

Procedure.—All absorption spectra were measured by means of a Beckman model DU spectrophotometer and 1-cm. silica cells at $26 \pm 1^\circ$. In the determination of the ionization constants the spectra of buffered solutions were measured in "50%" aqueous ethanol (100 ml. of the solution contained 50 ml. of water; remainder of the solution consisted of 95% ethanol). The concentration of all compounds was $5 \times 10^{-5} M$. The ionization constants were calculated⁷ by the equation $pK_a = pH_m + \log (E_B - E_m / E_m - E_{BH^+})$ where E_B , E_{BH^+} are the optical densities of base and salt and E_m is the optical density of a mixture of base and salt at an intermediate pH_m fairly close to the value of the pK_a of the compound.

(7) L. A. Flexser, L. P. Hammett and A. Dingwall, *THIS JOURNAL*, **57**, 2106 (1935).

CANCER RESEARCH LABORATORY
UNIVERSITY OF FLORIDA
GAINESVILLE, FLA.

The Preparation of S-Succinyl Coenzyme A¹

By ERIC J. SIMON² AND DAVID SHEMIN

RECEIVED FEBRUARY 27, 1953

We have previously concluded that a succinyl derivative is a precursor of protoporphyrin.³ This succinyl intermediate may be identical with S-succinylcoenzyme A. We have found that this compound is readily formed by succinylating coenzyme A with succinic anhydride, as shown by the disappearance of the sulfhydryl group (nitroprusside reaction),⁴ the formation of a hydroxamic acid,⁵ and an increased light absorption of 232 $m\mu$.⁶ When the product is warmed for a few minutes on a steam-bath, the sulfhydryl group reappears, the reaction with hydroxylamine no longer occurs and there is a decreased light absorption at 232 $m\mu$. Also, this synthetic preparation behaved as succinyl-coenzyme A in enzymatic systems.⁷

Thirty-five mg. of a coenzyme A preparation (Pabst) was dissolved in 30 ml. of ice cold water. To this solution 3 mg. of succinic anhydride was added, followed by sodium bicarbonate until the pH was 7–7.5. The mixture was kept in an ice-bath and shaken frequently. The reaction appeared to be completed within 30 minutes at which time over 90% of the sulfhydryl groups had disappeared. At 0° the succinyl coenzyme A is stable at pH 7–7.5 for several hours, at room temperature it is half hydrolyzed in about 1–2 hours, as measured by the nitroprusside and hydroxamic acid methods. However, at pH 1, at room temperature, the succinyl coenzyme is much more stable than at neutrality. The hydroxamic acid test was carried out on the formed succinyl coenzyme A after the complete hydrolysis of any unreacted succinic anhydride.

Succinyl coenzyme A has previously been enzymatically prepared from α -ketoglutarate.^{8,9} Acetic anhydride has

been used for the synthesis of acetyl-coenzyme A.¹⁰ This anhydride method may be applicable for the preparation of other acyl coenzyme A derivatives.

(10) I. B. Wilson, *THIS JOURNAL*, **74**, 3205 (1952).

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK 32, N. Y.

On Some Partial Molal Volumes of Gases in Solution¹

By RICHARD H. SCHUMM AND OLIVER L. I. BROWN²

RECEIVED JANUARY 3, 1953

This note reports measurements of the partial molal volumes of carbon tetrafluoride and methane in some non-polar solvents at 27.0° .

Carbon tetrafluoride, obtained from the Minnesota Mining and Manufacturing Company, was led through a Dry Ice trap. Methane, Research Grade from the Phillips Petroleum Company, had been analyzed by mass spectrometer as 99.7% pure. Benzene was obtained thiophene-free or was freed of thiophene by washing with sulfuric acid, sodium carbonate and water, and dried over calcium chloride. Reagent grade (A.C.S.) carbon tetrachloride was used without further purification. *n*-Hexane, *n*-heptane and "isooctane" (2,2,4-trimethylpentane) from the Phillips Petroleum Company were all of 99 mole per cent. purity.

The apparatus and procedure were essentially the same as those of Horiuti³ and of Gjaldbaek and Hildebrand.⁴ The volume of the bulb was approximately 150 cc. The capillary stems had a capacity of about 8.5 cu. mm. per cm. Two dilatometers were used during each run; gas was dissolved in one and the other was used as a blank to correct for small temperature variations in the bath. Compression of the solution by the increased head of mercury in the capillaries was avoided by pulling a sufficient vacuum in one capillary to return the mercury in the other capillary to its original height.

To check the procedure against that of Horiuti and of Gjaldbaek and Hildebrand the partial molal volume of methane in benzene was determined at 25.0° . Values of 53.51 and 51.22 cc. per mole were found, which agree well with Horiuti's value of 52.0 and Gjaldbaek and Hildebrand's value of 52.5 cc. per mole. The results of the measurements are summarized in Table I. It will be noted that the value determined for methane in *n*-hexane at 27° differs considerably from the value of 60.0 cc. per mole at 25° found by Gjaldbaek and Hildebrand and that it does not vary in the direction to be expected from the two-degree temperature difference. Horiuti found that the partial molal volume of methane in carbon tetrachloride increased 6.8% in going from 0 to 25° .

As shown by Gjaldbaek and Hildebrand, the partial molal volumes of methane, ethane and nitrogen decrease consistently with increasing solubility parameter of the solvent except for solutions in carbon disulfide. This exception they attribute to the effect of differences in the size and shape of the component molecules. However, if the data for these gases, as given here and in

(1) This work was supported by grants from the National Institutes of Health, United States Public Health Service, from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, and from the Rockefeller Foundation.

(2) Aided by a fellowship from the National Foundation of Infantile Paralysis. Present address: Payne Whitney Clinic, Cornell Medical College, New York, N. Y.

(3) (a) D. Shemin and J. Wittenberg, *J. Biol. Chem.*, **192**, 315 (1951); (b) D. Shemin and S. Kumin, *ibid.*, **198**, 827 (1952).

(4) R. R. Grunert and P. H. Phillips, *Arch. Biochem.*, **30**, 217 (1951).

(5) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(6) E. R. Stadtman, 122d Meeting, Am. Chem. Soc., Atlantic City, N. J., Sept. 1952, Abs. 32C.

(7) We are indebted to S. Kaufman, C. Gilvarg, M. J. Coon and J. R. Stern for these enzymatic experiments.

(8) D. R. Sanadi and J. W. Littlefield, *Science*, **116**, 327 (1952).

(9) H. Beinert, 122d Meeting, Am. Chem. Soc., Atlantic City, N. J., Sept. 1952, Abs. 34C.

(1) This note is based on the Master's Thesis of R. H. Schumm, 1952.

(2) Department of Chemistry, Connecticut College, New London, Conn.

(3) J. Horiuti, *Sci. Papers, Inst. Phys. Chem. Res., Tokyo*, **17**, 125 (1931).

(4) J. Chr. Gjaldbaek and J. H. Hildebrand, *THIS JOURNAL*, **72**, 1077 (1950).