Toluene-1-C^{14}.—The dehydrogenation apparatus, similar to that described by Ruzicka,[13] consisted of a pot, catalyst tube (90 cm. long attached to a side arm equipped with a stopcock so as to allow the product to be recycled), and a reflux condenser. A 3-l. gas buret connected to the condenser served to measure the volume of evolved hydrogen, which was collected over water.

The catalyst tube, charged with a 70-cm. column of pellets of dehydrogenation catalyst,[14] was heated slowly to 450° while a stream of dry hydrogen was passed slowly through the system. After 20 hours the hydrogen flow was interrupted and the system was evacuated to 0.1 mm. until no more water was evolved. Finally dry hydrogen was admitted to bring the system to atmospheric pressure.

1-Methylcyclohexene-1-C^{14}, 2.11 g. (22.0 mM.) was added to the pot and slowly distilled through the catalyst bed at 450°. Hydrogen evolution, rapid at first, practically ceased after the second or third cycle over the catalyst. In all 926 ml. (41.5 mM.) of dry hydrogen (S.T.P.) was collected; calculated 985 ml. (44.0 mM.); the pure toluene obtained in the dehydrogenation amounted to 1.91 g. (95% yield) including a small quantity isolated from the hot catalyst bed by evacuation at 0.1 mm.

The toluene isolated in a typical dummy run had a refractive index n^{29}D 1.4908 as compared with n^{29}D 1.4892 observed for an authentic sample.

Benzoic-1-C^{14} Acid.—Oxidation of 1.91 g. of toluene-1-C^{14} with 7.80 g. of potassium permanganate[15] in 96 ml. of distilled water and reduction of the manganese dioxide with sulfur dioxide in the usual manner afforded 1.87 g. (15.4 mM., 74% yield) of benzoic-1-C^{14} acid, m.p. 122.6–123.0° cor.; a sample of pure benzoic acid melted at 123.2–123.6°. The product had a specific activity of 1.07 mc./mM., in excellent agreement with the specific activity of the initial carbon dioxide.

In the small scale run the over-all radiochemical and weight yields of benzoic acid from carbon dioxide amounted to 24.2%; the actual yield is somewhat higher, however, since quantitative recovery of methylcyclohexanol and methylcyclohexene was not attempted here and the remaining active products were recovered from the distillation apparatus and residues by the carrier technique. In two other runs, 131 and 149 mmoles of carbon dioxide containing 53.0 and 68.0 mc. of C^{14} were converted to benzoic-1-C^{14} acid in 36.7 and 24.6% yields, resp., when the syntheses were performed without further dilution. In the three preparations the over-all yield of benzoic-1-C^{14} acid obtained from 191 mc. of carbon dioxide C^{14} amounted to 64.6 mc. (33.8% yield) including the benzoic acid of lower specific activity isolated by processing all of the stripping fractions.

Benzene-C^{14}.—Heated under nitrogen in a distilling flask with 20 ml. of freshly distilled quinoline with 4.0 g. of powdered copper oxide, 10.0 g. of benzoic-1-C^{14} acid underwent smooth decarboxylation at 260°. After purification by drying over phosphorus pentoxide and distillation there was obtained 5.5 g. of benzene, $n^{24.5}$D 1.4978; observed for an authentic sample, $n^{24.5}$D 1.4971. The product from a representative dummy run had a b.p. 80–81°.

Acknowledgment.—The assistance of Miss Alice McCarthy in performing the radioanalyses is gratefully acknowledged.

BOSTON, MASS.

(13) L. Ruzicka and M. Stoll, *Helv. Chim. Acta*, **7**, 84 (1924).

(14) Universal Oil Products "Dehydrogenation Catalyst."

(15) F. Ullmann and J. B. Uzbachian, *Ber.*, **36**, 1797 (1903).

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

Preparation of Peptides Containing Cysteine

BY L. CARROLL KING AND FRED H. SUYDAM

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N-(N-Phenylacetyl-L-cysteinyl)-glycine and N-(N-phenylacetyl-L-cysteinyl)-D-valine were prepared. The procedures employed and some of the properties of the intermediates are described.

N-(N,N'-Dicarbobenzoxy-L-cystinyl)-diglycine (III) was prepared[1] and converted to N-(S-benzyl-L-cysteinyl)-glycine (IV).[2] IV could be crystallized from water as recommended by Loring and du Vigneaud[2] providing the temperature of the water was kept below 80°. Above 80° IV was converted rapidly to L-benzylmercaptomethyldiketopiperazine (V). Compound IV reacted with phenylacetyl chloride to give N-(N-phenylacetyl-S-benzylcysteinyl)-glycine (VI). On treatment of VI with sodium in liquid ammonia N-(N-phenylacetyl-L-cysteinyl)-glycine (I) was obtained. The homogeneity of I was established by analysis and mercapto group determination.[3]

In a similar series of reactions, N-(N-phenylacetyl-L-cysteinyl)-D-valine (II)[4,5] was prepared. Thus, N,N'-dicarbobenzoxy-L-cystinyl chloride[1] was converted to N-(N,N'-dicarbobenzoxy-L-cystinyl)-di-D-valine (VII). VII was converted to N-(S-benzyl-L-cysteinyl)-D-valine (VIII), and VIII was converted to N-(N-phenylacetyl-S-benzyl-L-cysteinyl)-D-valine. This latter substance was obtained only as a sirup, but the sirup on reduction with sodium and liquid ammonia gave crystalline II.

Attempts to prepare I or II directly from N-acylated cysteine or N,N'-diacylated cystine were not successful. The step involving formation of the acid chloride or azlactone always failed to give a characterizable product. Some of the difficulties involved are of general interest and are reported herein.

N-N'-Diphenylacetyl-L-cystine (IX) was prepared from phenylacetyl chloride and L-cystine by the method of Shiple and Sherwin.[6] When crystallized from water the product melted at 119–121°, in agreement with the report of Shiple and Sherwin.[6] However, when crystallized from ethyl acetate by addition of ligroin the product melted at 159–161°. Each form of the compound had a neutralization equivalent in agreement with that calculated for IX and the two forms had identical specific rotations.

(1) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(2) H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **111**, 387 (1935).

(3) J. W. Kimball, R. L. Kramer and E. E. Reid, THIS JOURNAL, **43**, 1199 (1921).

(4) It was of interest to use D-valine since penicillamine is of the D-series. See H. T. Clarke, J. R. Johnson and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 6.

(5) The resolution of valine using the method of E. Fischer, *Ber.*, **39**, 2320 (1906), was improved by using toluene and distilling the azeotrope. With this technique the reaction time was reduced from 12 hours to three hours and 90% formic acid could be used.

(6) G. J. Shiple and C. P. Sherwin, *J. Biol. Chem.*, **55**, 671 (1923).

Furthermore, the two forms were interconvertible simply by recrystallization from the appropriate solvent. This is a case of dimorphism, and difficulty in duplicating the results of Shiple and Sherwin[6] are resolved by this observation. Attempts to prepare an acid chloride or azlactone from IX were unsuccessful.

Compound IX was reduced with sodium in liquid ammonia and the product benzylated to give N-phenylacetyl-S-benzyl-L-cysteine (X) in 72% yield. This constitutes a new method of preparation for this compound.

S-Benzyl-L-cysteine was prepared by the method of du Vigneaud, Audrieth and Loring.[7] This compound was then allowed to react with phenylacetyl chloride according to the procedure of Shiple and Sherwin.[6] Two separate products were isolated from this reaction, the first and least water soluble of the two melted sharply at 130–130.5° and constituted about 40% of the total yield. The second compound, 60% of the total yield, melted at 88–89° and was identical with X described above. The high melting compound obtained in the above reaction was optically inactive and was shown to be the racemic compound N-phenylacetyl-S-benzyl-DL-cysteine.

The preparation of X by reduction of IX is to be preferred over the old method in that one obtains a better yield and partial racemization of the product is avoided.

Attempts to convert compound X to the acid chloride using thionyl chloride or phosphorus trichloride did not give characterizable compounds. Treatment of X with phosphorus pentachloride in anhydrous ether gave the racemic compound, N-phenylacetyl-S-benzyl-DL-cysteine. This racemization is probably due to formation of an azlactone salt which lost hydrogen chloride during filtration and hydrolyzed on crystallization.[8]

Acknowledgment.—The authors wish to thank Swift and Company for a research grant to support this work.

Experimental[9]

N,N'-Diphenylacetyl-L-cystine (IX).—This compound was prepared by the method of Shiple and Sherwin[6] and crystallized from water; yield 47%, m.p. 119–121°, after sintering at 107–108°, $[\alpha]^{30}D$ −124.5° in ethanol.

Anal. Calcd. for $C_{22}H_{24}O_6N_2S_2$: C, 55.44; H, 5.08; N, 5.88; neut. equiv., 238. Found: C, 55.85; H, 5.14; N, 5.76; neut. equiv., 237, 240.

Interconversion of Crystalline Modifications of IX.—N,N'-Diphenylacetyl-L-cystine (m.p. 119–121°) was dissolved in an excess of ethyl acetate. The solution was heated to boiling and hot ligroin was added to cloudiness. Cooling of the solution gave a precipitate of long, white needles, melting at 159–161°, $[\alpha]^{30}D$ −124.7° in ethanol.

Anal. Calcd. for $C_{22}H_{24}O_6N_2S_2$: C, 55.44; H, 5.08; N, 5.88; neut. equiv., 238. Found: C, 55.78; H, 4.62; N, 5.54; neut. equiv., 239.

Recrystallization of the high melting modification from water gave long, narrow plates; m.p. 119–121°, sintering at 107–108°. The mixed melting point of this material with the low melting product previously obtained was 119–121°, $[\alpha]^{30}D$ −124.8° in ethanol. Calcd.: neut. equiv., 238. Found: neut. equiv., 239.

A mixture of the high and low melting modification melted at 137–139°.

N-Phenylacetyl-S-benzyl-L-cysteine (X). (a) From IX.—Compound IX was reduced with sodium in liquid ammonia and benzylated. The crude solid product was taken up in benzene, the benzene evaporated and the product crystallized from a large volume of water; yield 72%, m.p. 88–89°, $[\alpha]^{32}D$ −47.3° in ethanol.

(b) From S-Benzyl-L-cysteine.—Six grams (0.03 mole) of S-benzyl-L-cysteine[7] was dissolved in a solution of 1.2 g. of sodium hydroxide in 70 ml. of water. To the cooled solution 6.2 g. (0.04 mole) of phenylacetyl chloride and a solution of 1.6 g. of sodium hydroxide in 50 ml. of water were added alternately in ten portions with vigorous shaking. The alkaline solution was then acidified to congo red with 50% sulfuric acid. A yellow gum precipitated, which solidified on standing overnight in the mother liquor. The crude product was dissolved in 3 liters of hot water, the solution filtered and the filtrate allowed to cool slowly, whereupon a precipitate of small, white needles was obtained. This product melted at 126–128°. Concentration of the mother liquor to approximately 2.25 l. and allowing it to cool gave a second batch of the same compound. Further concentration of the mother liquor to about 1 liter, followed by cooling, gave a second product of long, white needles, melting at 88–89°.

The low melting product was N-phenylacetyl-S-benzyl-L-cysteine (X). No depression in melting point was observed when this compound was mixed with X prepared from N,N'-diphenylacetyl-L-cystine (IX); yield 2.7 g., 28% of theoretical, $[\alpha]^{32}D$ −47.5° in ethanol. Calcd. for $C_{18}H_{19}O_3NS$: neut. equiv., 329. Found: neut. equiv., 330.

The compound melting at 126–128° was recrystallized from water, giving bunches of fine needles which melted sharply at 130–130.5°; yield 1.7 g. This material gave no rotation in ethanol or in methanol and was apparently N-phenylacetyl-S-benzyl-DL-cysteine.

Anal. Calcd. for $C_{18}H_{19}O_3NS$: C, 65.63; H, 5.81; N, 4.25; neut. equiv., 329. Found: C, 66.09; H, 5.64; N, 4.23; neut. equiv., 325, 328.

N-Phenylacetyl-S-benzyl-DL-cysteine, from X.—One gram (0.003 mole) of finely powdered X was suspended in 20 ml. of anhydrous ether, and the suspension cooled in an ice–salt-bath. There was added 0.64 g. (0.003 mole) of powdered phosphorus pentachloride, and the mixture was shaken vigorously. After about three minutes all the solid had dissolved. Shaking was continued, with intermittent cooling in the freezing mixture, for 10 minutes, during which time white crystals precipitated from the solution. The solution was filtered rapidly with suction, and the solid tested for chloride with negative results; m.p. 126–127°. Recrystallization from water raised the melting point to 130–130.5°. The compound was without optical activity and showed no depression in melting point when mixed with the N-phenylacetyl-S-benzyl-DL-cysteine obtained as a side product in the preparation of N-phenylacetyl-S-benzyl-L-cysteine, as described above.

Anal. Calcd. for $C_{18}H_{19}O_3NS$: C, 65.63; H, 5.81; N, 4.25; neut. equiv., 329. Found: C, 65.60; H, 5.81; N, 4.23; neut. equiv., 328.

N,N'-Dicarbobenzoxy-L-cystine.—This compound was prepared by the method of Bergman and Zervas[1]; yield 82%, m.p. 119–120°, $[\alpha]^{24}D$ −51.6°.

Anal. Calcd. for $C_{22}H_{24}O_8N_2S_2$: C, 52.05; H, 4.76; N, 5.52; neut. equiv., 254. Found: C, 51.76; H, 5.07; N, 5.35; neut. equiv., 255, 255.

N,N'-Dicarbobenzoxy-L-cystinyl Chloride.—By the procedure of Bergman and Zervas,[1] this compound had a m.p. 68–69°. Because of instability the compound was prepared only as needed.

N-(N,N'-Dicarbobenzoxy-L-cystinyl)-diglycine (III).—This substance was prepared by the method of Loring and du Vigneaud[2]; yield 55%, m.p. 176–177°, $[\alpha]^{24}D$ −129° in methanol.

Anal. Calcd. for $C_{26}H_{30}O_{10}N_4S_2$: C, 50.10; H, 4.85; N, 9.00; neut. equiv., 311. Found: C, 49.81; H, 4.84; N, 8.67; neut. equiv., 312.

N-(S-Benzyl-L-cysteinyl)-glycine (IV).—Prepared from III using the method of Loring and du Vigneaud.[2] The crude

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

(8) H. E. Carter and J. W. Hinman, *J. Biol. Chem.*, **178**, 403 (1947); Karrer and dalla Vedova, *Helv. Chim. Acta*, **11**, 368 (1928).

(9) All melting point data were observed on a Fisher–Johns melting point apparatus. Analyses by J. Sorensen.

product was recrystallized from water after treatment with decolorizing charcoal, care being taken to keep the temperature below 80°; yield 75%. The compound melted with decomposition at 165–166°; $[\alpha]^{30}D$ 74° in water.

Anal. Calcd. for $C_{12}H_{16}O_3N_2S$: C, 53.71; H, 6.01; N, 10.44; neut. equiv., 268. Found: C, 54.27; H, 6.08; N, 10.48; neut. equiv., 267.

L-Benzylmercaptomethyldiketopiperazine (V).—Two grams of IV was dissolved in the minimum necessary amount of boiling water, and the solution was boiled 10 minutes. On cooling, the product separated as small white plates, which melted sharply at 198°; yield 1.7 g. The compound was not soluble in dilute acid, dilute base, ether, acetone, carbon disulfide, chloroform, ethanol or cold water, but was somewhat soluble in pyridine, and very soluble in hot water; $[\alpha]^{30}D$ 65.4° in pyridine.

Anal. Calcd. for $C_{12}H_{14}O_2N_2S$: C, 57.57; H, 5.64; N, 11.19. Found: C, 57.77; H, 5.67; N, 11.14.

N-(N-Phenylacetyl-S-benzyl-L-cysteinyl)-glycine (VI).—Compound IV was treated with sodium hydroxide and phenylacetyl chloride. The product was crystallized from ethyl acetate; yield 73%, m.p. 129–131°, $[\alpha]^{24}D$ −42.5° in methanol.

Anal. Calcd. for $C_{20}H_{22}O_4N_2S$: C, 62.15; H, 5.74; N, 7.25; neut. equiv., 386. Found: C, 62.73; H, 5.89; N, 7.34; neut. equiv., 382, 386.

N-(N-Phenylacetyl-L-cysteinyl)-glycine (I).—Compound VI was reduced with sodium in liquid ammonia. The product was crystallized from water. An inert atmosphere, nitrogen, was maintained around all the operations; yield 75%, m.p. 134–135°, $[\alpha]^{27}D$ −35° in ethanol.

Anal. Calcd. for $C_{13}H_{16}O_4N_2S$: C, 52.69; H, 5.48; N, 9.46. Found: C, 52.72; H, 5.62; N, 9.62.

Determination of the mercapto group by the method of Kimball, Kramer and Reid[3] gave an equivalent weight of 300; calcd. 296.

N-(N,N'-Dicarbobenzoxy-L-cystinyl)-di-D-valine (VII).—A solution of 8.0 g. (0.068 mole) of D-valine ($[\alpha]^{27}D$ −28.4° in 20% hydrochloric acid) in 80 ml. of normal sodium hydroxide was allowed to react with the acid chloride from 15.0 g. (0.03 mole) of N,N'-(dicarbobenzoxy)-L-cystine.[10]

For purification the product was dissolved in an excess of dioxane, the solution was heated to boiling, and hot water added carefully until the solution was barely cloudy. Slow cooling of this hot solution yielded 12.5 g. (60%) of fine white needles, melting at 145–147°, $[\alpha]^{29}D$ −42.5° in methanol.

Anal. Calcd. for $C_{32}H_{42}N_4O_{10}S_2$: C, 54.41; H, 5.99; N, 7.93; neut. equiv., 353. Found: C, 53.54; H, 5.37; N, 7.89; neut. equiv., 355, 356.

N-(S-Benzyl-L-cysteinyl)-D-valine (VIII).—The procedure was similar to that used for preparation of IV. From 5.0 g. (0.007 mole) of VII, 3.0 g. (70%) of VIII, m.p. 200–202°, was obtained; $[\alpha]^{29}D$ 14.5° in 50% ethanol.

Anal. Calcd. for $C_{15}H_{22}N_2O_3S$: C, 58.04; H, 7.15; N, 9.03; neut. equiv., 310. Found: C, 58.10; H, 7.12; N, 8.96; neut. equiv., 311.

N-(N-Phenylacetyl-S-benzyl-L-cysteinyl)-D-valine.—Compound VIII was treated with phenylacetyl chloride and sodium hydroxide. The crude product was dissolved in ethyl acetate from which it separated as an oil. This product was not obtained as a solid. It was used directly for the following step.

N-(N-Phenylacetyl-L-cysteinyl)-D-valine (II).—The procedure used was similar to that used for preparation of I. Two grams of the sirup described above gave 1.0 g. (62%) of II, m.p. 178–180°, $[\alpha]^{30}D$ −46.0° in ethanol.

Anal. Calcd. for $C_{16}H_{22}N_2O_4S$: C, 56.78; H, 6.55; N, 8.28; equiv. wt., 338. Found: C, 54.05; H, 6.70; N, 7.86; equiv. wt., 344.[3]

(10) The reaction conditions were identical with those described for preparation of III.

EVANSTON, ILLINOIS

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Thieno[3,2-b]pyridine. I. The Preparation and Properties of an S-Isosteric 8-Hydroxyquinoline[1]

BY JOHN T. SHEEHAN AND GEORGE J. LEITNER[2]

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The synthesis of 3-hydroxythieno[3,2-b]pyridine, an isoester of 8-hydroxyquinoline, from 3-(carboxymethylmercapto)-picolinic acid is described. In addition, a number of derivatives of 3-hydroxythieno[3,2-b]pyridine have been prepared.

Aside from the thianaphthene derivatives relatively little has been reported about other condensed ring systems of thiophene. For the preparation of some S-isosteric analogs[3] of Pamaquine as antimalarials the route chosen was *via* 3-hydroxythieno(3,2-b)pyridine, an isoster of 8-hydroxyquinoline.[4] The formation of this compound is intimated by Plazek and Sucharda[5] in their synthesis of δ-thiopyrindigo (Fig. 1); however, its isolation was not described nor was it characterized.

In the present work, following the method employed by Koenigs and Kantrowitz[6] in their synthesis of 3-hydroxy-4,6-dimethylthieno(3,2-c)pyridine, 3-hydroxythieno(3,2-b)pyridine was initially prepared directly by heating 3-(carboxymethylmercapto)-picolinic acid in acetic anhydride. The yields obtained by this method were only about 15%; however, it was found that by first isolating the intermediate acetate derivative and subsequently hydrolyzing it, the over-all yield of 3-hydroxythieno(3,2-b)pyridine could be raised to about 50%. An attempt to effect cyclization of 3-(carboxymethylmercapto)-picolinic acid in neutral medium like mineral oil proved unsuccessful, decarboxylation occurring to form 3-(carboxymethylmercapto)-pyridine.[7]

The 3-(thieno(3,2-b)pyridyl) acetate, unlike its isoster 8-quinolyl acetate is a stable oil which can be kept indefinitely in the absence of moisture. It is readily hydrolyzed by heating in water and the hydrolysis proceeds smoothly in an atmosphere of

(1) Presented in part before the Medicinal Section of the XIIth International Congress, New York, N. Y., Sept. 10-13, 1951.

(2) Nuodex Products Inc., Elizabeth, N. J.

(3) J. T. Sheehan, THIS JOURNAL, **74**, 5504 (1952).

(4) 4-Hydroxybenzothiazole, another isoster of 8-hydroxyquinoline has been investigated by H. Erlenmeyer, *et al.*, *Helv. Chim. Acta.*, **21**, 709, 1695 (1938).

(5) E. Plazek and E. Sucharda, *Ber.*, **59**, 2282 (1926).

(6) E. Koenigs and H. Kantrowitz, *ibid.*, **60**, 2097 (1927).

(7) The failure of 3-(carboxymethylmercapto)-pyridine to cyclize in acetic anhydride as described by A. E. Chichibabin and N. N. Vorozhtzov, Jr., *ibid.*, **66**, 364 (1933), would seem to suggest that the cyclization occurs through the carboxyl on carbon atom two of the pyridine ring.