of the ligands coördinated to the central chromium atom in these complexes, but only a slow substitution for at least one of the attached ligands. In general, this latter question is best answered by experiments in which the ligands are labeled rather than the central metal atom.

The results suggest that the three chromium(III) complexes would be suitable compounds for the Szilard–Chalmers recoil separation of radioactive chromium from inactive target chromium in which radiochromium may be produced by the (n,γ) and other reactions. Recoil separations have been successfully applied to the tris-(ethylenediamine) complexes of cobalt(III), rhodium(III), iridium (III) and platinum(II), for example.[9]

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(9) J. Steigman, *Phys. Rev.*, **59**, 498 (1941).

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Crystalline Acetoacetic Acid

BY ROBERT C. KRUEGER

RECEIVED JUNE 7, 1952

There is apparently no record in the literature of any attempts to prepare pure acetoacetic acid. Ceresole[1] reported the preparation of a concentrated solution but no analytical figures were given. Other workers have found dilute solutions of the free acid or the sodium salt suitable for their needs.

Recently acetoacetic acid has been crystallized in this Laboratory. Initially, the procedure follows that of Davies.[2] Twenty-six ml. of redistilled acetoacetic ester is hydrolyzed at room temperature in 200 ml. of 1 *N* sodium hydroxide. The alkaline solution is extracted several times with ether and then chilled and acidified with cold, dilute sulfuric acid. The solution is saturated with sodium chloride and extracted with ether until the ether extract gives only a faint test with ferric ions. This requires about 1500 ml. The ether extract is dried for several hours over anhydrous sodium sulfate and evaporated under diminished pressure in a 30–50° water-bath. When the concentration of the acetoacetic acid reaches about 85% as judged by titration with standard base, the viscous liquid will solidify when set in the deep freeze. These crystals are dissolved in 2–3 ml. of ether and the solution cooled as before. The crystals that separate are filtered quickly by suction and rapidly transferred to a desiccator containing silica gel. Use of concentrated sulfuric acid as drying agent should be avoided as this eventually causes darkening of the acetoacetic acid. On moist days the crystals tend to melt during this process but may be dried by evacuating the desiccator. By evaporation of the mother liquors and handling in similar fashion several batches of crystals can be obtained. Recrystallization from ether affords several crops of large, colorless crystals. Yields have been of the order of 10%. The material keeps dry and stable over silica gel but melts immediately when exposed to the air. The sulfuric acid content was of the order of 0.05% as judged by turbidity tests using barium chloride solution. Its melting point, 36–37°, was determined in a dried test-tube capped with a silica gel drying tube.

Anal. Calcd. for $C_4H_6O_3$: neut. equiv., 102; CO_2, 1.00 vol. Found: neut. equiv., 103; CO_2 (by decarboxylation using aniline citrate as catalyst), 1.01 vol.

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(1) M. Ceresole, *Ber.*, **15**, 1326 (1882).

(2) R. Davies, *Biochem. J.*, **37**, 230 (1943).

An Investigation of the Production of Acetylene by Flames of Methane and Oxygen[1]

BY PAUL H. KYDD

RECEIVED JUNE 5, 1952

The purpose of the experiments to be described was to determine how the amount of acetylene produced in rich flames of methane and oxygen varied with the distance above the burner port, and to obtain some idea as to the total amount of acetylene produced by flames of different feed-gas compositions. Similar experiments on flames of illuminating gas containing methane have been performed by Lewes[2] while Eltenton[3] detected acetylene in flames of oxygen in methane and measured its concentration mass spectroscopically.

Experimental

A water-cooled metal burner 1.6 cm. i.d. was used. The inner and outer cones of the flame were separated with a Pyrex mantle 3.7 cm. in diameter and the gases of the inner cone were sampled with a Haber type water cooled probe constructed of brass.[4] The probe was inserted through a side-arm on the mantle and was inclined at an angle of 20 degrees to the horizontal to disturb the flame as little as possible. Samples of 300 cc. were withdrawn over mercury, and 100-cc. portions were analyzed by precipitating the acetylene as cuprous acetylide with Ilosvay reagent[5] and determining the copper by spectrophotometric titration with versine reagent.[6] The results are calculated as volume per cent. of the dry gas sampled. To determine the total amount of gas produced by the flame and thus the total conversion of methane to acetylene, the flame gases were led from the top of the mantle through an air-cooled condenser and soot trap to a dry gas meter.

The variation in acetylene concentration along the central axis of the flame as the probe was raised from 0.5 to 12 cm. above the burner port is plotted in Fig. 1 with an outline of the inner cone for comparison. The size of the points indicates the analytical accuracy. A mixture of 59.6% methane and 43.1% oxygen was burned, the gases being used just as they came from the cylinders. The methane was 99% pure reagent grade and the oxygen was standard grade. The inner cone of the flame consisted of two parts, a bright blue combustion zone 0.15 cm. above the burner port and 0.15 cm. high, and a streamer of incandescent carbon which faded out at about 4.5 cm. It was not possible to sample the gases within 0.2 cm. of the combustion zone without violently distorting it. The results from 4.0 to 5.0 cm. are not guaranteed because the tip of the luminous streamer was not stable and the samples were inhomogeneous. A series of three flames of varying composition was studied to obtain the difference in the amounts

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(1) Research performed as part of a senior thesis for the Department of Chemistry, Princeton University, under the supervision of Professor Robert N. Pease.

(2) V. B. Lewes, *J. Chem. Soc. Trans.*, **61**, 322 (1892).

(3) G. C. Eltenton, *J. Chem. Phys.*, **15**, 455 (1946).

(4) F. Haber and R. Le Rossignol, *Z. physik. Chem.*, **66**, 181 (1909).

(5) L. Ilosvay, *Ber.*, **32**, 2698 (1899).

(6) Method developed by Prof. C. E. Bricker and P. Sweetser of this Laboratory. Details will be published later.

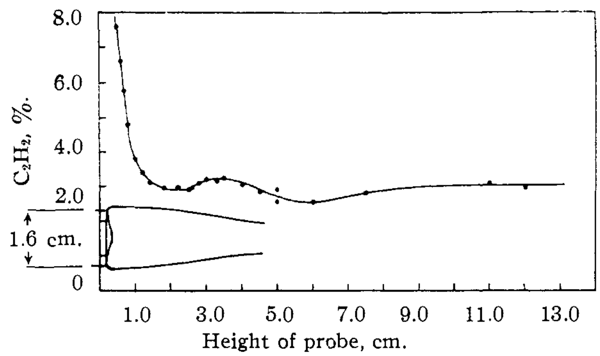

Fig. 1.—Variation of acetylene concentration with height above burner port compared to an outline of the inner cone.

of acetylene in the cooled products. The results are given in Table I.

TABLE I

Composition of feed gas, methane, %	Feed gas flow rate, cc./sec.	Composition dry off gas, C_2H_2, %	Dry off gas flow rate, cc./sec.	Methane converted to acetylene, %
56.3	45.3	2.67	50.4	5.30
56.55	45.05	3.05	50.0	5.92
57.05	44.7	3.60	48.6	6.90

Discussion

On the basis of an equilibrium calculation of the products of a flame of 56.9% methane the amount of acetylene formed should be vanishingly small; therefore, it is evident from the relatively large amounts of acetylene that are found even in the cooled products that equilibrium with the elements is never established in the flame.

The sharp drop in acetylene concentration which occurs as the probe is raised is considered to be due to the decomposition of the acetylene formed in the combustion zone to give solid carbon and hydrogen. The subsequent rise in acetylene concentration is due to a competing reaction which is not clearly understood. It is doubtful if this rise is due to spurious effects of the probe, since the time taken to withdraw the sample varied widely with the diameter of the probe orifice without seeming to affect the reproducibility of the results. The second minimum is also unexpected, but the most reasonable explanation is that further reactions involving acetylene are taking place at quite low temperatures.

The author wishes to thank Dr. Richard S. Brokaw for his assistance and advice during the progress of the research.

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Interactions of Metal Ions with the Sulfhydryl Group of Serum Albumin

BY IRVING M. KLOTZ, JEAN M. URQUHART AND HAROLD A. FIESS

RECEIVED MAY 2, 1952

It has been shown by Hughes[1,2] that the sulfhydryl group of serum albumin combines with mercuric ion and by Benesch and Benesch[3] that it forms complexes with silver. It seems pertinent to describe some experiments which demonstrate that other metals such as copper, zinc, cadmium and lead also form albumin mercaptides. These interactions occur even under acidic or neutral conditions where –SH groups may compete with other side chains of albumin, such as those of histidine, for metallic ions.[4]

In aqueous solutions, between pH 5 to 8, containing 0.2 M sodium acetate or sodium nitrate, an unusual absorption band appears at 375 mμ when bovine serum albumin[5] is added to cupric ions[6] (Fig. 1). It has been possible to prove that a copper–sulfhydryl interaction is responsible for this

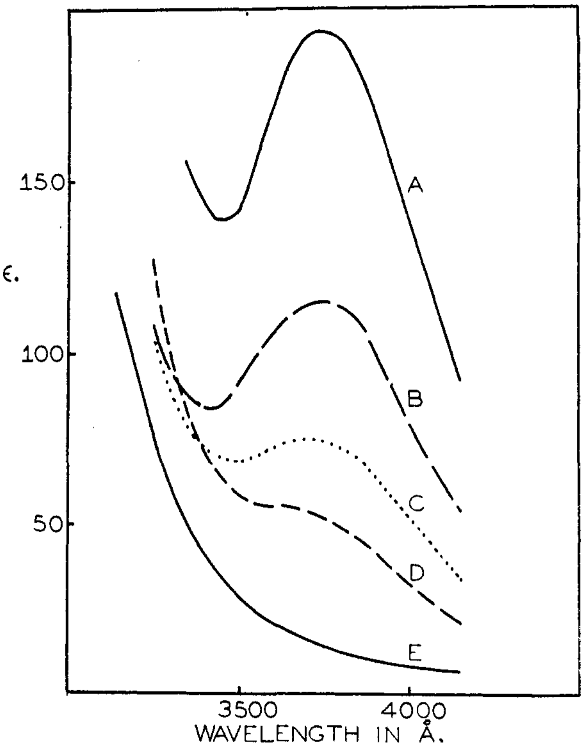

Fig. 1.—Effect of various metallic ions on absorption of copper–albumin complex: A, 0.003 M Cu^{++} and 0.0003 M bovine serum albumin, pH 6.0; B, same as A but with 0.003 M Zn^{++}; C, same as A but with 0.003 M Cd^{++}; D, same as A but with 0.003 M Pb^{++}; E, same as A but with 0.0003 M Hg^{++}. Solution A was made up to 0.2 ionic strength with sodium nitrate; the peak is slightly lower if sodium acetate is used. Solutions B to E were made up to 0.2 ionic strength with sodium acetate. The molecular extinction coefficients, ϵ, were calculated from the equation: $\log I_0/I = \epsilon cd$, where I_0 is the intensity of the light emerging from the pure protein solution, I the intensity of the light emerging from the metal ion–protein solution, c the molar concentration of the metal ion, and d the cell thickness in centimeters.

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(1) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1836 (1947).

(2) W. L. Hughes, Jr., *Cold Spring Harbor Symposia on Quantitative Biology*, **14**, 79 (1950).

(3) R. Benesch and R. E. Benesch, *Arch. Biochem.*, **19**, 35 (1948).

(4) E. Barbu, J. Lessiau and M. Macheboeuf, *Bull. soc. chim. Biol.*, **31**, 1254 (1949), have shown that treatment of proteins with strong alkali produces sulfhydryl groups which then combine with copper.

(5) This band has not been found with the samples of human serum albumin at our disposal.

(6) Attention should be drawn to the presence of an absorption band in this same region, as well as in the visible range, in the natural copper-protein, oxyhemocyanin.