which was alongside the 800-mg. samples during each irradiation.

Since the success of the Szilard–Chalmers reaction is known to be dependent on the level of the γ-radiation in the pile it was desirable that all samples be irradiated under the same conditions.[2] The tetraphenyltin samples used in experiments 1 and 2 were alongside each other in the pile during the irradiation as were the samples used in experiments 3 and 4. It is believed that the γ-fluxes during these two irradiations were approximately the same because the beta activity of the samples used in experiments 3 and 4 was only 15% higher than that of the samples used in experiments 1 and 2.

The β-activity of the 1.1-day Sn^{121} was detected in all experiments. During the two-hour irradiation the activities of Sn^{113} and Sn^{123} produced are negligible compared to that of Sn^{121}. The 2.7-year Sb^{125} activity that is produced through decay of the 10-minute Sn^{125} is also negligible compared to the Sn^{121} activity. The tin was precipitated as stannic phenylarsonate,[3] suspended in 5 ml. of 95% alcohol and then prepared for counting by filtration on a tared, 24 mm. diameter, Whatman #50 filter paper, dried for 30 minutes at 110°, and weighed. The counting samples measured 14 mm. in diameter and were mounted on 2 × 2.5 in. cardboard cards with scotch tape placed directly over the sample. These cards were placed in a holder which fixed the position of the sample relative to an Amperex #100 C Geiger–Muller tube. All counting samples had activities within the range from 600 to 6000 counts per minute, and in this range the response of the counter was linear. The thickness of the samples, which was of the order of 28 mg./cm.^{2}, varied as much as 2 mg./cm.^{2}, necessitating corrections for self-absorption of the β-particles. The self-absorption correction amounted to a 1.15% increase in the total counting rate of a sample for each milligram increase in sample weight.

In order to determine the tin activity in the aqueous phase a 5-ml. aliquot was warmed slightly to remove traces of benzene, and then treated with a drop of liquid bromine so that all the tin present would be in the stannic state. The excess bromine was removed by gentle heating, and 1 ml. of a solution of stannic chloride containing 10 mg. of tin per ml. was added. The tin was precipitated as stannic hydroxide which was then dissolved in a minimum of 6 *f* hydrochloric acid. One-half ml. of 6 *f* hydrochloric acid was added in excess, and the sample diluted to 5 ml. with water. The sample was heated to 90° and then 5 ml. of a saturated aqueous solution of phenylarsonic acid was added. After a digestion period of ten minutes at 90°, the sample was cooled, allowed to stand at room temperature for 15 minutes, and then mounted for counting. The percentage of tin in the precipitate formed under these conditions was equal to that calculated from the formula of stannic phenylarsonate within a probable error of 1%.

The tin activity in the benzene phase was determined by evaporating an aliquot to dryness, ashing the tetraphenyltin residue with concentrated sulfuric acid and 30% hydrogen peroxide, and finally precipitating the tin as stannic phenylarsonate which was then mounted for counting. The tin activity in the 36-mg. sample of tetraphenyltin was determined in the same way.

In all the experiments only about 80% of the total tin activity was recovered. It turned out that approximately 80% of the activity lost was adsorbed on the walls of the beaker in which the irradiated solid tetraphenyltin was originally dissolved. In one experiment[4] an irradiated sample of tetraphenyltin was dissolved directly in a separatory funnel prior to extraction with 3 *f* hydrochloric acid and accordingly a larger enrichment factor (3400) and a higher yield (61%) were obtained. These results suggest the formation of tin radiocolloids in benzene.

The total tin present in the aqueous phase was determined colorimetrically by observing the molybdenum-blue color produced by the action of stannous tin on a molybdate reagent.[5] After reduction to the stannous state the color produced by the tin present in an aliquot of the aqueous phase was compared, visually, with that produced in several standard solutions of tin.

Acknowledgment.—We wish to express our appreciation to Dr. Roderick W. Spence and Mr. James E. Sattizahn, Jr., of the Los Alamos Scientific Laboratories for irradiation of the samples of tetraphenyltin.

(2) R. R. Williams, *J. Phys. Colloid Chem.*, **52**, 603 (1948).

(3) E. G. Meyer, Ph.D. Thesis, University of New Mexico, 1950.

(4) On the basis of the β-activity of this irradiated sample, the γ-flux during the irradiation of this sample was the same as that during the irradiation of the samples used in experiments 3 and 4.

(5) F. D. Snell and C. T. Snell, "Colorimetric Methods of Analysis," 3rd. ed., D. Van Nostrand Co., New York, N. Y., 1949, p. 217.

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ALBUQUERQUE, NEW MEXICO RECEIVED AUGUST 9, 1951

COMMUNICATIONS TO THE EDITOR

THE SYNTHESIS OF BUTATRIENE[1]

Sir:

Butatriene has been synthesized in high yield by the action of powdered zinc on 1,4-dibromobutyne in purified diethylene glycol diethyl ether at 70°. The reaction product was collected as a low boiling liquid in a Dry Ice–acetone trap, or as a solid in a liquid nitrogen trap. The material polymerized upon being warmed to room temperature in the absence of air and with hydroquinone or other inhibitors present. The monomer showed negative tests with acetylenic hydrogen reagents such as alkaline mercuric iodide. With bromine in carbon tetrachloride it gave 1,2,3,4-tetrabromobutene-2 (80% yield), m.p. 68–69.5°, identical with an authentic sample prepared by bromination[2] of 1,4-dibromobutyne-2.

The butatriene exhibited the following physical properties: C/H ratio on two different samples, 92.29/7.71 and 91.82/8.18; mol. wt. (Dumas method at 250 mm. pressure), 52.6; mass spectrum (relative intensities): mass 52 (100), mass 51 (72), mass 50 (54); u.v. max. (95% ethanol), 241 mμ (20,300), apparent max. 310 mμ (250). In the infrared[3] (gas, 100 mm., 10 cm. cell), strong bands were shown at 2990, 1708, 1610, and 860 cm.$^{-1}$ (broad); medium bands at 2030, 1358, 1206, and 1065 cm.$^{-1}$ (broad).

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RECEIVED NOVEMBER 23, 1951

(1) Supported in part by the Research Corporation.

(2) A. Valette, *Ann. chim.*, [12] **3**, 644 (1948).

(3) For aiding in obtaining the data we wish to thank Dr. David Eggers, who is now working on a more detailed interpretation of the infrared spectrum.

OXYGEN INDUCED ELECTROREDUCTION OF HYDROGEN PEROXIDE AT THE ROTATED PLATINUM ELECTRODE

Sir:

Based upon the following considerations the effect of hydrogen peroxide upon the diffusion current of oxygen has been investigated. It is assumed that the electroreduction of oxygen to hydrogen peroxide occurs in two steps

$$O_2 + e^- \longrightarrow O_2^- \quad (1)$$
$$O_2^- + e^- \longrightarrow O_2^= \quad (2)$$

At the surface of the electrode the O_2^-—or its corresponding acid HO_2—may react according to the Haber–Weiss[1] mechanism with hydrogen peroxide

$$O_2^- + H_2O_2 \longrightarrow OH^- + OH\cdot + O_2 \quad (3)$$
$$HO_2 + H_2O_2 \longrightarrow H_2O + OH\cdot + O_2 \quad (3a)$$
$$OH\cdot + H_2O_2 \longrightarrow HO_2 + HO_2 \quad (4)$$

Reactions (1) and (3) account for an exalted oxygen wave in the presence of hydrogen peroxide. Reactions (1), (3) and (4) also account for the same effect.

Terminators of the above chains are reaction (2) and

$$OH\cdot + e^- \longrightarrow OH^- \quad (5)$$

No information on the rate of electroreduction of $OH\cdot$ and HO_2 under the experimental conditions being available, the effect of hydrogen peroxide upon the oxygen reduction was studied empirically. Experimentally it was found that hydrogen peroxide causes a very large increase in the limiting current of oxygen at the rotated platinum electrode. Figure 1 illustrates the effect in 0.1 M sodium perchlorate solution. The oxygen concentration of the original solution was less than 10^{-6} M and the solution yielded a diffusion current of the order of 0.2 microampere at 25°. In the presence of 3.2×10^{-4} M hydrogen peroxide the limiting current became 2 μ and in 16×10^{-4} M hydrogen peroxide, 10 μ. Under the experimental conditions the exaltation was proportional to the hydrogen peroxide concentration. Analytical application of this exaltation for the determination of traces of oxygen is now being made.

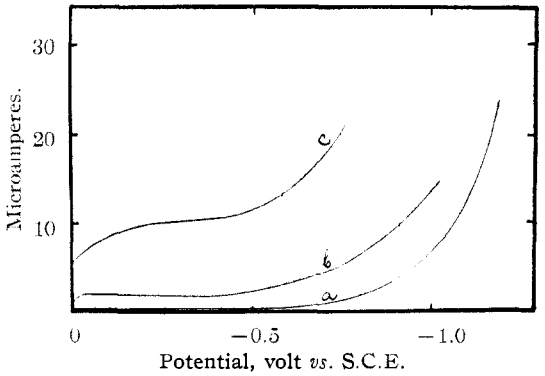

Fig. 1.—Current–voltage curves in 0.1 M sodium perchlorate: $[O_2] < 10^{-6}M$; (a) no H_2O_2, (b) $3.2 \times 10^{-4}M$ H_2O_2, (c) $16 \times 10^{-4}M$ H_2O_2.

(1) F. Haber and J. Weiss, *Naturwiss*, **20**, 948 (1932); *Proc. Roy. Soc.* (*London*), **A147**, 332 (1934).

The effect was also observed at the stationary platinum electrode. However, at the dropping mercury electrode the exaltation was obscured by maxima.

Substances which react very rapidly with $OH\cdot$, like acrylonitrile, allyl acetate, and other monomers did not affect the exaltation at the rotated electrode. From this it was concluded[2] that reaction (4) does not occur to a measurable extent and that reaction (5) is the main terminating reaction. Thus the electroreduction of oxygen induces the electroreduction of hydrogen peroxide, at potentials at which hydrogen peroxide is not (or very slowly) reduced, according to the over-all reaction

$$H_2O_2 + 2e^- \longrightarrow 2OH^-$$

This was confirmed by electrolysis experiments with a large platinum cathode in which the decrease of the hydrogen peroxide concentration during the electrolysis was determined.

The effect of various factors, such as pH, kind of supporting electrolyte, concentration of oxygen and hydrogen peroxide and of the temperature are now being investigated. A detailed account will be given at a later date.

(2) See I. M. Kolthoff and E. P. Parry, THIS JOURNAL, **73**, 3718 (1951).

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RECEIVED DECEMBER 26, 1951

CRYSTALLINE PYRIDOXAMINE PHOSPHATE

Sir:

Phosphorylated derivatives of vitamin B_6 are believed to play an important role in a number of enzyme systems, including transaminases, amino acid decarboxylases, and tryptophanase. It appears probable that pyridoxamine phosphate (2-methyl - 3 - hydroxy - 4 - aminomethyl - 5 - pyridylmethylphosphoric acid,[1,2] which has been reported to occur naturally,[3,4] is involved in biological transamination. Although this system has received considerable attention, the cofactors have thus far been available only in impure condition and the mechanisms of their action have not been established. Very recently, Viscontini, *et al.*,[5] have reported the preparation of calcium pyridoxal phosphate of high purity. We report at this time the preparation of crystalline pyridoxamine phosphate which gives theoretical analytical values and a very high order of activity.

Commercial pyridoxamine dihydrochloride was converted to the free amine and purified by recrystallization from 50% ethanol. To the colorless amine was added 10 times its weight of anhydrous phosphoric acid (1 part P_2O_5 to 2.5 parts 85% H_3PO_4)[6] and the mixture was heated at 100° for

(1) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **73**, 3436 (1951).

(2) A. N. Wilson and S. A. Harris, *ibid.*, **73**, 4693 (1951).

(3) J. C. Rabinowitz and E. E. Snell, *J. Biol. Chem.*, **169**, 643 (1947).

(4) W. S. McNutt and E. E. Snell, *ibid.*, **173**, 801 (1948).

(5) von M. Viscontini, C. Ebnöther and P. Karrer, *Helv. chim. acta*, **34**, 1834 (1951).

(6) R. H. A. Plimmer and W. J. N. Burch, *Biochem. J.*, **31**, 308 (1937).

24 hours. Nine volumes of absolute ethanol was added slowly with stirring to the cooled reaction mixture to yield a white precipitate, which, after being washed successively with absolute ethanol and ether, was dissolved in a minimal amount of water and brought to about *p*H 6 with concentrated ammonia. The mixture was applied to the top of an Amberlite XE-64 (a fine mesh, weak cation exchange resin) column in the washed free acid form. The effluent fractions from the column, on elution with water, were examined spectrophotometrically and by paper chromatography.[7] Two components, one of which was very small, gave positive ninhydrin tests. The major and slower moving of these, which showed a maximum absorption at 3250 Å., was concentrated *in vacuo* to a white residue which upon treatment with a small amount of water soon became crystalline. The product was sparingly soluble in water and was recrystallized by the addition of an equal volume of ethanol to the aqueous solution. The crystals appeared as systems of rhombic plates.

Anal. Calcd. for $C_8H_{13}N_2O_5P \cdot 2H_2O$: C, 33.8; H, 6.0; N, 9.9; P, 10.9. Found: C, 34.1; H, 5.9; N, 10.2; P, 11.1. When dried *in vacuo* (P_2O_5), the compound lost the theoretical amount of weight.

Ultraviolet absorption in 0.01 *M* buffers: $\lambda_{max.}$ (*p*H 2.0) 2935 Å., E_m 9600; $\lambda_{max.}$ (*p*H 7.2) 2535 Å., E_m 5200, 3265 Å., E_m 9400; $\lambda_{max.}$ (*p*H 10.0) 2440 Å., E_m 7500, 3120 Å., E_m 8300.

The crystalline pyridoxamine phosphate was oxidized with MnO_2[2] yielding NH_3 stoichiometrically. The oxidized preparation (pyridoxal phosphate) exhibited a high order of catalytic activity in a transaminase system of *Lactobacillus arabinosus*,[8] the tyrosine decarboxylase system of *Streptococcus faecalis*,[9] the aspartic acid β-decarboxylase system of *Clostridium welchii*,[10] and the tryptophanase system of *Escherichia coli*.[11] Within experimental error, the determination of relative purity yielded values of 100% based on assay with the tyrosine decarboxylase system[9] and by comparison with an impure preparation of pyridoxal phosphate assayed in another laboratory.[12] Assay of the crystalline pyridoxamine phosphate as described by Hendlin, *et al.*,[13] yielded similar results.

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RECEIVED DECEMBER 1, 1951

(7) Pyridoxamine and pyridoxamine phosphate were detected by the orange color formed after reaction with ninhydrin.

(8) A. Meister, *Federation Proceedings*, **10**, 223 (1951).

(9) W. W. Umbreit, W. D. Bellamy and I. C. Gunsalus, *Arch. Biochem.*, **7**, 185 (1946).

(10) A. Meister, H. A. Sober and S. V. Tice, *J. Biol. Chem.*, **189**, 577 (1951).

(11) W. A. Wood, I. C. Gunsalus and W. W. Umbreit, *J. Biol. Chem.*, **170**, 313 (1947).

(12) This preparation was generously provided by Dr. W. W. Umbreit.

(13) D. Hendlin, M. C. Caswell, V. J. Peters and T. R. Wood, *J. Biol. Chem.*, **186**, 647 (1950).

(14) Postdoctoral Fellow, National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency.

BOOK REVIEWS

Progress in Chromatography 1938–1947. By L. ZECHMEISTER, California Institute of Technology, Pasadena, California. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1951. xviii + 368 pp. 14 × 22 cm. Price, $8.00.

Professor L. Zechmeister, well known as a superb experimentalist both before and after his migration from Hungary to the California Institute of Technology, performed a distinct service to the science by writing, with L. Cholnoky, the authoritative "Principles and Practice of Chromatography," published in 1941 as a translation of a second German edition of the original work.

In the present volume Professor Zechmeister has presented a progress report consisting of a survey of the literature on the technique and application of chromatography in the period 1938–1947. The field is currently expanding and developing at so rapid a pace that the author felt that at this time a supplement would be more appropriate than a revision of the original monograph. He has thus presented a meticulously prepared survey of literature on principles and methods of chromatography, and on specific applications.

The 57-page survey of advances in the principles and methodology is a worthy extension of the original book as far as it goes, but the long gap between literature coverage (through 1947) and publication (1950) is hardly excusable in a book dealing with this rapidly developing and enormously useful technique. Thus partition chromatography, particularly as applied in the paper strip method, has become so familiar a tool that the papers of 1941–1947 now seem like early history.

The major part of the book is devoted to a review of the literature on specific applications of chromatography to organic compounds of some twenty structural types and to inorganic compounds. In the preface, the author states that "even hints about methods, adsorbents or solvents which can be used within a certain class of compounds may be welcome and time-saving for the experimenter." The objective is worthy, and perhaps the plan of citing applications classified according to type of compound was the best expedient for this interim work. However, the citation of chromatographic experiences and accomplishments in various fields seems to me to fall somewhat short of the mark. One working in a given field will soon learn from the specific literature the methods of chromatography traditionally used for the class of compounds concerned and might derive more stimulation from a cross-sectional discussion that perhaps would suggest trial of methods found useful for separation of compounds of other types. Chromatographic processing of reaction mixtures and mother liquors by the empirical elution technique is now practiced in many laboratories so frequently—to an extent at least comparable to