C. Comparison of Rate of Splitting of the Phenyl Glycosides of Lyxose, Mannose and Glucose

1. **Preparation of Enzyme Solutions.**—Two solutions were used for this work. The one, called "unheated," was the same as that used in the above work. The second, called "heated," was prepared by heating the first solution in a soft glass bottle for thirty-six hours at 50° and then centrifuging. Some evaporation occurred during this process. The dry residue determined in the same manner as for the "unheated" solution was 0.1765 g. in 10 cc.

2. **Preparation of Substrate Solutions.**—Equivalent solutions were prepared by dissolving the following amounts in 25 cc. of 0.1 *N* acetate buffer (*p*H 5.0): 0.240 g. α-phenyl-*d*-mannoside, 0.273 g. β-phenyl-*d*-glucoside-$2H_2O$ and 0.212 g. α-phenyl-*d*-lyxoside.

3. **Method of Making Measurements.**—To 1 cc. of the enzyme solutions, diluted 1:4 for the mannoside and glucoside experiments, there was added 2 cc. of the substrate solution and after the reaction was allowed to take place at 30° for the required time, it was stopped by the addition of 20 mg. of potassium carbonate. The optical rotation (D line of sodium) was then measured in 2-dm. tubes at 20° and the degree of hydrolysis calculated from the observed value and the known specific rotations of the unhydrolyzed glycosides and the corresponding sugars. The results are given in Table IV. The measurements on the lyxoside were carried out as described for the hydrolysis experiments reported in Table III and are given in that table.

TABLE IV

Enzyme solution	Time, min.	α_t	% Hydrolysis	$k \times 10^3$
β-Phenyl-*d*-glucoside, $\alpha_0 = -0.94$; $\alpha_\infty = +0.48$				
Heated	30.5	−0.60	23.9	3.90
Heated	80.0	− .22	50.7	3.84
Unheated	33.4	− .33	43.0	7.30
α-Phenyl-*d*-mannoside, $\alpha_0 = 1.39$; $\alpha_\infty = 0.12$				
Heated	33.0	1.10	22.8	3.41
Heated	79.8	0.87	40.9	2.87
Unheated	78.7	.79	47.2	3.53

Acknowledgment.—The writer wishes to express his appreciation of the coöperation, assistance and advice given by Professor B. F. Helferich during the progress of this investigation. The assistance given by the Lalor Foundation of Wilmington, Del., is also gratefully acknowledged.

Summary

The preparation of α-phenyl-*d*-lyxoside is reported. This substance is hydrolyzed by almond emulsin. The rate is the slowest reported for a hydrolyzable phenyl glycoside. The enzyme responsible for the hydrolysis is shown to be α-mannosidase. A prediction is made of the specificity to be expected of the enzymes found in almond emulsin and of other similar enzymes.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Laccase from the Wild Mushroom, *Russula Foetens*

BY DONALD C. GREGG[1] AND WILBUR H. MILLER

Much of the early information regarding the enzyme, laccase, resulted from the researches of Bertrand and his students.[1a] Bertrand reported that laccase, the enzyme responsible for the blackening of lac tree juices, could be distinguished from tyrosinase by its ability to catalyze the oxidation of polyphenols such as hydroquinone, pyrogallol and guaiacol. In recent years, the enzymatic oxidation of these substrates, and in addition *p*-phenylenediamine, has become a criterion of laccase activity.[2–5] Most of this work has been concerned with an enzyme prepared from lac tree juices. In 1939, Graubard[5] reported limited studies on an enzyme that he obtained from the wild mushroom, *Russula foetens*. This enzyme was called laccase because of its ability to catalyze the aerobic oxidation of hydroquinone and *p*-phenylenediamine. In view of the present limited knowledge regarding the action of the plant oxidases, a more extensive study on the laccase from *Russula foetens* seemed desirable.

The enzyme preparations were obtained by the general method used in these laboratories for the preparation of oxidases from mushrooms. In a typical preparation, 100 g. of dried mushroom powder was suspended in 500 cc. of water in a refrigerator for seventy-two hours. To the resulting crude extract, solid ammonium sulfate was added to make the solution 0.5 saturated. The dark colored, gummy precipitate was triturated with a small quantity of disodium phosphate and dissolved in water. For each volume of solution, two volumes of acetone were added. At room temperature, the acetone not only precipitates the

(1) Geo. W. Ellis Fellow, 1939–1940.
(1a) G. Bertrand, *Compt. rend.*, **118**, 1215 (1894).
(2) K. Suminokura, *Biochem. Z.*, **224**, 292 (1930).
(3) G. Books, *La Nature*, No. 3011, 359–363 (1937).
(4) D. Keilin and T. Mann, *Nature*, **143**, 23 (1939).
(5) M. Graubard, *Enzymologia*, *V*, 332 (1939).

laccase, but apparently inactivates the tyrosinase. Three or more adsorptions to alumina, each followed by elution and subsequent dialysis, removed much inert protein and all color. Such preparations had a dry weight of about 5 units (defined below) per mg. which corresponds to a Q_{O_2} of about 3000. Because of a very limited supply of this wild mushroom, it was not practical to further concentrate and purify the enzyme. For the same reason, no extensive attempt was made to correlate the copper content with enzyme activity. These laccase preparations were found to be unstable to heat treatment and were markedly inhibited in their action by cyanide.

The activity of the laccase preparations was determined by measuring the rate of oxygen uptake during the enzymatic oxidation of hydroquinone. This substrate was chosen since its oxidation by laccase is not measurably affected by the presence of other common plant oxidases. The measurements were made in Warburg respirometer flasks of 30 cc. capacity.[6] The rate of shaking was 120 complete oscillations per minute, and the temperature was maintained at 25°. The reaction mixture consisted of 1 cc. of an aqueous solution of hydroquinone (10 mg.) added from the side arm at zero time, 3 cc. of (0.2 *M*) citrate–(0.4 *M*) phosphate buffer (*p*H, 6.1), 1 cc. of an aqueous solution containing 5 mg. of gelatin, enzyme solution and sufficient water to make a total volume of 8 cc. The amount of enzyme causing an oxygen uptake of 10 cu. mm. per minute is defined as one "hydroquinone" unit. Under these conditions, the rate of oxygen uptake is directly proportional to the amount of laccase used provided the amount of enzyme in the flask is not much in excess of two "hydroquinone" units. Furthermore, the rate of oxygen uptake remains constant for periods of at least thirty minutes.

As previously mentioned, the rate of oxidation of hydroquinone by laccase is not significantly affected by the presence of other plant oxidases. This has been demonstrated by the introduction of varying quantities of other oxidases into the reaction mixture containing a fixed amount of laccase (0.90 "hydroquinone" units). The introduction of tyrosinase (*campestris*) preparations with catecholase activity varying from 10 to 60 units and cresolase activity ranging from 1 to 33 units[7] caused less than a 5% variation in the rate of oxidation of hydroquinone by the laccase. Ascorbic acid oxidase also was found to have very little effect, 142 units[5] causing a variation of less than 8%. These experiments were performed according to the procedure for measuring hydroquinone units of laccase except that the additional enzyme solutions replaced part of the water in the flask.

Since tyrosinase is without effect on the oxidation of hydroquinone by laccase, it cannot be detected by this procedure. However, with the catechol–hydroquinone mixture used for measuring catecholase activity,[7] the activity of these two enzymes appears to be additive. Thus, in a typical experiment, 0.68 "hydroquinone" units of laccase gave an oxygen uptake of 6.8 cu. mm. per minute with the catechol–hydroquinone mixture. 0.80 tyrosinase catecholase unit gave no measurable activity toward hydroquinone alone, but did cause an oxygen uptake of 8 cu. mm. per minute with the substrate mixture. Upon mixing the 0.68 laccase unit and 0.80 tyrosinase (*campestris*) catecholase unit, an oxygen uptake of 14.7 cu. mm. per minute was observed with the catechol–hydroquinone mixture. Therefore, if tyrosinase is present in the laccase preparations, the rate with the catechol–hydroquinone mixture should be greater than with hydroquinone alone. No such differences were observed with the laccase preparations used in this paper.

The marked stability of this enzyme to changes in *p*H over a wide range (*p*H, 2 to 9.5) is of considerable interest. In comparison with other oxidases of this type it appears to be more stable, the contrast in stability being especially marked in the lower *p*H regions. This is illustrated by the data in Table I in which tyrosinase has been used for comparison.

TABLE I[a]

SHOWING THE DIFFERENCE IN RESISTANCE OF LACCASE AND TYROSINASE PREPARATIONS TO *p*H INACTIVATION

Enzyme	Prep. no.	Units[a] cc. at start	*p*H	Exposed to indicated *p*H	Activity remaining, %
Laccase	Crude juice	0.18	2.20	20 days	70
Laccase	L-64	.40	1.95	13 days	65
Laccase	L-64	.20	1.50	13 days	17
Laccase	L-64	.20	1.10	30 min.	15
Tyrosinase	C-7-25-10	1.50	2.50	3 min.	2.2
Tyrosinase	C-7-25-10	2.25	2.20	1 min.	3.5
Tyrosinase	C-7-25-10	0.92	2.00	1 min.	0.0

[a] The tyrosinase units in the table are catecholase units.[7] All activities were measured at *p*H 6.10. Low *p*H values were obtained by using citric acid and acetic acid, the latter being used for all values below *p*H 2.0.

(6) M. Dixon, "Manometric Methods," Univ. Press, Cambridge, 1934.

(7) M. H. Adams and J. M. Nelson, THIS JOURNAL, **60**, 2479 (1938).

When laccase activities of equal quantities of the same enzyme preparation were determined at different *p*H values over a range of *p*H 2.6 to 8.1, a marked *p*H optimum was observed in the vicinity of *p*H 6.1 (Fig. 1, Curve I). With other substrates such as catechol the same *p*H optimum effect was observed (Curve II, Fig. 1). The apparent decrease in laccase activity observed on both sides of the *p*H optimum is a reversible effect. This was demonstrated by bringing the *p*H to 6.10 after a fifteen to twenty minute reaction period at the indicated *p*H. In all cases the optimum rate of oxidation was observed within experimental error. The reason for measuring laccase activity at a *p*H 6.10 is now apparent.

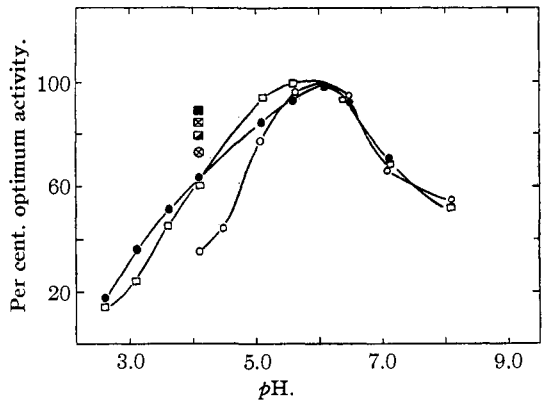

Fig. 1.—Showing the combined effect of *p*H and substrate concentration on the percentage optimum activity of laccase based on the maximum obtainable rate at *p*H 6.10. Oxygen uptake determined by means of Warburg respirometer (Barcroft differential manometers used for *p*H 7.1 and 8.1), temperature 25°. Citrate–phosphate buffers were used in all cases. ○, Curve I, reaction mixture: 3 cc. buffer, 1 cc. gelatin solution (5 mg.), 1 cc. hydroquinone solution (10 mg.) (added from side arm at zero time), 1 cc. laccase preparation containing 1 unit, and sufficient water to make final volume in reaction vessel 8.0 cc. ●, Curve II, reaction mixture same as for Curve I except 1 cc. catechol solution (25 mg.) in place of hydroquinone. □, Curve III, reaction mixture same as for Curve I, 1 cc. hydroquinone solution containing 25 mg. instead of 10 mg. The additional data noted at *p*H 4.10 were obtained using the same reaction mixture as for Curve I except for the following substrate concentrations: ■, IV, 75 mg. catechol; ⊠, V, 1.5 cc. hydroquinone solution (75 mg.); ◭, VI, 50 mg. catechol; ⊗, VII, 40 mg. hydroquinone.

The activity of laccase (*Russula foetens*) is dependent on substrate concentration as well as hydrogen-ion concentration. Thus, for each substrate, a concentration has been observed above which further increase in substrate caused no appreciable increase in the rate of oxidation. This value of concentration varies considerably with the choice of substrate (Fig. 2). After a consideration of the results shown in Fig. 2, 10 mg. of hydroquinone was chosen as a suitable concentration for the measurement of laccase units. For the measurement of maximum activity on catechol, 25 mg. has been used.

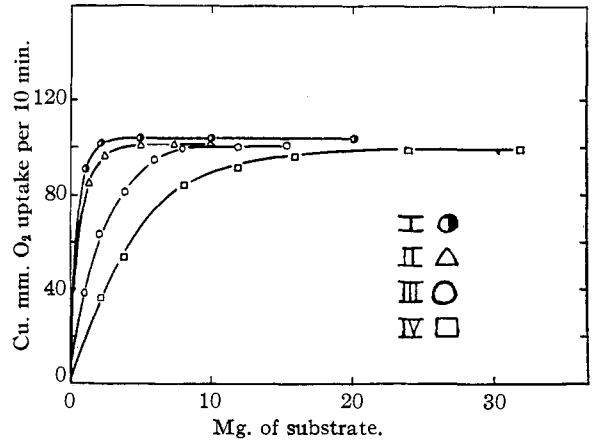

Fig. 2.—The effect of concentration of substrate on laccase activity, using four different substrates at *p*H 6.10. The rate of oxidation was determined by means of Warburg respirometers; temperature 25°. ◑, Curve I, reaction vessels contained: 1 cc. enzyme solution (1.0 unit), buffer, gelatin, aqueous *p*-phenylenediamine solutions to give indicated concentrations, and sufficient water to make the final volume 8.0 cc. △, Curve II, reaction mixtures same as for Curve I except dimethyl catechol was used as substrate. ○, Curve III, same as for Curve I, hydroquinone as substrate. □, Curve IV, same as for Curve I, catechol as substrate.

Curve III, Fig. 1, represents the results of a *p*H optimum study of laccase using 25 mg. of hydroquinone. It will be noted that this curve is similar to that obtained using 25 mg. of catechol (Curve II, Fig. 1). A comparison of Curve III with Curve I (dataf rom 10 mg. hydroquinone) demonstrates that the effect of substrate concentration is more pronounced at *p*H values below the optimum. Additional data obtained at *p*H 4.10 have been included in Fig. 1 to emphasize this point. The maximum rate obtainable at *p*H 4.10 is that obtained using 75 mg. of catechol or hydroquinone. It is interesting to note that this rate does not equal that obtained at the optimum *p*H (6.10), using much smaller concentrations of substrate (Fig. 2). In the case of catechol at *p*H 4.10, an increase in substrate from 25 mg. to 75 mg. caused about a 25% increase in rate, yet this rate was less than the optimum value at *p*H 6.10 by about 12%. A similar

increase in rate was observed with hydroquinone, the maximum rate being about 18% less than the optimum rate. Due to the fact that on the alkaline side of the *p*H optimum, both hydroquinone and catechol show an increasing tendency to undergo autoxidation, enzymatic studies become less reliable in this region. This is especially true with substrate concentrations larger than those used to obtain maximum activity at *p*H 6.10 (Fig. 2). For this reason, the alkaline side of the *p*H optimum has not been studied as extensively as in the acid regions. Regardless of this fact, the data in Fig. 1 clearly support the contention that laccase exhibits a *p*H optimum, and the nature of the *p*H effect is dependent on the substrate concentration used during the study.

Laccase preparations from *Russula foetens* are unusual in their ability to catalyze the aerobic oxidation of a wide variety of substrates. Because of this ability it is probable that these activities will be confused with those of other plant oxidases. This has been the case with laccase preparations reported from other sources. Before this confusion in classification can be eliminated, careful comparisons of these activities must be made. For future study of the relative abilities of these enzymes to react with various substrates, it is the writers' contention that unless the maximum activity of the enzyme toward the substrate at any given *p*H is obtained the results are of little comparative value. Comparable studies of the catalytic effect of these laccase preparations on the oxidation of certain common substrates follow.

Hydroquinone.—The aerobic oxidation of hydroquinone by laccase involves one atom of oxygen per molecule of substrate (Table II), the oxidation of the product *p*-quinone being immeasurably small. The effect of substrate concentration on the rate has been discussed. No appreciable inactivation was noted under normal conditions over periods of one to two hours. Chlorohydroquinone was similar in its behavior (Table II).

***p*-Phenylenediamine.**–This substrate has been used for the measurement of laccase activity by other investigators.[4] Since *p*-phenylenediamine is not as water soluble, is more easily autoxidized, and apparently has a more complex reaction mechanism than hydroquinone, it is felt that the latter substrate is a better choice for the measurement of laccase activity. While the rate with *p*-phenylenediamine approximates that of hydroquinone and remains constant over a concentration range of 2 to 20 mg. of substrate (Fig. 2), the total oxygen uptake is indefinite (Table II).

TABLE II

SHOWING THE TOTAL OXYGEN UPTAKE CORRESPONDING TO THE TOTAL OXIDATION OF 1 MG. OF SUBSTRATE BY TWO UNITS OF LACCASE FOR FIVE DIFFERENT SUBSTRATES AT *p*H 6.10

Substrate	Total uptake in cu. mm. corresponding to 1 atom oxygen per mg. substrate	Total observed oxygen uptake in cu. mm. using 1 mg. of substrate	No. of atoms of oxygen per molecule of substrate
Hydroquinone	102	106	1
Chlorohydroquinone	77	80	1
p-Phenylenediamine	104	145+	1.4+
Catechol	102	207	2
Dimethylcatechol	81	83	1

Catechol.—The enzymatic oxidation of catechol by laccase (*Russula foetens*) occurs at nearly the same rate as *p*-phenylenediamine and hydroquinone (Fig. 2). Some laccase preparations described in the literature,[4] however, are reported to oxidize *p*-phenylenediamine twice as rapidly as catechol. No substrate concentrations were given and so no conclusion concerning the similarity or dissimilarity of the laccase preparations may be made. The aerobic oxidation of catechol by our laccase preparations involves two atoms of oxygen per molecule of substrate (Table II). This behavior is similar to that of tyrosinase.[8] However, the apparent absence of inactivation of laccase in the oxidation of catechol in contrast to the marked inactivation experienced by tyrosinase (*campestris*) indicates the dissimilarity of the two oxidases. According to Ludwig and Nelson[9] one unit of purified *campestris* tyrosinase is completely inactivated after an oxygen uptake of approximately 100 cu. mm. According to their definition of a catecholase unit, this means that one unit of tyrosinase remains active with catechol for only about ten minutes. The writers have found that one unit of laccase with 24 mg. of catechol caused an oxygen uptake of 3100 cu. mm. during a period of eight hours. During this eight hour period the gradual drop in rate of oxidation was little more than that to be expected due to the constantly decreasing concentration of catechol. In other words, there appeared to be little inactivation of the laccase during this long period of oxidation.

Dimethylcatechol.—The behavior of dimethylcatechol with laccase contrasted to that of the

(8) H. Wagreich and J. Nelson, THIS JOURNAL, **60**, 1545 (1938).
(9) B. Ludwig and J. Nelson, *ibid.*, **61**, 2601 (1939).

closely related substrate catechol is of interest from two viewpoints. First, the mode of oxidation differs from that of catechol in that the reaction proceeds rapidly to an oxygen uptake of one atom per molecule (Table II). Further oxidation is very slow. Second, the same rate of oxidation was observed over a concentration range of 2 to 10 mg. of substrate (Curve II, Fig. 2).

Potassium Ferrocyanide.—Laccase preparations (*Russula foetens*) have been found to have the ability to catalyze the oxidation of potassium ferrocyanide. This enzymatic oxidation of an inorganic salt is rather unusual. In measuring the activity of the enzyme on this substrate, Barcroft differential manometers[6] were used to avoid any possible errors due to autoxidation of the salt. One "hydroquinone" unit of laccase with 5 mg. of potassium ferrocyanide resulted in an oxygen uptake of 10 cu. mm. per ten minutes. With this concentration of potassium ferrocyanide, the enzyme appeared to have very little catalytic effect. However, when the concentration of this substrate was increased to 100 mg., one "hydroquinone" unit of laccase caused an oxygen uptake of 70 cu. mm. per ten minutes. This rate of oxidation did not appear to be further increased by larger concentrations of substrate. From these results, potassium ferrocyanide seems to compare favorably with certain organic substrates.

Less detailed studies have been made on some other substrates. More work on these is being carried out along the lines of the above discussion.

Discussion

Measurement of Laccase Activity.—A practical method for the measurement of enzyme activity should be specific, reproducible, and convenient. The method that has been proposed herein for the measurement of laccase activity meets these requirements. The convenience of using the Warburg respirometer in following oxidase activity is well established. The high reproducibility of the method is a result of the fact that during the laccase-catalyzed oxidation of hydroquinone, the rate of oxygen uptake remains constant over periods of a half hour to an hour, thus allowing several observations on the rate to be made. The specificity of the method is demonstrated by the fact that relatively large concentrations of other plant oxidases have no appreciable effect on the measurement of laccase activity.

Effect of *p*H.—In defining the experimental conditions for the measurement of laccase activity, it was stated that the reaction mixture should be buffered to about *p*H 6. This is necessary since it was found experimentally that the same quantity of enzyme caused lower rates of oxygen uptake at higher and lower *p*H values than the one indicated. The fact that this *p*H optimum effect is reversible shows that the enzyme is not inactivated. This *p*H optimum effect is partially offset by increasing the substrate concentration. The marked stability of laccase over a wide *p*H range, and especially at the low *p*H values, is in contrast to the behavior of many plant oxidases, in particular tyrosinase with which it has been compared.

Substrate Concentration Effects.—The apparent activity of a fixed quantity of laccase varies with the substrate concentration up to a certain value above which further increase in substrate concentration causes no greater rate of oxygen uptake. It has been shown that this optimum substrate concentration varies with different substrates. Likewise, different enzymes which act on the same substrate show a difference in the amount of that substrate needed for optimum activity. Because of these facts, the substrate concentrations used have been fixed to ensure maximum activity at a definite *p*H. It is felt that this general procedure of fixing conditions of substrate concentrations and *p*H relative to a definite quantity of enzyme for maximum activity is necessary in all cases where a differentiation between the laccase preparations and other oxidase preparations is to be made.

Substrate and Enzyme Behavior.—The reproducibility of measuring laccase units has been found to result largely from the stability of the enzyme during the oxidation of hydroquinone. This stability has been observed with other substrates, especially catechol. In contrast to tyrosinase, which is completely inactivated by catechol after a short period of time, laccase does not appear to be inactivated to any appreciable extent even after a long reaction period. The oxidation of substrates considered here appears to proceed in a manner similar to that with other enzymes, except for the extent of enzyme inactivation.

Acknowledgment.—The authors wish to express their appreciation to Professor J. M. Nelson for his interest and friendly advice during this investigation. They are grateful to Dr. C. R.

Dawson for his help and constructive criticism in preparing the material for publication.

Summary

1. A method is described for the preparation of the enzyme, laccase, from the wild mushroom, *Russula foetens*. A typical preparation has a Q_{O_2} of 3000.

2. A measurement of units using hydroquinone as the substrate is outlined. Common plant oxidases do not appear to interfere in this procedure.

3. The unusual stability of the laccase preparations over a wide *p*H range is demonstrated.

4. The optimum *p*H for measurement of laccase activity is shown to be in the vicinity of *p*H 6.0.

5. The enzymatic oxidation of hydroquinone, *p*-phenylenediamine, catechol, dimethylcatechol, and potassium ferrocyanide has been described.

6. The behavior of the laccase preparations is contrasted when possible with tyrosinase (*campestris*).

7. The importance of substrate concentration in studying the behavior of the enzyme has been emphasized.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WASHINGTON]

The Ionization Constant of Monoethanolammonium Hydroxide at 25° from Electrical Conductance Measurements

BY V. SIVERTZ, R. E. REITMEIER AND H. V. TARTAR

In this investigation use has been made of the work of several investigators[1-5] who have shown that accurate conductance measurements of solutions of weak electrolytes are adequate for the determination of limiting equivalent conductance and of thermodynamic ionization constants. The method finally developed is based upon the solution of three simultaneous equations. The first is

$$K = \frac{Cx^2f_{\pm}^2}{1-x} = K'f_{\pm}^2$$

where K and K' are the thermodynamic and stoichiometric ionization constants, respectively, x is the degree of dissociation, C is the concentration of base in equivalents per liter of solution and $f_{\pm}$ is the mean activity coefficient of the ions. The activity coefficient of the undissociated base is assumed to be unity. The second equation is

$$-\log f_{\pm} = a\sqrt{Cx}$$

where a is the Debye and Huckel coefficient. Lastly

$$x = \frac{\Lambda}{\Lambda_0}\left[1 + \frac{(\alpha\Lambda_0 + \beta)}{\Lambda_0}\sqrt{Cx}\right]$$

where α and β are the Onsager coefficients.

The authors have determined the electrical conductance at 25° of aqueous solutions of monoethanolammonium hydroxide. Since we were unable to obtain from the literature an accurate value for the equivalent conductance of the hydroxyl ion, the conductances of sodium and potassium hydroxide solutions have also been measured over a limited range of concentrations.

Experimental

Apparatus and Technique.—A Leeds and Northrup conductivity bridge[6,7] was used. The conductance cell, used with nitrogen, was similar in design to a cell described by Shedlovsky[8] and made of Pyrex glass. The electrode chamber met the specifications given by Jones and Bollinger.[9] Because of the magnified effects due to adsorption at very dilute concentrations, platinization was limited to the minimum value to prevent polarization. The electrodes were given a light gray coating, using the procedure suggested by Jones and Bollinger.[9] The cell constant at 25° was of the order of 0.26228 and was determined by comparison with a cell which had been calibrated with 0.01 demal potassium chloride, the specific conductance of which was taken from the recent work of Jones and Prendergast.[10] The cell was cleaned before each run with redistilled alcohol and ether and dried under nitrogen. This procedure produced no undesirable effects either on the character of the platinized electrodes or in the operation of the cell.

The current was supplied to the bridge by a Leeds and Northrup a. c. oscillator, the frequency of which could be varied in steps over a range of 500 to 2000 cycles. Several trials in each series of measurements showed that the conductance was essentially independent of the frequency. A Leeds and Northrup two-stage amplifier and telephone formed the detector circuit.

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(2) Fuoss and Kraus, *ibid.*, **55**, 476 (1933).
(3) Shedlovsky and Uhlig, *J. Gen. Physiol.*, **17**, 549 (1934).
(4) Fuoss, THIS JOURNAL, **57**, 488 (1935).
(5) Shedlovsky, *J. Franklin Inst.*, **255**, 739 (1938).
(6) Dike, *Rev. Sci. Instruments*, **2**, 379 (1931).
(7) Jones and Josephs, THIS JOURNAL, **50**, 1049 (1928).
(8) Shedlovsky, *ibid.*, **54**, 1411 (1932).
(9) Jones and Bollinger, *ibid.*, **53**, 411 (1931).
(10) Jones and Prendergast, *ibid.*, **59**, 731 (1937).