.0192	80	47	.00745	.246	

correction for addition of ascorbic acid, +79 lines. Herefrom: $[s] = 0.0141\ M$. The concentration of the total dye, $[a] = 0.0178\ M$; $[s/a] = 0.794$.

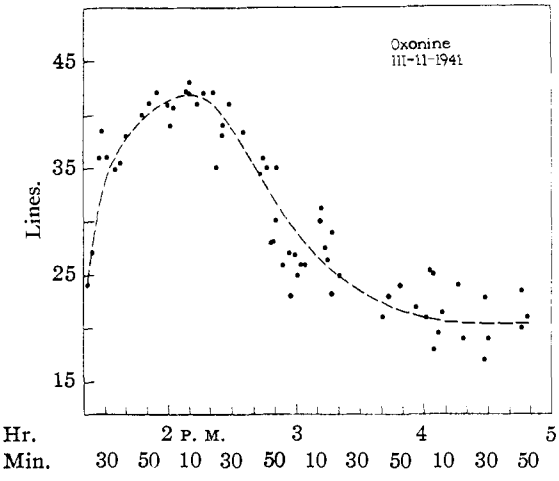

Fig. 3.

(2).—0.0400 g. of aminothiazine (preparation described in (1), $C_{12}H_9NSCl + {}^1/_2H_2O$) is dissolved in 10.4 cc. of 22.6 *N* sulfuric acid; pull, −20.8 lines. After addition of 0.025 g. of ascorbic acid, pull, +55.6 lines; difference, +76.4 lines; after correction, 71.4 lines. Hence, $[s] = 0.0119\ M$. Total concentration of dye, $a = 0.0159\ M$; $[s/a] = 0.750$.

The next example shows the development of the semiquinone radical of oxonine in alkaline solution as described recently by the potentiometric method.[7] Here the method of slow reduction by glucose with successive readings during the reduction can be used. The maximum concentration of the radical is rather low. It is practically limited by the solubility of the compounds, since the experiment has to be arranged so that a precipitate never arises. The results are shown in Table I and in Figs. 3 to 7.

(7) L. Michaelis and S. Granick, THIS JOURNAL, 63, 1636 (1941).

The change in susceptibility plotted against time is, in presence of a sufficient excess of glucose, an approximately parabolic curve. The beginning of the ascending branch cannot be observed because of the lapse of time from the mixing of the solution to the first reading. The descending branch reaches the abscissa at a sharp angle, and from here on no change takes place any more. However, when a smaller amount of glucose is used, the end of the reduction may be reached rather asymptotically, as can be seen by comparing Figs. 3 to 7, which are in order according to increasing amounts of glucose used. The table shows distinctly the limits of reproducibility. There can be no doubt that the final result of the experiments 1 to 5 in Table I, $[s/a]_{max.} = 0.18$, is uncertain to at least ±0.03. In order to compare this result with that previously obtained by the potentiometric method one has to start from the index potential obtained in strongly alkaline solution. This has been found to be 19.0 mv. (at 30°) and

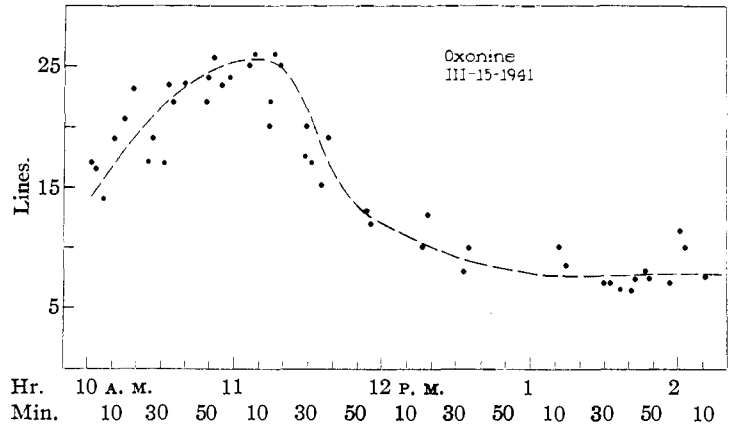

Fig. 4.

the probable error may be put = ±0.3 mv. From this we obtain $[s/a]_{max.} = 0.24 \pm 0.02$. Considering that the magnetic experiment was carried out at a concentration a hundred times

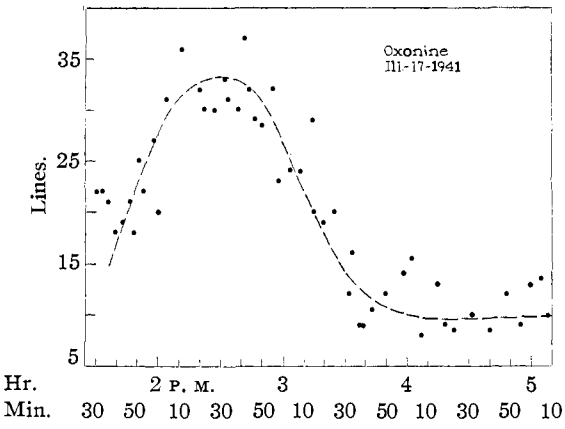

Fig. 5.

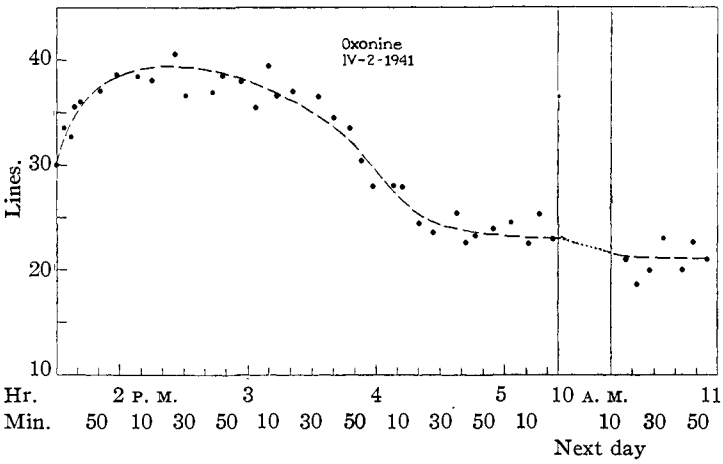

Fig. 6.

greater than the potentiometric one, the agreement may be said to be quite satisfactory. The discrepancy is not decidedly beyond the limits of error. One might imagine that it may be due to the fact that at such high concentrations as are used in the magnetic experiment any one of the molecular species involved has undergone a partial association to a polymeric molecule. However, even a very small tendency of association should have an enormous effect when varying the concentration a hundred times. At any rate it is safe to assume that the free radical even at the highest concentration used is not dimerized to any distinctly measurable extent to a dimeric non-paramagnetic molecule. As regards the molecular polymerization of the quinonoid form, it is true that the quinonoid dye, in the form of its singly charged cation, does undergo a partial polymerization at higher concentration,[8] although this effect is particularly small just for oxonine.[9] However, the uncharged base of oxonine, as used in these experiments, in a solution of 20% pyridine, containing 0.01 *M* sodium hydroxide, showed, according to spectrophotometric measurement, no evidence of polymerization. The molar absorption coefficient remains constant for all visible wave lengths at least on varying the concentration of the dye from 10^{-5} to 10^{-3} *M*. (The same is true for the uncharged base of thionine, of which the univalent cation very decidedly disobeys Beer's law.)

The result of experiment 6 in Table I agrees still better with the value obtained potentiometrically. The concentration of the dye was higher in this experiment, hence the limits of error are probably smaller. However, in order to attain a homogeneous solution of such concentration, 30% instead of 20% pyridine had to be added. It may not be entirely justifiable to compare this experiment with the others, and not too much emphasis will be put on this value.

Attempts to carry out similar experiments with thionine have failed so far. Not only is the maximum ratio of radical to total dye, according to the potentiometric experiments, much smaller than with oxonine (only one-third of the latter), but also the solubility conditions are less favorable

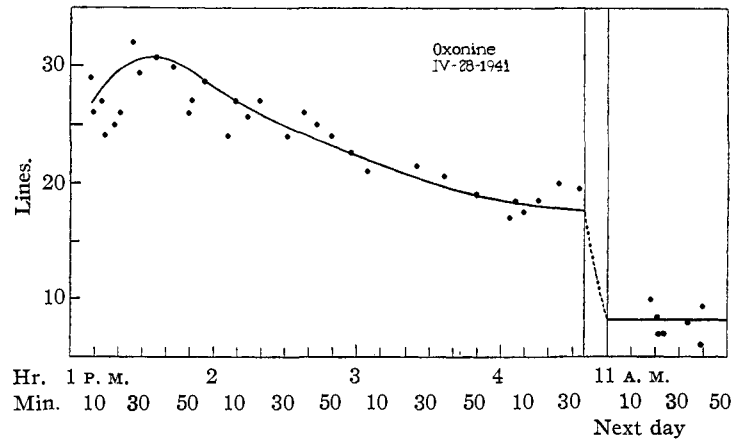

Fig. 7.

(8) E. Rabinowitch and L. F. Epstein. THIS JOURNAL. **63**, 69 (1941).

(9) *Cf.* Fig. 9 in Granick, Michaelis and Schubert, *ibid.*, **62**, 1802 (1940).

than with oxonine. No concentration of the dye could be reached high enough to preserve the homogeneity of the solution during the experiment and yet to raise the change in magnetic pull during the reduction above the limits of error of the method in its present state. Thus the magnetic measurement with thionine in alkaline solution at least confirms the result obtained potentiometrically, that the semiquinone formation constant is distinctly smaller than for oxonine, but no quantitative result could be obtained.

Summary

A differential magnetometric method is described by which the development of a semiquinone radical on partial reduction of organic dyestuffs can be recognized owing to the increment of susceptibility caused by the free radical. In some cases the difference in susceptibility is measured before and after adding the reducing agent (ascorbic acid), namely, in strongly acid solution of thiazine dyestuffs. In other cases the method of slow reduction is used, for example, in alkaline solution of oxonine, where glucose is added at the beginning, and the change of susceptibility is measured during the slowly progressing reduction. In the case of oxonine, the paramagnetic increment passes through a maximum.

The maximum concentrations of the free radical, calculated from the magnetic data, agree fairly well with those values previously obtained by the potentiometric method. In addition the data show that for the dyestuffs examined here no noticeable dimerization of the radicals takes place although the concentrations of the dyes are more than a hundred times higher than in the potentiometric experiments formerly described.

NEW YORK, N. Y. RECEIVED JUNE 3, 1941

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY]

The Exchange Reaction between Chloride Ion and Tertiary Butyl Chloride

BY WALTER KOSKOSKI,* HOWARD THOMAS AND ROBERT DUDLEY FOWLER

Introduction

Several ionization mechanisms have been studied by the method of radioactive indicators. Tuck[1] measured the rate of electrolytic dissociation of *t*-butyl iodide in sulfur dioxide solution and Nevell, de Salas and Wilson[2] studied exchanges in camphene hydrochloride. The latter case is of considerable interest since solvent effects and the effect of heavy metal chlorides[3] indicate that the ionization is the rate controlling step in the isomerization of camphene hydrochloride to isobornyl chloride. However, the exchange work was reported to indicate that the ionization as measured by radio chlorine exchanges was much too rapid to be the rate controlling step. Prior to any possible detailed investigation into the camphene hydrochloride exchanges it was decided to study exchanges in a similar but simpler compound. The similarities in the reactivities of *t*-butyl chloride and camphene hydrochloride have been pointed out by Meerwein and van Emster[3] so *t*-butyl chloride was chosen as an interesting compound in which to study exchanges. Formic acid was chosen as the solvent since the rate of hydrolysis[4] of *t*-butyl chloride has been studied in this solvent and it offers a convenient check of the exchange work.

Experimental

Materials.—*t*-Butyl chloride was prepared from *t*-butyl alcohol and concentrated hydrochloric acid. It was distilled and then redistilled from phosphorus pentoxide and the fraction boiling at 49.7° (742.4 mm.) was used. The formic acid was distilled from anhydrous copper sulfate and then further purified by repeated fractional freezing until the freezing point (8.4°) no longer changed. Solutions of the butyl chloride in formic acid were standardized by permitting a measured amount to hydrolyze overnight in water and then titrating the resulting chloride by the Volhard method. Preliminary experiments with weighed amounts of butyl chloride indicated that this was a satisfactory method for standardizing the organic chloride solutions. The radio-chlorine was prepared by bombarding chloroform with neutrons from the deuteron–deuteron disintegration apparatus in this Laboratory. After irradiation the active chloride was removed from the chloroform by shaking with water containing a little

* This work constitutes a portion of a thesis to be submitted by W. Koskoski in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Johns Hopkins University.

(1) J. L. Tuck, *Trans. Faraday Soc.*, **34**, 222 (1938).

(2) T. P. Nevell, E. de Salas and C. L. Wilson, *J. Chem. Soc.*, 1188 (1939).

(3) Meerwein and van Emster, *Ber.*, **53**, 1815 (1920).

(4) L. C. Bateman and E. D. Hughes, *J. Chem. Soc.*, 1187 (1937).