which only histidine could be demonstrated by the dye, or by the ninhydrin, after the paper had been dipped in alcohol saturated with $HgCl_2$ (containing no dye), washed and dried. Nor is solubility in the alcohol involved, since almost all the amino acids are visible early in the rinsing process, only to be washed out eventually by the water.

In order to visualize all the amino acids along with histidine, it has been found practical to first spray the papers with ninhydrin, and, after development of the color, to apply the dye. In this way, all the amino acids and peptides may still be located. After this dual treatment, histidine and leucyl-histidine gave dark-brown areas which were changed to blue by NH_3 vapor, whereas histamine was green. All the other acids were salmon or pink in color.

The sensitivity of the dye reaction is less than that of the ninhydrin reaction with histidine. However, a combination of these two reactions offers a specific, sensitive test for histidine and its peptides.

The reaction with brom phenol blue has been used in conjunction with paper chromatographic and electrophoretic runs. The dye reaction with paper chromatograms obtained with partial hydrolysates of proteins is more revealing than that obtained with ninhydrin. Thus, some areas of peptide-containing material gave a blue color even though the ninhydrin reaction was negative.

Experimental

Brom phenol blue was made up as a 0.1% solution in 95% ethanol saturated with mercuric chloride. Ninhydrin was employed as a 0.1% solution in 95% ethanol. The latter solution was used as a spray, while the former was used as a spray (caution!) or as a dip. Color development with ninhydrin was carried out in a 70° oven.

Whatman No. 1 paper was used exclusively in these experiments. Later experiments have shown that Whatman No. 52 is preferable, for its greater tenacity is advantageous during the rinsing process. The amino acids and glutathione were obtained from commercial sources. The following peptides were used.[4,5] G–A, G–L, G–ϕ, G–Tyr, G–S, G–Asp, GSH, G–G–G, G–L–G, G–L–L, A–G, A–A, A–S, L–G, L–H, L–Sarc, L–L, L–Glut, L–Asp, L–G–L, S–A, S–S, S–G, S–A–Glut, S–G–L, S–G–Glut, Prol–ϕ, and V–G.

The paper chromatograms of amino acids and peptides were developed according to a standard procedure[6] in the following solvents: collidine–lutidine–H_2O (1:1:2), phenol–H_2O, and butanol–acetic acid–H_2O (4:1:5). The peptides were developed additionally in 2 N NH_3–lutidine (1:1). Phenol–H_2O together with one of the other solvent systems was used for two-dimensional chromatography. With the basic solvents employed, it was necessary first to remove the solvent before attempting to stain with the dye. This was accomplished by one of two procedures. In the first, the paper was placed in a long cylinder into which ether had been poured, and the solvents were removed from the paper by repeated extraction with fresh ether. The second procedure involved placing the paper in a drying oven, through which a stream of warm air was continually blown. After 1 to 2 days, the paper was removed and placed in a vacuum desiccator over H_2SO_4. Usually, these procedures were sufficient to remove most of the basic volatile solvents, which otherwise gave the paper a dark blue background with the dye. When neither of these procedures was completely effective, it was found useful to repeat as often as needed, after the addition of the dye, the process of passing the paper through HCl fumes and the subsequent rinsing in water.

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UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA RECEIVED SEPTEMBER 4, 1951

(4) We are indebted to Prof. H. O. L. Fischer for the gift of peptides from the Emil Fischer collection, and to Dr. J. I. Harris for the serine peptides.

(5) G, glycine; A, alanine; L, leucine; ϕ, phenylalanine; Tyr, tyrosine; S, serine; Asp, aspartic acid; H, histidine; Sarc, sarcosine; Glut, glutamic acid; Prol, proline; and V, valine; GSH is glutathione.

(6) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

Calculated Values for the Solubility Product Constants of the Metallic Sulfides

BY J. REX GOATES, MARVIN B. GORDON AND NEAL D. FAUX

Recent determinations of the second ionization constant of H_2S[1,2] (K_{2H_2S}) show that the standard free energy of formation of the sulfide ion ($\Delta F_f°_{S^-}$) is considerably lower than it was previously thought to be. When the value of K_{2H_2S} given by Konopik and Leberl[2] (7.9×10^{-14} at 20°) is corrected to 25° by means of heat content data from reference 3, one obtains $K_{2H_2S} = 1.2 \times 10^{-13}$. Use of this value for K_{2H_2S} and 3.01 kcal.[3] for $\Delta F_f°_{HS^-}$ gives $\Delta F_f°_{S^-} = 20.64$ kcal. at 25°. This figure is 2.7 kcal. lower than the value calculated from Knox's[4] work, which has been used in previous calculations of solubility product constants of the metallic sulfides, and hence makes necessary a recalculation of these constants. The correction in the $\Delta F_f°_{S^-}$ together with smaller changes made in the last few years in the free energy data of the metallic sulfides and metallic ions produce changes of approximately two orders of magnitude in the values of the solubility product constants. The K_{sp} values given in the table below have been calculated from free energy data by means of the relationship $\Delta F° = -RT \ln K_{sp}$, in which $\Delta F°$ is the value of the standard free energy change of the reaction $M_2S_{(S)} \rightleftarrows 2M^+_{(aq)} + S^{=}_{(aq)}$ or the corresponding equation when the metallic ions are di- or trivalent. The data in Table I are for the crystalline forms that are stable at 25°.

Three values for the solubility product constant of Bi_2S_3 may be found in the literature: 1×10^{-91}, 1.6×10^{-72} and 7.1×10^{-61}. The first value is found in the tables of Bruner and Zawadski[5] and was calculated from electrochemical data taken by I. Bernfeld[6] on the cell Bi, $Bi_2S_3|S^{--}||$ calomel reference electrode, the Bi, $Bi_2S_3|S^{--}$ half-cell reaction for which was assumed to be $2Bi + 3S^{--} \rightleftarrows Bi_2S_3 + 6e$. In 1931, Kolthoff[7] recalculated Bernfeld's data to get the value of 1.6×10^{-72}(?), which has been very widely quoted (minus his question mark) in both texts and source books.

Inasmuch as Bernfeld's work was old (1898), we repeated his experiments and obtained consistent reproducible voltages near those reported by him. The value of the solubility product constant calculated from these data is near 10^{-72}, but the value of the $\Delta F_f°_{Bi_2S_3}$ that corresponds to the e.m.f. data is the unreasonable value of 1 kcal., indicating that the reaction taking place in the cell is not the one postulated by the earlier investigators. Hence,

(1) H. Kubli, *Helv. Chim. Acta*, **29**, 1962 (1946).

(2) N. Konopik and O. Leberl, *Monatsh.*, **80**, 781 (1949).

(3) F. D. Rossini, D. D. Wagman, W. H. Evans, S. Levine and I. Jaffe, *Natl. Bur. Standards Circ.*, 500 (1950).

(4) Knox, *Trans. Faraday Soc.*, **4**, 29 (1908).

(5) L. Bruner and J. Zawadski, *Anorg. Chem.*, [2] **67**, 454 (1910).

(6) I. Bernfeld, *Z. physik. Chem.*, **25**, 46 (1898).

(7) I. M. Kolthoff, *J. Phys. Chem.*, **35**, 2712 (1931).

TABLE I

SOLUBILITY PRODUCT CONSTANTS OF 16 METALLIC SULFIDES AT 25° AND THE DATA[a] USED IN THEIR CALCULATIONS

Compound	ΔF_f°, kcal. Metallic sulfide	Metallic ion	K_{sp}
PbS	−22.15	− 5.81	8×10^{-28}
Tl_2S	−21.0[b]	− 7.755	7×10^{-20}
ZnS	−47.4	−35.184	8×10^{-25}
CdS	−33.6	−18.58	7×10^{-27}
HgS	−10.22[c]	39.38	3×10^{-52}
Cu_2S	−20.6	12.0	1×10^{-48}
CuS	−11.7	15.53	8×10^{-36}
Ag_2S	− 9.56[d]	18.430	7×10^{-50}
NiS	−18.8[b]	−11.1	2×10^{-21}
CoS	−21.8[b]	−12.3	8×10^{-23}
Co_2S_3	−47.6[b]	29.6	10^{-124}
FeS	−23.32	−20.30	5×10^{-18}
MnS	−47.6[b]	−53.4	1×10^{-11}
Ce_2S_3	−293.0[b]	−170.5	6×10^{-11}
La_2S_3	−301.2[b]	−172.9	2×10^{-13}
Bi_2S_3	−39.4	15	10^{-96}

[a] Except where otherwise noted the free energy data are from F. D. Rossini, *et al.*, *Natl. Bur. Stds. Circ.*, 500 (1950). [b] Calculated from heat content data taken from reference "a" and estimates of entropy made by the method recently proposed by W. M. Latimer, THIS JOURNAL, **73**, 1480 (1951). [c] J. R. Goates, A. G. Cole and E. L. Gray, *ibid.*, **73**, 3596 (1951). [d] J. R. Goates, A. G. Cole , E. L. Gray and Neal D. Faux, *ibid.*, **73**, 707 (1951).

the values of 1×10^{-91} and 1.6×10^{-72}(?) should certainly be discarded.

The value of 7.1×10^{-61} was reported by A. F. Kapustinsky and I. A. Makolkin[8] and was supposed to have been calculated from the $\Delta F_f°_{Bi_2S_3}$ given by Kelley (−39.14 kcal.).[9] It appears, however, that an error was made in these calculations, for the $\Delta F_f°_{Bi^{+++}}$ which corresponds to Kapustinsky and Makolkin's solubility product constant value is the unreasonable value of −9.5 kcal.

The value given in the table was calculated from the $\Delta F_f°_{Bi_2S_3}$ given by the Bureau of Standards[3] and a value for the $\Delta F_f°_{Bi^{+++}}$ that was calculated from data reported by Feitknecht.[10] There is some question as to the accuracy of this last value, but it appears reasonable, and since its effect on the solubility product constant is much less than that of the sulfide ion, the value of the solubility product constant given seems to be a reasonable approximation.

(8) A. F. Kapustinsky and I. A. Makolkin, *Acta Physiochim., U. R. S. S.*, **10**, 259 (1939).
(9) K. K. Kelley, *U. S. Bur. of Mines, Bul.* 406, 63 (1937).
(10) W. Feitknecht, *Helv. Chim. Acta*, **16**, 1307 (1933).

DEPARTMENT OF CHEMISTRY
BRIGHAM YOUNG UNIVERSITY
PROVO, UTAH
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The Chlorination of Diisopropyl Ether at Low Temperatures[1,2]

BY GEORGE E. HALL AND ICLÂL SIREL

The chlorination of diethyl ether at −20° or lower yields α,α'-dichlorodiethyl ether rather than the α,β-dichlorodiethyl ether obtained at higher temperatures.[3] The present investigation was made to determine whether this low temperature orientation to the α-position is also found with diisopropyl ether. Chlorination of diisopropyl ether under the conditions used with diethyl ether gave no more than traces of α-chlorinated ethers, as determined by hydrolysis of the products and Volhard chloride determinations. The chlorination mixture distilled over a wide range and fractional distillation, both at atmospheric and reduced pressures, failed to give sharp fractions. 1,3-Dichloropropanone was the only substance isolated. This product indicates a cleavage during chlorination not found with diethyl ether.

Henry's method,[4] using isopropyl alcohol and acetone, failed to give α-chlorodiisopropyl ether, desired for comparison with the chlorination products.

Experimental

Chlorination of Diisopropyl Ether.—Seventy-two grams (0.710 mole) of purified[5] anhydrous diisopropyl ether were placed in a Pyrex flask fitted with a thermometer, mechanical stirrer and gas inlet tube and protected from moisture. The flask was immersed in a Dry Ice–acetone-bath and irradiated with a 275-watt reflector sun lamp at a distance of 30 cm. Dry chlorine was slowly passed into the ether at −20 to −25° until 53.0 g. (0.746 mole) had been absorbed, requiring 8.5 hours. Gases escaped as the reaction mixture warmed to room temperature, leaving a net gain of 30.8 g. Distillation and redistillation under reduced pressure gave 12.0 g. of material boiling at 83–88° (33 mm.) which solidified in the ice-box. Repeated crystallization from chloroform gave colorless needles with the following properties: m.p. 42.0–43.0° (cor.); b.p. 172–172.5°; n_D^{25} 1.4773; volatile at room temperature; lachrymatory; soluble water, alcohol, ether; reduces Fehling solution. These properties are in agreement with those reported for 1,3-dichloropropanone.[6]

Anal. Calcd. for $C_3H_4Cl_2O$: C, 28.35; H, 3.18; Cl, 55.91; mol. wt., 127.0. Found: C, 28.56; H, 3.18; Cl, 55.40; mol. wt.,[7] 128.4.

(3) G. E. Hall and F. M. Ubertini, *J. Org. Chem.*, **15**, 715 (1950).
(4) L. Henry, *Bull. acad. roy. Belg.*, [3] **25**, 439 (1893); *Ber.*, **26**, Referate, 933 (1893).
(5) A. I. Vogel, *J. Chem. Soc.*, 618 (1948).
(6) E. H. Huntress, "Organic Chlorine Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 91.
(7) Cryoscopic method, *dl*-camphor solvent.

MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MASS.
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The Preparation and Spectrophotometric Estimation of 2-Amino-7-hydroxyfluorene[1]

BY HELMUT R. GUTMANN

The preparation and properties of 2-amino-7-hydroxyfluorene are of considerable interest since this phenolamine is a likely intermediate in the metabolism of the carcinogen 2-aminofluorene.

The synthesis of this compound from 2-amino-7-nitrofluorene was first reported by Bielschowsky.[2] 2-Amino-7-hydroxyfluorene melting at 271° was obtained in unrecorded yield. Goulden and Kon[3] prepared 2-amino-7-hydroxyfluorene starting with 2-aminofluorenone.

(1) This investigation was supported by Research Grant C-1066 from the National Cancer Institute of National Institutes of Health, Public Health Service.
(2) F. Bielschowsky, *Biochem. J.*, **39**, 287 (1945).
(3) F. Goulden and G. Kon, *J. Chem. Soc.*, 930 (1945).