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THE SYNTHESIS AND RESOLUTION OF S-PHENYLHOMOCYSTEINE[1]

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Many aromatic hydrocarbons and halogenated hydrocarbons are biologically conjugated with the amino acid, cysteine, and are excreted as mercapturic acids, which are acetylated S-aryl derivatives of cysteine. As a consequence of their biological occurrence many of these compounds have been prepared synthetically. There appears to be no report, however, of the occurrence or synthesis of any of the corresponding derivatives of homocysteine.

A considerable number of S-alkyl derivatives of both of these two amino acids were prepared in this laboratory (1) and it was noted that the specific rotations of most of the thioether derivatives of L-homocysteine, when measured for a 1% solution in *N* hydrochloric acid, were about +23° and that the numerical value for the rotations did not appear to be very dependent upon the nature of the alkyl group. In the case of the alkyl and ω-phenylalkyl derivatives of L-cysteine, however, increasing the chain length of the alkyl group resulted in a progressively more dextrorotatory compound by small, fairly constant increments. The previously prepared S-phenyl-L-cysteine represents a striking exception to this behavior; whereas the S-benzyl-, β-phenylethyl-, and γ-phenylpropyl-L-cysteines exhibit specific rotations of −1.9°, +3.5°, and +4.5° respectively when measured in 1% solution in *N* hydrochloric acid, S-phenyl-L-cysteine shows a specific rotation of +82.0° under the same conditions (2). It was considered of interest to undertake the synthesis and resolution of S-phenylhomocysteine in order to determine whether it, too, showed an "abnormal" rotation.

The procedure of du Vigneaud, *et al.* (3), and of Zbarsky and Young (4) for the synthesis of the aromatic thioethers of cysteine by the condensation of the cuprous mercaptide of cysteine and aryldiazonium chlorides was first tried for the preparation of S-phenylhomocysteine, using homocystine instead of cystine as a starting material. Two preliminary runs were made using cystine for the preparation of S-phenylcysteine in order to establish that the details of the procedure were followed satisfactorily. Several attempts to prepare S-phenylhomocysteine by the same procedure were unsuccessful. The cuprous mercaptide of homocysteine appeared to be formed readily and when the solution of the diazonium salt was added a copious evolution of nitrogen occurred, just as in the preparation of phenylcysteine. The product obtained when the reaction mixture was worked up, however, appeared to be highly contaminated with homocystine and to contain an amorphous material polymeric in nature. No S-phenylhomocysteine could ever be isolated from this product by any of a variety of different methods which were tried, even when the properties of the desired compound were known after its successful synthesis by the alternative method described below.

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An attempt to cause methionine and iodobenzene to react to form a sulfonium compound which could be decomposed to form S-phenylhomocysteine in a manner analogous to the procedure for the formation of S-benzylhomocysteine from methionine and benzyl chloride also was unsuccessful. Another experiment, in which an attempt was made to form a sulfonium derivative by the decomposition of benzenediazonium chloride in an acidic solution containing methionine was likewise fruitless.

A total synthesis of S-phenylhomocysteine was then undertaken following the general procedure reported by Patterson and du Vigneaud (5) for the synthesis of S-benzylhomocysteine using thiophenol, instead of benzyl mercaptan, as a starting material. The intermediate phenyl-β-chloroethyl sulfide was condensed with ethyl acetamidomalonate and the resulting substituted malonic ester was hydrolyzed to form S-phenylhomocysteine directly; the over-all yield was 20%, based on the thiophenol used a starting material.

The synthetic amino acid was resolved readily through the formation of the crystalline strychnine salt of the N-acetyl derivative of what proved to be the D-isomer. A configuation could not be assigned to the resolved isomers on the usual basis of the change in rotation in solutions of different acid concentration (6), since the possibility of an abnormality in optical behavior was one of the factors which prompted the preparation of this compound. It was expected, however, that the isomer which proved to have a specific rotation of $+30°$ in N acid was of the L configuration, since all of the other known thioether derivatives of L-homocysteine have positive rotations ranging between $+15.0°$ (γ-phenylpropyl-L-homocysteine) and $+25.8°$ (D-allocystathionine[2]). That this was, indeed, the case was demonstrated by the ready formation of an optically active phenylhydrazide by the action of papain-cysteine upon a buffered solution of the active N-acetyl derivative of the dextrorotatory isomer in the presence of phenylhydrazine (8). Under precisely the same experimental conditions, the other (levorotatory) isomer formed no phenylhydrazide and the starting material was recovered in good yield. It has been shown previously in many laboratories that papain-cysteine forms substituted anilides or phenylhydrazides with predominantly or only the L or natural isomers of the acylated α-amino acids.

Thus, although the value of $[\alpha]_D$ for S-phenyl-L-homocysteine may represent one limit for the thioether derivatives of homocysteine, it cannot be regarded as having an anomalous behavior in the same sense as S-phenyl-L-cysteine, which exhibits properties radically different from those of closely related compounds.

EXPERIMENTAL

Phenyl β-chloroethyl sulfide. Sodium thiophenoxide solution prepared from 102 g. of thiophenol and 21.3 g. of sodium in 140 ml. of methanol was added to 350 ml. of ethylene chloride. The general procedure reported by Patterson and du Vigneaud (5) for the preparation of benzyl β-chloroethyl sulfide was followed in working up the reaction mixture The 161 g. of crude product so obtained was used without further purification for the next step of the synthesis.

[2] L-Allocystathionine, which has the D configuration on the four-carbon side of the thioether bond, has $[\alpha]_D^{24} -25.8°$ (c, 1, N HCl) (7).

*S-Phenyl-*DL*-homocysteine.* The crude product from above was dissolved in an equal volume of absolute ethanol and the solution was added dropwise to a well-stirred and refluxing solution of 160 g. of ethyl acetamidomalonate and 17 g. of sodium in 1400 ml. of absolute ethanol. After the addition was completed, the stirring and refluxing was continued 7½ hours. The hot reaction mixture was filtered to remove the precipitated sodium chloride and the filtrate was concentrated *in vacuo* until most of the solvent was removed.

The oily residue was suspended in 1400 ml. of 6 *N* HCl and the mixture was heated under reflux for 20 hours. A viscous oil that remained in the bottom of the flask was separated and weighed (83.5 g.); examination showed this material to be a mixture of thiophenol and 1,2-*bis*-(phenylmercapto)ethane. The acidic aqueous layer was concentrated to dryness *in vacuo*, 500 ml. of water was added to the residue, and the concentration was repeated. The residue was dissolved in 200 ml. of hot water, the hot solution was treated with Norit and filtered, and the clear filtrate was adjusted to *p*H 4 by the careful addition of concentrated NH_4OH. The suspension that resulted was cooled in the refrigerator overnight and the product was collected and washed with cold water and absolute ethanol. The yield of crude material was 39.6 g. (20% based on the amount of thiophenol originally used); m.p. 236–238° d.[3] This material was recrystallized by suspending it in 200 ml. of hot water, adding enough concentrated HCl to take it into solution, adding Norit, and reprecipitating the compound by neutralizing the hot solution with concentrated NH_4OH; 35.2 g., m.p. 232–233° d. was obtained. An analytical sample was prepared by three more recrystallizations from hot water; m.p. 229–232° d.

Anal. Calc'd for $C_{10}H_{13}NO_2S$: C, 56.87; H, 6.17; N, 6.63; S, 15.18.
Found: C. 56.99; H, 6.25; N, 6.50; S, 14.86.

*N-Acetyl-S-phenyl-*DL*-homocysteine.* A solution of 30.0 g. of S-phenylhomocysteine in 47.4 ml. of 3 *N* NaOH was cooled to 5° and was acetylated by the simultaneous dropwise addition of 16.1 ml. of acetic anhydride and 59.5 ml. of 3 *N* NaOH to the well-stirred and cooled solution. After the addition was completed, the ice-bath was removed and the stirring was continued for one hour while the solution warmed to room temperature. It was then made acid to Congo Red by the addition of concentrated HCl, the resulting suspension was cooled in an ice-bath, and the product was collected, washed with 3 portions of cold water, and dried; yield, 35.8 gm. (99%); m.p. 163–164°. An analytical sample was prepared by recrystallizing a small portion of the compound from aqueous ethanol; m.p. 164–165°.

Anal. Calc'd for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.96; N, 5.53; S, 12.62.
Found: C, 57.38; H, 6.09; N, 5.50; S, 12.82.

*Resolution of N-acetyl-S-phenyl-*DL*-homocysteine.* A suspension of 28 g. of N-acetyl-S-phenylhomocysteine and 37.5 g. of strychnine in 1 l. of water was heated on a steam-bath until a clear solution resulted. The hot solution was allowed to cool slowly and a partially crystalline oil separated. The oil redissolved when the mixture was warmed, leaving some undissolved crystals, and when the solution was again allowed to cool slowly, more oil and crystalline material separated. This procedure was repeated until the solution could be cooled to room temperature without the separation of oil. The solution was then allowed to stand at room temperature overnight, was cooled in an ice-bath for two hours, and the crystalline precipitate was collected, washed once with cold water, and dried; 33.5 g. of crude salt was obtained; m.p. 206–212°.

*N-Acetyl-S-phenyl-*D*-homocysteine.*[4] The strychnine salt obtained above was dissolved in 900 ml. of hot water and the solution was treated with Norit and filtered. The hot filtrate was seeded with a crystal saved from the original crop and was allowed to cool slowly to room temperature. After it had stood at room temperature overnight, it was cooled in an

[3] All melting points were made on the micro hot stage and are corrected.

[4] The configuration was established, as shown later, by the formation of a phenylhydrazide from the N-acetyl derivative of the dextrorotatory isomer and phenylhydrazine by the action of papain-cysteine.

ice-bath for four hours, and the salt was collected, washed two times with cold water, and dried; 28.8 g. (89%) was obtained, m.p. 216–218°, $[\alpha]_D^{26.5}$ −30.7° (*c*, 1, H_2O). A small sample was recrystallized two more times from hot water, but showed no change in appearance, melting point, or rotation.

The pure strychnine salt (25.0 g.) was dissolved in 1 l. of hot water and the solution was brought to *p*H 8 by the addition of concentrated NH_4OH. The strychnine that separated was collected and the filtrate was further extracted with three 250-ml. portions of chloroform to complete the removal of strychnine. The clear aqueous layer was made acid to Congo Red with concentrated HCl, the solution was cooled in an ice-bath, and the N-acetyl-S-phenyl-D-homocysteine that separated was collected, washed with water, and dried. Yield, 10.4 g. (96%); m.p. 175–176°; $[\alpha]_D^{24}$ −10.1° (*c*, 1, 95% EtOH).

A sample was recrystallized two more times from aqueous ethanol for analysis; m.p., 178–179°; $[\alpha]_D^{22}$ −9.9° (*c*, 1, 95% EtOH).

Anal. Calc'd for $C_{12}H_{15}NO_3S$: N, 5.53; S, 12.66.

Found: N, 5.21; S. 12.10.

N-Acetyl-S-phenyl-L-homocysteine. The filtrate from the first crystallization of the strychnine salt of the D-isomer was treated for the removal of strychnine and the acetyl derivative of the L-isomer was prepared in the same manner as was the D; 12.7 g. was obtained; $[\alpha]_D^{25}$ +8.9° (*c*, 1, 95% EtOH). Two recrystallizations from aqueous ethanol yielded 10.5 g. (75%); m.p. 178–179°; $[\alpha]_D^{25}$ +9.6° (*c*, 1, 95% EtOH).

Anal. Calc'd for $C_{12}H_{15}NO_3S$: N, 5.53; S, 12.66.

Found: N, 5.29; S, 12.29.

S-Phenyl-D-homocysteine. N-Acetyl-S-phenyl-D-homocysteine (5.5 g.) was suspended in 400 ml. of 2 *N* HCl and the mixture was heated under reflux for three hours. The resulting solution was concentrated to dryness *in vacuo*. The residue was redissolved in a small amount of water, and the procedure was repeated. The residue was then dissolved in 50 ml. of hot water, the solution was adjusted to *p*H 4 by the addition of concentrated NH_4OH, cooled in an ice-bath, and the product was collected, washed with cold water, and dried. Yield, 4.3 g. (93%); $[\alpha]_D^{26}$ −28.7° (*c*, 1, *N* HCl). Two recrystallizations from water yielded material, $[\alpha]_D^{24}$ −30.2° (*c*, 1, *N* HCl); m.p. 233–236° d.

Anal. Calc'd for $C_{10}H_{13}NO_2S$: C, 56.85; H, 6.22; N, 6.63; S, 15.18.

Found: C, 56.94; H, 6.33; N, 6.46; S, 14.71.

S-Phenyl-L-homocysteine. N-Acetyl-L-homocysteine (4.5 g.) was hydrolyzed with 2 *N* HCl and worked up by the above procedure; yield, 3.6 g. (96%); $[\alpha]_D^{24}$ +30.2° (*c*, 1, *N* HCl). One recrystallization from water yielded material, $[\alpha]_D^{20}$ +30.3° (*c*, 1, *N* HCl); m.p. 234–236° d.

Anal. Calc'd for $C_{10}H_{13}NO_2S$: C, 56.85; H, 6.22; N, 6.63; S, 15.18.

Found: C, 56.81; H, 6.14; N, 6.60; S, 15.11.

Enzymatic synthesis of N-acetyl-S-phenyl-L-homocysteine phenylhydrazide. N-Acetyl-S-phenyl-L(+)-homocysteine (1 g.) was dissolved in 45 ml. of 0.6 *M* (*p*H 4.6) acetate buffer and 214 mg. of cysteine hydrochloride and 0.42 ml. of phenylhydrazine were added. Enzyme solution was prepared by triturating crude papain (Merck) with 12 ml. of cold water, centrifuging the mixture, and adding the supernatant to the above solution. The solution was adjusted to *p*H 4.75 by the careful addition of 1 *N* NaOH and it was incubated at 38°. Rosettes of needles began to form after a few hours had elapsed, and at the end of three days the crystalline precipitate that had formed was collected, washed with cold water, and air-dried. The crude product weighed 0.75 g. It was dissolved in 20 ml. of hot ethanol, a small amount of cystine was removed by filtration, and the ethanol was diluted with 2 volumes of hot water. The solid that formed when the solution had cooled was collected; 0.50 g., $[\alpha]_D^{25}$ +3.5° (*c*, 1, 95% EtOH); m.p. 178–180°. Two more recrystallizations did not change the melting point or rotation.

Anal. Calc'd for $C_{18}H_{21}N_3O_2S$: N, 12.33; S. 9.34.

Found: N, 11.80; S, 9.32.

Acidification of the original mother liquor yielded 0.45 g. of starting material.

N-Acetyl-S-phenyl-D(−)-homocysteine (1 g.) was treated with phenylhydrazine in the presence of papain in the same manner as in the preceding experiment. No phenylhydrazide was formed and 0.92 g. of starting material was recovered from the reaction mixture.

The microanalyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

SUMMARY

S-Phenyl-DL-homocysteine has been synthesized and resolved into its optical antipodes. Configurations have been assigned to the resolved isomers on the basis that papain-cysteine forms an active phenylhydrazide from the N-acetyl derivative of the dextrorotatory isomer but not from the other.

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