K_S and k_3 values of nicotinyl-L-tryptophanamide at 25° and pH 7.9 in aqueous systems 0.02 M in respect to the amine component of a tris-(hydroxymethyl)-aminomethane-hydrochloric acid buffer.[3] Thus with these relatively precise values at hand and also because this specific substrate had been used in inhibition experiments to evaluate the K_I values of a large number of competitive inhibitors[14] it was selected for use in this investigation. For comparison with the above Armour preparation a sample of α-chymotrypsin originally obtained as a twice recrystallized filter cake from the Worthington Biochemical Laboratories, which had been further recrystallized and then dialyzed in the cold, first against dilute aqueous hydrochloric acid of pH 3.5, then exhaustively against water, and finally lyophilized, was kindly placed at our disposal by Mr. E. F. Jansen of the Western Regional Research Laboratory. The experimental conditions and the analytical methods used in this investigation were identical with those employed previously.[3]

The results of the present study are summarized in Fig. 1 wherein eight determinations of the initial velocity at seven different initial substrate concentrations and a single enzyme concentration are expressed as a $[S]_0$ *versus* $[S]_0/v_0$ plot.[15] From the intercept of this plot, *i.e.*, $-K_S$, and the slope, *i.e.*, V, the K_S and k_3 values for nicotinyl-L-tryptophanamide were found to be 2.7×10^{-3} M and 1.5×10^{-3} mole/liter/min./mg. protein-nitrogen/ml., respectively.[16] These values are in excellent agreement with the previously determined K_S and k_3 values of 2.7×10^{-3} M and 1.6×10^{-3} mole/liter/min./mg. protein-nitrogen/ml., respectively.[3]

It can be concluded from the above results that the kinetic constants reported previously for systems containing bovine α-chymotrypsin[3-8,14] are those of systems containing a reproducible characteristic catalytic species of considerable stability. However, the agreement noted above adds little to what is already known about the accuracy of these values,[3] since an error that can still be present is the operational one involved in the determination of initial velocities and this has in a sense been standardized by using approximately the same procedure in all cases. An investigation is now in progress in which it is hoped that initial velocities can be estimated with much greater accuracy than has previously been possible.

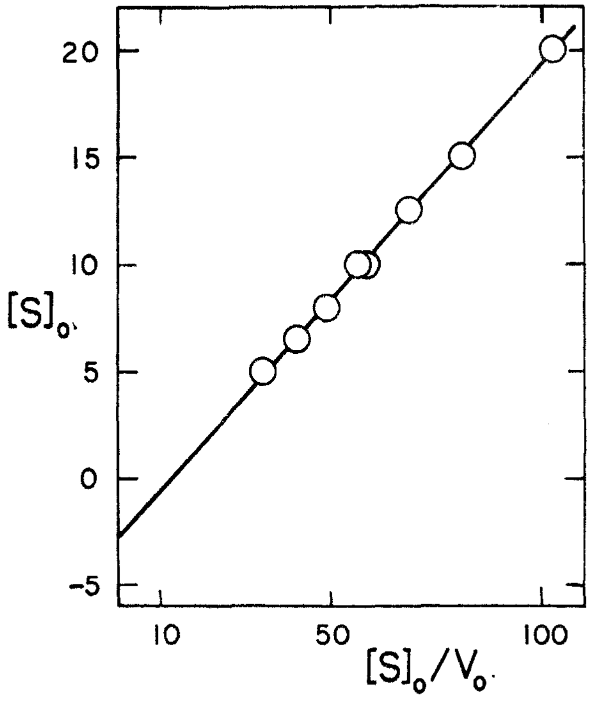

Fig. 1.—α-Chymotrypsin catalyzed hydrolysis of nicotinyl-L-tryptophanamide at 25° and pH 7.9; v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M, [E] equivalent to 0.144 mg. protein-nitrogen per ml., 0.02 M tris-(hydroxymethyl)-aminomethane-hydrochloric acid buffer.

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[14] H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1555, 3223, 3228, 4039 (1951); **74**, 101 (1952).

[15] H. Lineweaver and D. Burk, *ibid.*, **56**, 658 (1934).

[16] An independent evaluation of these data by Dr. D. W. Thomas and based upon a least squares treatment gave $K_S = 2.8 \times 10^{-3}$ M and $k_3 = 1.5 \times 10^{-3}$ mole/liter/min./mg. protein-nitrogen/ml.

Anion Exchange of Niobium in 7.0 Molar Hydrochloric Acid

BY E. H. HUFFMAN AND G. M. IDDINGS

RECEIVED MAY 15, 1952

It has recently[1] been reported that the elution of titanium from a cation exchange resin with citrate solution has resulted in broad elution bands with several peaks. This behavior was attributed to the probable partial separation of the isotopes of titanium. Work in this Laboratory on the elution of niobium with hydrochloric acid from an anion exchange resin, subsequent to that previously reported,[2] has shown a somewhat similar behavior, but under conditions which precluded any possible isotope separation.

When carrier-free Nb^{95}, prepared as described before,[2] was adsorbed from a 10.0 M hydrochloric acid solution on a Dowex 2 anion exchange resin column, 8.0 cm. long and 3.0 mm. in diameter, and then eluted with 7.0 M hydrochloric acid at the rate of about 2.4 ml. per hour, the elution curve shown in Fig. 1 was obtained. The possibility of any foreign activity in the purified Nb^{95} accounting for three peaks was eliminated by obtaining the decay rates of the samples taken at the top of each

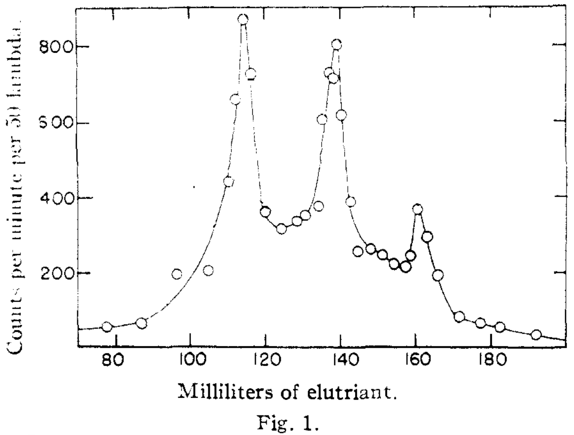

Fig. 1.

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[1] William E. Brown and William Rieman, III, THIS JOURNAL, **74** 1278 (1952).

[2] E. H. Huffman, G. M. Iddings and R. C. Lilly, *ibid.*, **73**, 4474 (1951).

peak. All three gave identical decay curves, corresponding to the disintegration rate of Nb^{95}. When this experiment was repeated a somewhat different curve was obtained. Again three peaks were found, but these were rounded, and the areas under the first and third were approximately equal and greater than that of the second. Brown and Rieman[1] also report that their elution bands were not exactly reproducible.

This departure from the expected type of elution band can probably be attributed to the slow establishment of equilibrium among various ionic species which are present. These ions would not necessarily have to have different charges, as in the case of the thiocyanate complexes of chromium,[3] but may contain different numbers of chloro-, oxy- and hydroxy-groups. Elution with 6.0 M hydrochloric acid gives the usual symmetrical curve.

This work was done under the auspices of the Atomic Energy Commission.

(3) E. L. King and E. B. Dismukes, *ibid.*, **74**, 1674 (1952).

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The State of Anthracene, *sym*-Trinitrobenzene and their 1 : 1 Complex in Liquid Sulfur Dioxide Solution

BY NORMAN N. LICHTIN, RALPH E. WESTON, JR., AND JUNE D. WHITE[1]

RECEIVED APRIL 3, 1952

Numerous investigations of molecular complexes between polynitroaromatic compounds and unsaturated hydrocarbons and their derivatives, both in the crystalline state and in solution, have led to varying interpretations as to the nature of the binding forces involved.[2] The investigation reported here was directed toward testing the proposal of Weiss[3] that such complexes are produced by complete transfer of an electron and are ionic in nature. The conductivity behavior and ultraviolet spectra of liquid sulfur dioxide solutions of *sym*-trinitrobenzene (TNB), anthracene and their 1:1 complex were accordingly investigated over a range of concentrations.

Experimental

Materials.—Reilly Tar and Chemical Corp. "Scintillation Grade" anthracene was employed, m.p. 215.4–215.5°,[4] strongly fluorescent. It was purified by sublimation at 120° and 1 mm. pressure to yield material melting sharply at 215.4°.[4] Eastman Kodak Co. "White Label" TNB, m.p. 122.2–122.4°,[4] was purified by sublimation at 100° and about 0.001 mm. pressure to yield slightly yellow material melting at 123.2–123.3°.[4] The complex was prepared from the vacuum sublimed components by the method of Briegleb and Schachowskoy.[5] The product consisted of long orange needles, m.p. 163.8–164.0°.[4] The purity of the sulfur dioxide employed in the conductivity measurements[6] and spectrophotometric work[7] is described elsewhere.

Measurements.—Conductivity measurements were carried out at 0.11 ± 0.03° using apparatus and procedures which are described elsewhere.[6] With anthracene and the complex the samples were pumped in the conductivity cell at about 0.001 mm. pressure and 0° for periods of at least 12 hours before sulfur dioxide was admitted. TNB was similarly pumped in only one run but this run did not differ significantly from others with this compound.

After some of the conductivity runs, the solute was recovered by pouring the solution into an evaporating dish and allowing the solvent to evaporate in air. Recovered anthracene melted at 215.2–216.8°.[4] Recovered TNB melted at 123°.[4] Recovered "complex" melted over the range 140–170°[4] even though it looked unchanged.

Spectrophotometric measurements were carried out at 1–2° using apparatus and procedures which are described elsewhere.[7]

Evaluation of Conductivity Measurements.—Solutions of the purified materials possess such low conductivities that, even though the specific conductivity of the solvent usually fell in the range 2.9×10^{-8} to 7.3×10^{-8}, this was normally more than 10% of the total specific conductivity of the most concentrated solutions (about 0.01 molar) and from 22 to 50% of the conductivity of the most dilute solutions (about 0.00005 molar). In this connection it must be emphasized that the measured conductivity of the solvent is highly sensitive to traces of electrolytes desorbed from the electrodes or the walls of the cell. This can be demonstrated by repeated redistillation of the solvent within the cell in the absence of added solute. Thus, in one case, six distillations reduced the solvent conductivity to one-fifth of its initial value. Correction of solution conductivities for solvent conductivity is therefore an approximate procedure. In the present case solute conductivities can be considered known to within a factor of two.

Data and Discussion

The molar conductances (Λ) of anthracene, TNB, and the complex were determined in the concentration range 4×10^{-4} M to 4×10^{-3} M. The Λ values ("corrected" for solvent conductivity) all fall in the range from 0.02 to 0.7 mho cm.^{2}/mole. The molar conductance of anthracene is somewhat lower than that of TNB at all concentrations. Within the reliability of the data, the conductance of the complex is equal to the sum of the conductances of its components.[8]

The spectral data are summarized in Fig. 1 where molar absorbancy index ($(1/bc) \log (I_0/I)$, b in cm., c in moles per liter) is plotted logarithmically *vs.* wave length. It is apparent that the spectrum of the complex is within experimental error identical with the sum of the spectra of its components.

Solutions of the complex were found to obey Beer's law at 430 mμ (in the concentration range 4.85×10^{-4} to 5.16×10^{-5} M) with a molar absorbancy index of 1350. At this wave length, anthracene solutions were also found to obey Beer's law (in the concentration range 1.16×10^{-3} to 5.06×10^{-5} M) with a molar absorbancy index of 1450. The agreement of these two values is within experimental error.

It is notable that, although the spectrum of TNB in sulfur dioxide solution closely resembles that in carbon tetrachloride,[5] the spectrum of anthracene in sulfur dioxide lacks the fine structure observed in the latter solvent,[5] and extends much

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(1) Taken in part from the A.M. Thesis submitted by June D. White to the Graduate School of Boston University.

(2) (a) G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 67–69; (b) M. J. S. Dewar, "The Electronic Theory of Organic Chemistry," Oxford University Press, London, 1949, pp. 184–185; (c) G. Briegleb, "Zwischenmolekulare Kräfte," G. Braun, Karlsruhe, 1949, pp. 12–15; (d) R. S. Mulliken, THIS JOURNAL, **74**, 811 (1952).

(3) J. Weiss, *J. Chem. Soc.*, 245 (1942).

(4) Capillary melting point, measured with Anschütz-type total immersion thermometer.

(5) G. Briegleb and T. Schachowskoy, *Z. physik. Chem.*, **19B**, 255 (1932).

(6) N. N. Lichtin and H. Glazer, THIS JOURNAL, **73**, 5537 (1951).

(7) P. D. Bartlett and R. E. Weston, Jr., *ibid.*, **74**, in press.

(8) The possibility that the observed conductivities are due to traces of electrolytic impurities cannot be ruled out. Presumably the impurities in the components would be carried into the complex.