β-Isopropylmercapto-L-alanine and Derivatives

BY OSCAR GAWRON AND JOHN A. LIEB[1]

β-Isopropylmercapto-L-alanine, its methyl ester hydrochloride and its N-tosyl and N-cinnamoyl derivatives have been synthesized by the following procedures.

β-Isopropylmercapto-L-alanine.[2]—To 20 g. (0.0833 mole) of cystine in 600 ml. of liquid ammonia in a 3-necked flask bearing a Dry Ice–acetone condenser and cooled in a Dry Ice–acetone-bath, 8.1 g. (0.35 gram-atom) of sodium was added slowly and with vigorous stirring. After completion of the reduction,[3] 20.9 g. (0.17 mole) of isopropyl bromide (the chloride does not react) was added in one portion. Stirring and cooling were then continued for 2 hours, after which time the cooling bath and condenser were removed and the ammonia allowed to evaporate (approx. 3 hours) with the stirrer in operation. Residual ammonia was removed by evacuation (water-pump) at 50°. The white residue was taken up in 150 ml. of water and extracted with 50 ml. of ether. Acidification of the aqueous solution with dilute hydrochloric acid to a *p*H of 4.5, followed by filtration, washing with cold water, and drying in a desiccator yielded 20 g. (72%) of crude product, m.p. 202–205°. The crude was recrystallized by dissolving in 300 ml. of boiling water, filtering, vacuum concentrating to one-half the volume and cooling in the refrigerator; yield, 8.5 g. of material melting at 223–224°.[4] Stoll and Seebeck[2] report 237–239°.

Anal. Calcd. for $C_6H_{13}O_2NS$: N, 8.6. Found: N, 8.5.

β-Isopropylmercapto-L-alanine Methyl Ester Hydrochloride.—The crude residue (after removal of the ammonia) from the isopropylation (above) of 24 g. (0.1 mole) of cystine was dissolved in 200 ml. of water. Concentrated hydrochloric acid was then added to excess, the precipitated β-isopropylmercapto-L-alanine redissolving. The acid solution was then concentrated *in vacuo* and the residue was dried *in vacuo* at 70°. The dry residue was then extracted with two 150-ml. portions of hot methanol and the combined methanol extracts after saturation with dry hydrogen chloride were allowed to stand overnight at room temperature. The methanol was then removed *in vacuo*, 300 ml. of fresh methanol added and the esterification procedure was repeated. After standing overnight the product was filtered off, washed with cold methanol and dried *in vacuo;* yield 20 g. (50%), m.p. 144–145°.

Anal. Calcd. for $C_7H_{16}O_2NSCl$: Cl, 16.6. Found: Cl, 16.4.

β-Isopropylmercapto-N-(*p*-tosyl)-L-alanine.—Essentially the procedure of Woolley[5] for the tosylation of amino acids was employed. To 3.3 g. (0.02 mole) of β-isopropylmercapto-L-alanine dissolved in 20 ml. of 2 *N* sodium hydroxide, 4.0 g. (0.021 mole) of *p*-toluenesulfonyl chloride was added in one portion. The mixture was shaken vigorously, and intermittently heated in a water-bath at 70° for 20 minutes. At the end of this period the reaction mixture was cooled under the tap, extracted once with 15 ml. of ether, filtered and the filtrate made acid to congo red with concentrated hydrochloric acid. The crude product after filtration, washing with water and drying in a desiccator weighed 3.0 g. (47%) and melted at 84–86°. After three recrystallizations from alcohol–water, 0.8 g. of analytically pure material, m.p. 116.5–117°, was obtained.

Anal. Calcd. for $C_{13}H_{19}O_9NS_2$: S, 20.3. Found: S, 20.3.

β-Isopropylmercapto-N-cinnamoyl-L-alanine.—To 3.6 g. (0.022 mole) of β-isopropylmercapto-L-alanine in 44 ml. of 1 *N* sodium hydroxide, 3.7 g. (0.023 mole) of cinnamoyl chloride was added in one portion. The reaction mixture was then vigorously shaken for 10 minutes, at the end of which time the exothermic reaction was complete. Following filtration and acidification of the filtrate, 5.1 g. (80%) of crude, air-dried product, m.p. 142–148°, was obtained. Recrystallization from alcohol–water gave pure material, m.p. 159–160°.

Anal. Calcd. for $C_{15}H_{19}O_3NS$: N, 4.8. Found: N, 4.7.

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(1) Abstracted in part from a thesis submitted by J. A. Lieb in partial fulfillment of the requirements for the Master's degree.

(2) During the course of this work A. Stoll and E. Seebeck (*Helv. Chim. Acta*, **32**, 866 (1949)) synthesized this compound in unreported yield by treating cysteine with isopropyl bromide in aqueous alcohol containing sodium hydroxide.

(3) V. du Vigneaud, L. F. Audrieth and H. S. Loring, THIS JOURNAL, **52**, 4500 (1930).

(4) Capillary melting points are uncorrected.

(5) D. W. Woolley, *J. Biol. Chem.*, **172**, 71 (1948).

The Reaction of Brom Phenol Blue with Amino Acids and Peptides[1]

BY IRVING I. GESCHWIND AND CHOH HAO LI

Brom phenol blue affords a sensitive indication of the presence of proteins in paper electrophoresis.[2,3] The paper is dipped in a 0.1–1% solution of the dye in ethanol saturated with $HgCl_2$. Rinsing of the strip in water, with or without prior fixation, allows the removal of excess dye, leaving the protein-containing areas a green-blue color.

In the course of experiments designed to demonstrate the mutual presence of peptides and proteins on paper chromatograms, it was discovered that certain natural and synthetic peptides and amino acids gave false positive tests for the presence of proteins under these conditions. A systematic study was then instituted, which revealed the findings reported below.

Among the naturally occurring amino acids (cysteine alone was not employed in this study) only histidine gave a definite positive reaction which could be accented by quickly passing the paper strip through ammonia vapor, in which case a deep blue color was obtained. Methionine gave a slightly positive reaction, and tryptophan a weak one. Histamine gave a reaction similar to that of histidine. Of all the peptides (28 were used, in all) only glutathione (GSH) and leucyl-histidine gave positive reactions. The former was the only cysteine-containing peptide, and the latter the only histidine peptide, used in this study. Glutathione gave a red-brown color with the dye, whereas the histidine peptide gave a green color.

In another series of experiments it was found that, if the chromatograms were treated very quickly with NH_3 vapor prior to the application of the dye, after the final washing with water, arginine, histidine, lysine and histamine showed up as royal blue spots on a light blue background. None of the other amino acids, with the exception of aspartic and glutamic acids, could be detected. These latter two could be detected by bright white areas left on the blue background.

The basic reaction of the amino acids and peptides would seem to depend upon the insolubility in the final rinsing process of the mercury complexes formed. Thus, after treatment with the dye, rinsing, and drying, spraying the paper with ninhydrin revealed none of the other amino acids originally present. That combination with the dye *per se*, was not involved, was shown by experiments in

(1) This work was supported in part by a grant from the Rockefeller Foundation.

(2) E. L. Durrum, THIS JOURNAL, **72**, 2943 (1950).

(3) H. D. Cremer and A. Tiselius, *Biochem. Z.*, **320**, 273 (1950).

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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(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).