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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(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]

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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

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

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