9-Dehydromanogenic Acid.—9-Dehydromanogenin diacetate, 203 mg., was hydrolyzed with 5% alcoholic potassium hydroxide and then oxidized with 210 mg. of chromic anhydride in 18 ml. of acetic acid for one hour at room temperature. Methanol and water was added and the mixture was extracted with ether. The acid fraction was recovered by a 5% aqueous potassium hydroxide wash followed by acidification and extraction with ether. The product, wt. 106 mg., from ether was crystallized from 80% acetic acid and then from glacial acetic acid, m.p. 265–267° (dec.), $[\alpha]^{24}D$ 0° (*c*, 0.99), λ_{max}^{alc} 240 mμ (log ε 4.0); wt. 24 mg.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.2; H, 8.0. Found: C, 67.7; H, 8.3.

The dimethyl ester was prepared by treating the diacid, 80 mg., with an excess of diazomethane in ether at 0° for three hours. The excess diazomethane was destroyed with acetic acid and the ethereal solution was washed with dilute sodium bicarbonate and water and then dried and evaporated. The residue was crystallized four times from methanol to raise the melting point from 171–177° to 178–180°, $[\alpha]^{26}D$ +4° (*c*, 1.10). A mixture with the dimethyl ester of manogenic acid (163°) melted at 170–176°.

Anal. Calcd. for $C_{29}H_{42}O_7$: C, 69.3; H, 8.4. Found: C, 69.0; H, 8.4.

Manogenic Acid (VI) (a) From 9-Dehydromanogenin.—9-Dehydromanogenin, 217 mg., was hydrogenated in ether with a trace of acetic acid as described for the reduction of the diacetate. The intermediate product showed no absorption maximum at λ240 mμ. It was then dissolved in acetic acid and oxidized at room temperature by the procedure described for the preparation of the unsaturated acid to give manogenic acid which was crystallized from acetic acid, m.p. and mixed m.p. with manogenic acid from hecogenin, 266–269° (dec.); wt. 64 mg. A mixture with 9-dehydromanogenic acid showed a melting point depression of 5°.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5. Found: C, 67.4; H, 8.4.

The dimethyl ester was prepared with excess diazomethane and crystallized from methanol as needles, m.p. and mixed m.p. with the dimethyl ester of manogenic acid from hecogenin, 161–163°.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 68.9; H, 8.5.

By a similar route, 9-dehydromanogenin was first reduced with sodium and alcohol in a manner described for the preparation of agavogenin and then oxidized at room temperature with chromic anhydride in acetic acid followed by methylation to yield the same dimethyl ester, m.p. and mixed m.p. 163–165°, $[\alpha]^{20}D$ +4 (*c*, 1.40), no maximum at λ240 mμ.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found C, 69.5; H, 8.9.

The dimethyl ester of chlorogenic acid (6-ketogitogenic acid) has m.p. 163° and $[\alpha]^{25}D$ −44° (*c*, 1.26). A mixture with the above dimethyl ester (VI) showed a melting point depression of 20°.

The dimethyl ester of digitogenic acid (15-ketogitogenic acid) has m.p. 155° and $[\alpha]^{25}D$ −48° (*c*, 2.07). A mixture with the above ester (VI) showed a melting point depression of 20°.

The dimethyl ester of 7-ketogitogenic melts at 189° and the keto diacid melts at 293° (dec.).[1] No samples were available for direct comparisons.

(b) From Hecogenin.—Hecogenin, m.p. 265–268°, isolated from *Agave toumeyana* Engelm. was oxidized at room temperature with chromic anhydride in acetic acid to give hecogenone, m.p. 237–240°, $[\alpha]^{26}D$ +17° (*c*, 1.30). To 1.6 g. of this material dissolved in 90 ml. of acetic acid was added a solution of 1.1 g. of chromic anhydride in 11 ml. of 90% acetic acid. The temperature was kept at 31° for 90 minutes and then heated at 55° for three hours. The excess oxidizing agent was destroyed with methanol and the mixture was evaporated to a small volume and extracted with ether. The ethereal solution was washed with aqueous sodium bicarbonate and evaporated to give 0.5 g. of unreacted hecogenone. The alkaline extract was partially acidified until a small acid fraction precipitated, wt. 53 mg., which was crystallized twice from acetic acid as white crystals, m.p. 269–272° (dec.).

The dimethyl ester was prepared with excess diazomethane and crystallized three times from methanol, m.p. 160–162°. This material did not exhibit an absorption peak at 240 mμ. A lack of sufficient material prohibited a rotation.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 69.2; H, 8.9.

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[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH, PHILADELPHIA]

The Preparation of the Diastereoisomers of Cystathionine and Homolanthionine[1]

BY SIDNEY WEISS AND JAKOB A. STEKOL

Homoserine or its lactone was resolved with brucine through its N-*p*-nitrobenzoyl derivative. The D- and L-α-amino-γ-butyrolactone hydrobromides were then converted to the corresponding optically active 3,6-bis-(β-hydroxyethyl)-2,5-diketopiperazines which, after chlorination, yielded D- and L-3,6-bis-(β-chloroethyl)-2,5-diketopiperazines. By a suitable choice of the isomers of cysteine or homocysteine, or their S-benzyl derivative, and of the isomers of the dichlorodiketopiperazines, L-cystathionine, L-allocystathionine and the L, D and *meso* forms of homolanthionine were prepared by condensation in liquid ammonia and sodium. This procedure is suitable for the direct preparation of radioactive isomers of methionine, homocystine, cystathionine, homolanthionine or any α-amino-γ-thioether of butyric acid.

Previous studies[2] have established that homolanthionine,[3] the next higher homolog of cystathionine, can be converted to cystine by the rat. The homolanthionine used in these experiments was a mixture of isomers and the question arose as to which of these are active *in vivo*. This paper presents the preparation of the individual isomers of homolanthionine as well as those of cystathionine.

The procedure for the preparation of cystathionine and homolanthionine[3] was based on the use of an extremely useful intermediate, 3,6-bis-(β-chloroethyl)-2,5-diketopiperazine of Snyder and co-workers who have used it for the syntheses of racemic methionine,[4] 3,6-bis-(β-benzylthioethyl)-2,5-diketopiperazine[5] and homocystine.[6]

The separation of mixtures of cystathionine (L- and L-*allo*) and homolanthionine (L, D and *meso*) was attempted by enzymatic resolution with pap-

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(2) J. A. Stekol and K. Weiss, *J. Biol. Chem.*, **175**, 405 (1948); **179**, 67 (1949).

(3) J. A. Stekol, *ibid.*, **173**, 153 (1948).

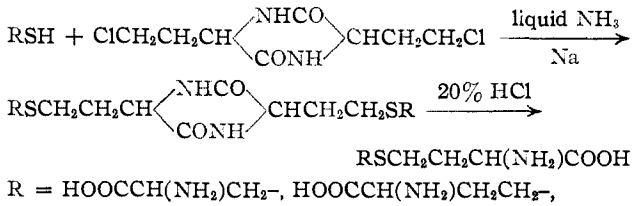
(4) H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, THIS JOURNAL, **64**, 2084 (1942).

(5) H. R. Snyder and M. F. Chiddix, *ibid.*, **66**, 1000 (1944).

(6) H. R. Snyder and G. W. Cannon, *ibid.*, **66**, 500 (1944).

ain[7] by means of their N,N-dibenzoyl and N,N-diacetyl derivatives. This type of resolution was partially successful only in the case of the N,N-dibenzoylcystathionine mixture where the L-isomer was obtained with a purity of 75%.

The direct chemical synthesis of these isomers involves the preparation of the optically active 3,6-bis-(β-chloroethyl)-2,5-diketopiperazines. The basic intermediates in their syntheses are the L and



D isomers of homoserine or of its lactone. DL-Homoserine has been resolved through the strychnine salt of O-phenyl-N-formyl-DL-homoserine[8] but the yields were low and a more practical resolution appeared desirable. We found that homoserine or its lactone could be directly resolved in good yields with brucine through its N-*p*-nitrobenzoyl derivative.

Armstrong,[8] starting from L-α-amino-γ-butyrolactone hydrobromide prepared L-3,6-bis-(β-hydroxyethyl)-2,5-diketopiperazine. In view of the possibility of racemization during the diketopiperazine formation and since only the L-isomer has been described,[8] it appeared necessary to establish that no loss of optical activity resulted during the diketopiperazine formation. This was accomplished by preparing both the L- and D-dihydroxydiketopiperazines with the same melting points and equal and opposite rotations. Furthermore, L-dihydroxydiketopiperazine was reconverted to the original starting material, L-α-amino-γ-butyrolactone without loss of optical activity.

The L- or D-dichlorodiketopiperazines, obtained from the dihydroxydiketopiperazines by chlorination with thionyl chloride, were then condensed in liquid ammonia and sodium with L-cysteine, S-benzyl-L-homocysteine or S-benzyl-D-homocysteine to yield L-cystathionine, L-allocystathionine, L-, D- and *meso*-homolanthionine, respectively, in about 64% yields. The homolanthionines appeared to be much more soluble in water than the cystathionines, the *meso* form being the least soluble of the three.

We also prepared L-ethionine from ethyl mercaptan and L-dichlorodiketopiperazine as a further illustration of the usefulness of the L and D-dichlorodiketopiperazine for the direct synthesis of any optically active γ-substituted thioether of α-aminobutyric acid. Since the condensation step of the diketopiperazine with a mercaptan or a thioamino acid in liquid ammonia results in good yields, from a manipulation standpoint the described procedure appears to be particularly suitable for the synthesis of radioactive optical isomers of γ-substituted thioethers of α-aminobutyric acid.

(7) C. A. Dekker and J. S. Fruton, *J. Biol. Chem.*, **173**, 471 (1948).

(8) M. D. Armstrong, THIS JOURNAL, **70**, 1758 (1948).

Experimental[9]

DL-Homoserine.—The procedure of Livak, *et al.*,[10] was employed for the preparation of this compound. From 516 g. of γ-butyrolactone, there was obtained 287 g. of DL-homoserine, m.p. 180° (dec.).

Anal. Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 11.83.

The mother liquors[10] yielded an additional 260 g. of α-amino-γ-butyrolactone hydrobromide, m.p. 223°. The over-all yield of amino acid was about 63%. Both compounds can be used for the preparation of N-*p*-nitrobenzoyl-DL-homoserine.

N-*p*-Nitrobenzoyl-DL-homoserine.—The preparation of this compound, with certain modifications, was carried out essentially by the procedure described for threonine.[11] One hundred and nineteen grams (1 mole) of DL-homoserine was dissolved in 1800 ml. of water and 574 ml. of 1.75 *N* sodium hydroxide and the solution cooled to 0°. With stirring, there were added simultaneously over a period of one hour 185.5 g. (1 mole) of *p*-nitrobenzoyl chloride and 574 ml. of 1.75 *N* sodium hydroxide. After being stirred for a total of one hour at 0° and then at room temperature, the solution, after cooling, was acidified with 265 ml. of concentrated hydrochloric acid and left overnight in the refrigerator. The precipitate was filtered, washed with ice-water, dried *in vacuo* over sulfuric acid and extracted 3 hours with ether in a Soxhlet. The white residue so obtained (201 g.), m.p. 142°, was of sufficient purity for the preparation of the brucine salt. Most of the unreacted homoserine was recovered from the original aqueous filtrate as the lactone hydrobromide[10] and then benzoylated to yield an additional 39 g. of N-*p*-nitrobenzoyl-DL-homoserine. Total weight of acid was 240 g. or 89% of theory. Comparable yields were obtained using the lactone hydrobromide.

Brucine Salt of *p*-Nitrobenzoyl-L-homoserine.—To 27.7 g. (0.059 mole) of brucine ($4H_2O$), dissolved in 150 ml. of hot absolute methanol (55–60°), was added 16 g. (0.059 mole) of N-*p*-nitrobenzoyl-DL-homoserine. After about 30 seconds, the precipitation of the brucine salt of the L-isomer commenced. The mixture, with stirring, was rapidly cooled to 30° in an ice-bath and filtered. The residue was washed with 50 ml. of cold absolute methyl alcohol and without further treatment was recrystallized from 600 ml. of boiling methanol to give, on drying (air), 18.7 g. (89% yield) of light orange needles, m.p. 119–121° (dec.). The brucine salt contained two molecules of water.

Anal. Calcd. for $C_{34}H_{38}O_{10}N_4 \cdot 2H_2O$: C, 58.44; H, 6.06; N, 8.02; H_2O, 5.16. Found: C, 58.46; H, 6.23; N, 8.26; H_2O, 5.30.

L-α-Amino-γ-butyrolactone Hydrobromide.—Forty-three and a half grams of the brucine salt of *p*-nitrobenzoyl-L-homoserine was shaken for 5 minutes with a mixture of 360 ml. of 2 *N* ammonium hydroxide and 150 ml. of chloroform. The aqueous layer was extracted with two portions of 75 ml. of chloroform and the alkaline solution was evaporated to about 100 ml. *in vacuo*. On acidification of the cooled solution with hydrochloric acid, N-*p*-nitrobenzoyl-L-homoserine precipitated. The mixture was allowed to stand overnight in the refrigerator, filtered and the precipitate washed with ice-cold water. The filtrates and washings were combined, concentrated *in vacuo* to about 25 ml., whereby additional quantities of the product were obtained. Total weight on different runs was 15.6–16.4 g. (94–98% of theory).

Twelve grams of *p*-nitrobenzoyl-L-homoserine was refluxed with 240 ml. of 16% hydrobromic acid for 4 hours and the mixture left overnight in the refrigerator. The *p*-nitrobenzoic acid was filtered off, the filtrate was evaporated to dryness *in vacuo* and the residue was transferred to a funnel with the minimum amount of ice-cold absolute methanol. The light yellow solid was washed 3 times with 3 ml. portions of cold methyl alcohol and the white crystalline product dried over P_2O_5 *in vacuo* at 60°; yield 6.6 g., m.p. 242° dec., $[\alpha]^{24}D$ −21.3 (1% in water) (lit.[8] −21.0).

(9) All melting points are uncorrected.

(10) J. E. Livak, E. C. Britton, J. C. VanderWeele and M. F. Murray, THIS JOURNAL, **67**, 2218 (1945).

(11) A. J. Zambito, W. L. Peretz and E. E. Howe, *ibid.*, **71**, 2541 (1949).

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.82.

Brucine Salt of *p*-Nitrobenzoyl-D-homoserine.—The filtrates from which the L-brucine salt had been precipitated plus the washings were placed on the refrigerator overnight. The light yellow solid was filtered and recrystallized from 40 ml. of boiling methyl alcohol to give 14.0 g. of yellow needles, m.p. 119–121° (dec.). By concentrating the filtrate further and cooling, additional 2 g. of the salt was obtained. Total weight was 16 g. or 77% of theory. The brucine salt crystallized with two molecules of water.

Anal. Calcd. for $C_{34}H_{38}O_{10}N_4 \cdot 2H_2O$: C, 58.44; H, 6.06; N, 8.02; H_2O, 5.16. Found: C, 58.85; H, 6.58; N, 8.27; H_2O, 5.12.

D-α-Amino-γ-butyrolactone Hydrobromide.—Forty-three and a half grams of the brucine salt was decomposed in the same way as described previously, yielding 16.0 g. of N-*p*-nitrobenzoyl-D-homoserine. From 60 g. of this compound there was obtained 33.5 g. of D-α-amino-γ-butyrolactone hydrobromide; m.p. 241° dec., $[\alpha]^{24}D$ +21.0 (1% in water) (lit.[8] +21.0).

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.68.

The over-all yield of the L-isomer was 60% while that of the D-isomer was 53%.

L-3,6-Bis-(β-hydroxyethyl)-2,5-diketopiperazine.—This compound was prepared by the procedure outlined by Livak, *et al.*[10] From 6.07 g. of L-α-amino-γ-butyrolactone hydrobromide, there was obtained 2.46 g. (73% yield) of the product; m.p. 188–189° dec., $[\alpha]^{24}D$ −29.8 (1% in water) (lit.[8] −30.0).

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.86. Found: N, 13.71.

D-3,6-Bis-(β-hydroxyethyl)-2,5-diketopiperazine.—Similarly, 6.10 g. of D-α-amino-γ-butyrolactone hydrobromide yielded 2.40 g. (71% yield) of the product, m.p. 189° dec., $[\alpha]^{24}D$ +29.9 (1% in water).

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.86. Found: N, 13.75.

L-3,6-Bis-(β-chloroethyl)-2,5-diketopiperazine.—Prepared from 14 g. of L-3,6-bis-(β-hydroxyethyl)-2,5-diketopiperazine according to the method of Snyder, *et al.*[4]; yield 14.8 g. (89% yield), m.p. 225–226 (dec.).

Anal. Calcd. for $C_8H_{12}O_2N_2Cl_2$: N, 11.71. Found: N, 11.60.

D-3,6-Bis-(β-chloroethyl)-2,5-diketopiperazine.—Prepared from 10.3 g. of D-3,6-bis-(β-hydroxyethyl)-2,5-diketopiperazine as described above; yield 10.8 g. (89% of theory); m.p. 225–226° (dec.).

Anal. Calcd. for $C_8H_{12}O_2N_2Cl_2$: N, 11.71. Found: N, 11.55.

S-Benzyl-L-homocysteine.—Prepared either from the enzymatic resolution of N-acetyl-S-benzyl-DL-homocysteine[12] or from L-methionine by condensing benzyl chloride with the reduction product of methionine with sodium in liquid ammonia[13]; $[\alpha]^{24}D$ +24.5 (1% solution in 1 *N* hydrochloric acid). The L-methionine was obtained by resolution of N-acetyl-DL-methionine by the procedure of Greenstein, *et al.*[14]

S-Benzyl-D-homocysteine.—Prepared by the resolution of N-formyl-S-benzyl-DL-homocysteine with brucine[15]; $[\alpha]^{24}D$ −24.4 (1% in 1 *N* hydrochloric acid).

L-Homolanthionine.—S-Benzyl-L-homocysteine (9.40 g.) was condensed with 5 g. of L-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine in 500 ml. of liquid ammonia by the method of Stekol[8] to yield after hydrolysis of the intermediate diketopiperazine 6.6 g. (68% yield) of L-homolanthionine; m.p. (*in vacuo*) 262° dec., $[\alpha]^{24}D$ +37.3 (1% in 1 *N* hydrochloric acid). One recrystallization from acid–alkali did not raise the optical rotation.

Anal. Calcd. for $C_8H_{16}O_4N_2S$: N, 11.86. Found: N, 11.63.

D-Homolanthionine.—The above reaction was repeated using 9.40 g. of S-benzyl-D-homocysteine and 5 g. of D-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine; yield 6.6 g. (68% yield); m.p. (*in vacuo*) 261° dec., $[\alpha]^{24}D$ −37.5 (1% in 1 *N* hydrochloric acid).

Anal. Calcd. for $C_8H_{16}O_4N_2S$: N, 11.86. Found: N, 11.89.

***meso*-Homolanthionine.**—Prepared from 1.88 g. of S-benzyl-D-homocysteine and 1 g. of L-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine as described above; yield 1.24 g. (64% yield); m.p. (*in vacuo*) 268° (dec.), $[\alpha]^{24}D$ 0 (1% in 1 *N* hydrochloric acid).

Anal. Calcd. for $C_8H_{16}O_4N_2S$: N, 11.86. Found: N, 11.60.

L-Cystathionine.—L-Cystine (1.25 g.) was condensed with 1.25 g. of L-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine in 100 ml. of liquid ammonia containing 0.24 g. of sodium to give 1.70 g. (80% yield) of the intermediate diketopiperazine. One gram of this product was hydrolyzed with 14 ml. of 20% hydrochloric acid for 3 hours, giving 0.86 g. of L-cystathionine; $[\alpha]^{24}D$ +23.5 (1% in 1 *N* hydrochloric acid) (lit.[16] +23.7). The over-all yield was 63%.

Anal. Calcd. for $C_7H_{14}O_4N_2S$: N, 12.60. Found: N, 12.35.

L-Allo-cystathionine.—Prepared from 0.5 g. of L-cystine and 0.5 g. of D-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine as described above; yield 0.6 g. (64% yield); $[\alpha]^{24}D$ −24.5 (1% in 1 *N* hydrochloric acid) (lit.[17] −25.0).

Anal. Calcd. for $C_7H_{14}O_4N_2S$: N, 12.60. Found: N, 12.38.

L-Ethionine.—Metallic sodium (0.21 g.) was dissolved in 100 ml. of liquid ammonia, and to the blue solution was added 0.68 ml. of ethyl mercaptan. Then 1 g. of L-3,6-bis-(β-chloroethyl)-2,5-diketopiperazine was added in small portions and the ammonia was allowed to evaporate. The residue was refluxed with 15 ml. of 20% hydrochloric acid for 3 hours, evaporated to dryness *in vacuo* and the residue was digested with 25 ml. of boiling absolute ethanol. The alcoholic solution was adjusted to *p*H 8 with ammonia and the white amorphous solid was filtered and recrystallized from 85% alcohol to yield 0.68 g. of white product; $[\alpha]^{24}D$ +20.5 (1% in 1 *N* hydrochloric acid) (lit.[18] +20.1).

Anal. Calcd. for $C_6H_{13}O_2NS$: N, 8.59. Found: N, 8.68.

Conversion of L-3,6-Bis-(β-hydroxyethyl)-2,5-diketopiperazine to L-α-Amino-γ-butyrolactone Hydrobromide.—Two grams of L-3,6-bis-(β-hydroxyethyl)-2,5-diketopiperazine were refluxed with 48 ml. of 16% hydrobromic acid for 4 hours and the solution was evaporated to dryness *in vacuo* to yield 3 g. of the product; m.p. 242°, $[\alpha]^{24}D$ −21.4. No depression of the melting point was obtained when mixed with an authentic sample of L-α-amino-γ-butyrolactone hydrobromide.

Anal. Calcd. for $C_4H_8O_2NBr$: N, 7.69. Found: N, 7.85.

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