Experimental

(A) **Preparation of 2-Octyl Nitrite by the Action of Nitrosyl Chloride on 2-Octanol.**—In a 500-cc., 3-necked flask fitted with a Hershberg-type tantalum stirrer, thermometer, and a calcium chloride drying tube was placed 78.0 g. (0.60 mole) of 2-octanol $n^{20}D$ 1.4264 (prepared by the action of methylmagnesium iodide on *n*-heptanal) and 240 cc. of dry pyridine (freshly distilled from barium oxide). Over a period of two and one-half to three hours 60 g. (0.95 mole) of liquid nitrosyl chloride[7] was allowed to evaporate into the stirred alcohol–pyridine solution; during this time the temperature of the mixture was kept between 0 and 10°. As soon as all the nitrosyl chloride had been introduced 100 cc. of water and 100 cc. of petroleum ether (35–37°) were run in at such a rate that the temperature of the mixture did not rise above 20°. The petroleum ether phase was separated, washed once with dilute hydrochloric acid, once with water and then dried over potassium carbonate. The solvent was removed and the residue rectified under reduced pressure through an 18″ modified Widmer column[8] fitted with a total reflux head. There was obtained 76.1 g. (80%) of a pale yellow-green liquid: b. p. 60–61° (15 mm.); d^{20}_4 0.8644; $n^{20}D$ 1.4082; M_d (calcd.) 45.48[9], M_d (found) 45.44.

Anal. Calcd. for $C_8H_{17}NO_2$: C, 60.24; H, 10.70; N, 8.80. Found: C, 60.44, 60.42; H, 10.67, 10.67; N, 8.74.

(B) **By the Action of Sodium Nitrite and Sulfuric Acid on 2-Octanol.**—Sixty-five grams (0.50 mole) of 2-octanol, $n^{20}D$ 1.4260 (Eastman Kodak Co. White Label material purified through the acid phthalate ester[10]) was converted to the nitrite ester according to the procedure of Pezold and Shriner[6] with but slight modifications. These were: (a) the temperature of the reaction mixture was kept between 0–5° during the addition of sulfuric acid, (b) the crude ester was dried over potassium carbonate, and (c) the crude ester was vacuum fractionated through a modified Widmer column fitted with a total reflux head. There was obtained 47 g. (59%) of pale yellow-green liquid having b. p. 50–51° (9 mm.); d^{20}_4 0.862; $n^{20}D$ 1.4083; M_d (calcd.) 45.48,[9] M_d (found) 45.48. Calcd. for $C_8H_{17}NO_2$: N, 8.80. Found: N, 8.60.

(7) Nitrosyl chloride may be obtained in small tanks from the Solvay Process Co., Hopewell, Va.

(8) Smith and Adkins, THIS JOURNAL, **60**, 657 (1938).

(9) Values for the calculation of the molecular refractivity were taken from Leermakers and Weissberger in Gilman's "Organic Chemistry," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 1751, and Cohen, "Organic Chemistry," Vol. II, p. 27, Edward Arnold and Co., London, 1928.

(10) Ingersoll, "Organic Reactions" (edited by Roger Adams), Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 400.

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Measurement of the Cresolase Activity of Tyrosinase

BY M. FRANK MALLETTE[1] AND CHARLES R. DAWSON

As the result of experience with highly purified preparations of mushroom tyrosinase it has been found necessary to develop a new method for determining the enzyme's catecholase activity[2] and now to modify the manometric method of Nelson and co-workers[3,4] for measuring the monophenolase (cresolase) activity of the enzyme. The changes have been made necessary by the observation that the highly purified enzyme rapidly loses activity when allowed to stand in a highly diluted condition such as is necessary for the usual activity measurement. The enzyme is much more stable, however, in relatively concentrated solutions or solutions containing other protein matter.

It has been the practice of earlier workers in this and other laboratories to equilibrate the manometer flasks and contents in the 25° thermostat for about fifteen to twenty minutes prior to mixing the enzyme and *p*-cresol to initiate the enzymatic oxidation of the latter. Recently it has been observed, however, that the sooner the reaction is started after the enzyme has been diluted, the higher is the measured rate of oxygen uptake. In other words, inactivation of the highly diluted enzyme occurs during the temperature equilibration period.

The data of Table I show how the measured cresolase activity of a purified tyrosinase preparation depends on the length of time that the enzyme stands in the highly diluted condition prior to measurement. In practice it was found difficult to start the activity measurements in much less than three minutes after making the final enzyme dilution. The data shown in Table I were obtained using the highly purified high catecholase enzyme C175BI prepared from the common mushroom, *Psalliota campestris*, by procedures described elsewhere.[5] The stock solution contained 34 cresolase units per ml. as measured by the modified procedure given below.

TABLE I

THE EFFECT OF STANDING IN COLD DILUTE SOLUTION (0 TO 2°) ON THE MEASUREMENT OF THE CRESOLASE ACTIVITY OF THE HIGHLY PURIFIED TYROSINASE PREPARATION C175BI (DILUTION FACTOR = 21)

Time between dilution and addition to reaction mixture, min.	3	28	60	95
Oxygen uptake, cu. mm./min.	16.3 ± 0.2	12.2 ± 0.2	5.6 ± 0.3	1.4 ± 0.1
Measured cresolase units in flask	1.63	1.22	0.56	0.14
Activity of stock solution, units per ml.	34.2	25.6	11.8	2.9

To obtain the rate data shown in the table, the stock solution of enzyme was diluted with 20 volumes of ice-cold water and 1.00 ± 0.01 ml. aliquots were used. The time intervals recorded represent the minutes elapsed between the dilution of the stock enzyme solution and its addition to the reaction mixture. As can be seen from the table, a marked inactivation of highly purified enzyme may occur when it is allowed to stand in the highly diluted state for long periods of time

(1) Present address: Department of Chemistry, Cornell University, Ithaca, N. Y.

(2) W. H. Miller, M. F. Mallette, L. J. Roth and C. R. Dawson, THIS JOURNAL, **66**, 514 (1944).

(3) M. H. Adams and J. M. Nelson, *ibid.*, **60**, 2472 (1938).

(4) D. C. Gregg and J. M. Nelson, *ibid.*, **62**, 2500 (1940).

(5) M. F. Mallette, Stanley Lewis, Stanley R. Ames, Charles R. Dawson and J. M. Nelson, to be published.

even though maintained at low temperature. For this reason the procedure for measuring the cresolase activity of tyrosinase has been modified to eliminate the fifteen to twenty minute temperature equilibration period prior to initiating the reaction by mixing the enzyme and substrate. The *p*-cresol reaction is characterized by a lag period that varies in length with a number of factors including the purity of the enzyme.[6] During this lag period the enzyme does not appear to be seriously inactivated. The presence of substrate and protective protein gelatin prevents the type of inactivation shown in Table I which occurs when the enzyme stands alone in highly diluted solution. The modified procedure, described below, utilizes the lag period as the temperature equilibration period prior to closing the manometers.

Experimental

The procedure developed for measurement of the cresolase activity is as follows: The cold stock solution of enzyme was diluted with ice-cold water to the proper volume for activity measurement and 1.00-ml. aliquots were added immediately to reaction mixtures already prepared from 2.0 ml. of McIlvaine buffer *p*H 7.0, 1.0 ml. of *p*-cresol solution (4 mg. per ml.), 1.0 ml. of gelatin solution (5 mg. per ml.) and enough water to make the final reaction volume 8.0 ml. (including the enzyme solution). The enzyme was added last and directly to this reaction mixture just prior to placing the respirometer flasks on the manometers (Warburg type). The flasks with attached manometers were then placed in a 25.00 ± 0.01° thermostat, and the shaker was started immediately (120 oscillations per minute). The reaction was allowed to proceed for five to ten minutes with the manometers open until temperature equilibrium was established. During this time, little oxygen was absorbed because of the lag period of the reaction. After closing the manometers, readings were taken at five-minute intervals throughout the linear course of oxygen uptake (usually twenty to thirty minutes). The units of activity were calculated from the rate of oxygen uptake during this linear period, using an uptake of 10 cu. mm. per minute as equal to one unit of cresolase activity. In accordance with the experience of previous workers in this Laboratory, it was found desirable to dilute the enzyme so that the reaction flask contained between 0.5 and 2.5 units. All activity measurements were made in triplicate using a fourth flask containing everything except enzyme as a barometric control.

(6) J. M. Nelson and C. R. Dawson, *Adv. Enzymology*, **4**, 99 (1944).

DEPARTMENT OF CHEMISTRY
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The Conductance of Tetraethylammonium Sulfamate in Liquid Hydrogen Sulfide[1]

BY EDGAR E. LINEKEN[2]

The original purpose of this investigation was to find the effect of varying the number of alkyl groups substituted for the hydrogen atoms of the ammonium ion on the conductance of substituted ammonium sulfamates in liquid hydrogen sulfide. This effect for substituted ammonium chlorides has been reported by Lineken and Wilkinson.[3] The conductance of a partially substituted ammonium sulfamate might be expected to be much lower than that of completely substituted ammonium sulfamate because of the probable attraction of the amino group present in the sulfamate ion for an unsubstituted hydrogen in the ammonium ion (in addition to the well-known attraction of an unsubstituted proton of an ammonium ion for the negative ion of a salt when dissolved in a liquid of low dielectric constant).

The conductance of tetraethylammonium sulfamate was found to be comparable in magnitude with the conductance of tetraethylammonium chloride. The solubility of trimethyl- and *n*-propylammonium sulfamates and of ammonium sulfamate each appeared to be nil and the conductance of liquid hydrogen sulfide did not increase upon agitation with either of these compounds. Thus the effect of incomplete substitution was to reduce greatly the solubility of the compounds and the purpose mentioned above was not realized.

Experimental

Sulfamates.—These were all prepared from sulfamic acid prepared from urea according to the method of Baumgarten.[4] The partially substituted ammonium sulfamates and ammonium sulfamate were prepared by the methods described by Butler and Audrieth[5] for those compounds. Tetraethylammonium sulfamate was prepared by adding sulfamic acid to an excess of the hydroxide (10% aqueous solution). The salt was recovered by evaporation and dried with dry air under reduced pressure. After washing with anhydrous ether, the drying was repeated. The resulting product was not sufficiently pure and it was then recrystallized from amyl acetate, washed with anhydrous ether, and dried at 110°.

Table I shows the calculated and determined sulfur values for these compounds and the melting points of some of them. Sulfur was determined as barium sulfate after converting the sulfamate to sulfate with nitrite in acid solution.[5] The purity of *n*-propylammonium sulfamate was not checked because a test showed that it appeared not to dissolve in the solvent and, if a trace did dissolve, it did not conduct. The other compounds which behaved similarly were analyzed before being so tested.

TABLE I

Compound, sulfamate	Sulfur, % Calcd.	Found	M. p.,[a] °C.
Ammonium	28.09	28.14, 27.90	.....
Trimethylammonium	20.51	20.27	150–152
Tetraethylammonium	14.17	14.17	152

[a] These melting points were determined with an uncalibrated thermometer. Sharpness of melting, as a criterion of purity, rather than the exact melting point, was of interest.

Solvent and Method of Measurement of Conductance.—Hydrogen sulfide was prepared, purified and liquefied as previously described.[3] The equipment and method used for conductance measurements were as outlined by

(1) The work described in this paper was done at the University of Vermont, Burlington, Vermont.

(2) Present address: Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey.

(3) E. E. Lineken and J. A. Wilkinson, THIS JOURNAL, **62**, 251–256 (1940).

(4) P. Baumgarten, *Ber.*, **69**, 1929–1937 (1936).

(5) M. Josetta Butler and L. F. Audrieth, THIS JOURNAL, **61**, 914–915 (1939).