576. *Partial Racemisation accompanying the Acid Hydrolysis of Dibenzoyl-D-cystathionine and -lanthionine.*

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It is shown that acid hydrolysis of dibenzoyl-D-cystathionine or -lanthionine is accompanied by partial racemisation. An unsuccessful attempt has been made to show that the enzymic synthesis of dibenzoyl-cystathionine dianilide is stereospecific for the L-isomer. Acid-hydrolysis of this dianilide and the similarly prepared dibenzoyl-L-lanthionine dianilide gives partially racemic products.

A NON-STEREOSPECIFIC enzymic synthesis of dibenzoyl-L-allocystathionine monoanilide (cf. Behrens, Doherty, and Bergmann [1] and Doherty and Popenoe [2]) or alternatively partial racemisation during its hydrolysis (cf. Murachi [3]) would account for Schöberl and Täuber's failure [4] to obtain pure L-allocystathionine from it. We have examined the similar reaction of dibenzoyl-DL-cystathionine with aniline in the presence of papain to ascertain whether this reaction is stereospecific for the L-isomer. An attempt has also been made to apply the enzymic synthesis of anilides to the separation of the lanthionines (cf. Izumi [5]).

DL-Methionine was reduced by sodium in liquid ammonia to DL-homocysteine which was converted [4] into a mixture of DL- and DL-allo-cystathionine and benzoylated. After extraction of the dibenzoyl-DL-allocystathionine with dry acetone, the dibenzoyl-DL-cystathionine was incubated with aniline in the presence of papain at pH 5. Dibenzoyl-L-cystathionine dianilide separated and was collected: acidification of the filtrate gave dibenzoyl-D-cystathionine.

DL-Methionine was resolved by Dekker and Fruton's method [6] and the D-isomer converted into a mixture of D- and L-allocystathionine which was separated by Armstrong's method [7] and then benzoylated. The separation was also achieved by fractional crystallisation of the dibenzoyl derivatives. Dibenzoyl-D-cystathionine obtained by both these routes had the same optical rotatory power as that obtained from dibenzoyl-DL-cystathionine. Dibenzoyl-L-cystathionine was obtained in a similar manner from L-methionine. Esterification with diazomethane and treatment with hydrazine hydrate gave dibenzoyl-L-cystathionine dihydrazide which was converted into the diazide and treated with aniline. Although the resulting compound gave a poor analysis for the dianilide, a mixture with a specimen of the dianilide, obtained by the papain route, melted without depression. The compound obtained by the azide route had a slightly higher optical rotatory power.

Acid-hydrolysis of the dianilide from the papain route gave a partially racemic product but, since the acid-hydrolysis of dibenzoyl-D-cystathionine was also accompanied by some racemisation, no conclusions as to the optical purity of the dianilide could be reached. This partial racemisation during the acid-hydrolysis of dibenzoyl-D-cystathionine was unexpected but it is interesting that Dekker and Fruton [6] obtained partially racemic D-methionine by the acid-hydrolysis of dibenzoyl-D-methionine and that Izumi's values [5] for the optical rotatory power of (+)- and (−)-diaminopimelic acid, obtained by acid-hydrolysis of (+)-di(benzamido)pimelic acid and (−)-di(benzamido)pimelic dianilide, were lower than those obtained by Work, Birnbaum, Winitz, and Greenstein [8] by resolution of DL-diaminopimelic diamide with hog-kidney amidase.

[1] Behrens, Doherty, and Bergmann, *J. Biol. Chem.*, 1940, **136**, 61.
[2] Doherty and Popenoe, *J. Biol. Chem.*, 1951, **189**, 447.
[3] Murachi, *Arch. Biochem. Biophys.*, 1956, **61**, 468.
[4] Schöberl and Täuber, *Annalen*, 1956, **599**, 23.
[5] Izumi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 1954, **75**, 1152.
[6] Dekker and Fruton, *J. Biol. Chem.*, 1948, **173**, 471.
[7] Armstrong, *J. Org. Chem.*, 1951, **16**, 433.
[8] Work, Birnbaum, Winitz, and Greenstein, *J. Amer. Chem. Soc.*, 1955, **77**, 1916.

Dibenzoyl-DL-allocystathionine, when treated with papain and aniline, gave a mixture of monoanilides which was not separable by fractional crystallisation.

A mixture of DL-lanthionine and *meso*-lanthionine was prepared from *S*-benzyl-DL-cysteine by Schöberl and Wagner's α-acetamidoacrylic acid route,[9] converted into the dibenzoyl derivatives, and incubated with aniline in the presence of papain at pH 5. Dibenzoyl-L-lanthionine dianilide and dibenzoyl-*meso*-lanthionine monoanilide were precipitated, and were separated by crystallisation: acidification of the filtrate gave a mixture of dibenzoyl-D-lanthionine and dibenzoyl-*meso*-lanthionine which was not completely separated on re-treatment with aniline and papain. Separation of this mixture was achieved by differential acidification of a solution in ammonia. Dibenzoyl-*meso*-lanthionine separated as a monohydrate, m. p. 127—128° and 200—201° (decomp.) (water was evolved at the first melting point and the sample resolidified). The formation of a hydrate accounts for the divergent reports in the literature on the melting point of dibenzoyl-*meso*-lanthionine (cf. Alderton and Fevold [10]).

As was the case with the cystathionines, acid-hydrolysis of both dibenzoyl-L-lanthionine dianilide and dibenzoyl-D-lanthionine gave partially racemic products.

A sample of pure dibenzoyl-D-lanthionine on hydrolysis and benzoylation lost 25% of its optical rotatory power.

Experimental

0·1N-Sodium dihydrogen phosphate buffer of pH 5 was used throughout. Unless otherwise stated, rotations were measured for N-sodium hydroxide solutions (*c* 1).

α-Acetamidoacrylic Acid.—Freshly distilled pyruvic acid (62 g.), acetamide (76 g.), and toluene (250 ml.), in a flask fitted with a Dean–Stark head, were stirred under reflux until water entrainment ceased (*ca.* 4 hr.). After cooling, the solid was collected and decolorised by washing with ethyl acetate. αα-Di(acetamido)propionic acid (74 g.), m. p. 181° (decomp.) [lit.,[11] m. p. 189—190° (decomp.)], remained and was converted into α-acetamidoacrylic acid (33·4 g.), m. p. 196—197° (decomp.) [lit.,[11] m. p. 198—200° (decomp.)], by suspension in cold acetic acid, boiling for 8 min., and rapid cooling.

Dibenzoyl-DL-cystathionine and Dibenzoyl-DL-allocystathionine.—Sodium (3·5 g.) was added with stirring to DL-methionine (10 g.) in liquid ammonia (160 ml.), and the resulting solution decolorised by addition of ammonium chloride. Evaporation of the ammonia gave a solid which was boiled in water (75 ml.) whilst nitrogen was passed through to remove the ammonia. The solution was then treated with α-acetamidoacrylic acid (17 g.), brought to pH 7—8 with N-sodium hydroxide, and heated under nitrogen on a steam-bath for 3 hr. Evaporation gave a syrup which was refluxed for 6 hr. with 5N-hydrochloric acid (300 ml.). The resulting solution was decolorised with charcoal and evaporated to dryness and the residue dissolved in ethanol and filtered. Excess of triethylamine was added to the filtrate and the precipitated solid (12·5 g.), m. p. 271—272°, collected. Benzoylation of this solid gave a mixture (20 g.), m. p. 213—214°, of dibenzoyl-DL- and DL-allo-cystathionine.

Separation of Dibenzoyl-DL- and -DL-allo-cystathionine.—The above mixture (18 g.) of dibenzoyl derivatives was extracted continuously with boiling dry acetone (150 ml.) for 2 hr. The insoluble portion on crystallisation from aqueous ethanol gave dibenzoyl-DL-cystathionine (8 g.), m. p. 221—222° (decomp.). Evaporation of the acetone extract and addition of light petroleum (b. p. 40—60°) gave dibenzoyl-DL-allocystathionine (8·5 g.), m. p. 177—178° (decomp.) (from propan-2-ol).

Attempted Resolution of Dibenzoyl-DL-cystathionine.—A solution of sodium cyanide (0·1 g.) in water (10 ml.), adjusted to pH 5 with acetic acid, and pH 5 buffer (20 ml.) were added to the filtered (1 hr.) extract of papain (1 g.) and water (20 ml.). This enzyme solution was added to a mixture of aniline (3 ml.) and dibenzoyl-DL-cystathionine (5 g.) in 2N-sodium hydroxide (30 ml.), brought to pH 5 with acetic acid and pH 5 buffer solution (40 ml.), and incubated at 37° for 24 hr. *Dibenzoyl-L-cystathionine dianilide* (3·7 g.) separated, having m. p. 291° (from aqueous dimethylformamide), $[\alpha]_D^{25}$ +26·7° (*c* 1 in dimethylformamide) (Found: C, 67·6; H, 5·5; N, 10·0. $C_{33}H_{32}O_4N_4S$ requires C, 68·2; H, 5·4; N, 9·7%). The filtrate was boiled with charcoal

[9] Schöberl and Wagner, *Z. physiol. Chem.*, 1956, **304**, 97.
[10] Alderton and Fevold, *J. Amer. Chem. Soc.*, 1951, **73**, 463.
[11] Bergmann and Grafe, *Z. physiol. Chem.*, 1930, **187**, 187.

and filtered. Acidification of the filtrate with concentrated hydrochloric acid gave dibenzoyl-D-cystathionine (1·8 g.), m. p. 229—230° (decomp.) (from acetic acid), $[\alpha]_D^{23}$ +44°.

Dibenzoyl-D-cystathionine.—D-Methionine [6] (5·0 g.) was converted by the above method into a mixture (4·0 g.) of D-cystathionine and L-allocystathionine. This mixture (2·0 g.) was separated by Armstrong's method [7] into D-cystathionine, $[\alpha]_D^{23}$ −23·8° (*c* 1 in N-HCl), and L-allocystathionine, $[\alpha]_D^{22}$ −25·2° (*c* 1 in N-HCl). Benzoylation of D-cystathionine and crystallisation from acetic acid gave dibenzoyl-D-cystathionine, m. p. 228—229°, $[\alpha]_D^{25}$ +43·8°.

Separation of Dibenzoyl-D- and -L-allo-cystathionine.—The mixture (2·0 g.) of D- and L-allocystathionine was benzoylated and the product extracted with boiling ethanol. Crystallisation of the insoluble portion (1·0 g.) from glacial acetic acid gave dibenzoyl-D-cystathionine, m. p. 227—228°, $[\alpha]_D^{22}$ +44°. Dilution of the ethanol extract with water gave dibenzoyl-L-allocystathionine (1·6 g.) which after crystallisation from 70% aqueous ethanol had m. p. 183—184° and $[\alpha]_D^{22}$ −17·4°.

Dibenzoyl-L-cystathionine.—L-Methionine [6] (2·3 g.) was converted by the above method into a mixture (1·8 g.) of L- and D-allo-cystathionine and benzoylated. The product (2·4 g.) was separated as above into dibenzoyl-L-cystathionine, m. p. 228—229°, $[\alpha]_D^{25}$ −43·3, and dibenzoyl-D-allocystathionine, m. p. 184—185°, $[\alpha]_D^{22}$ +15°.

NN′-*Dibenzoyl-L-cystathionine Dimethyl Ester.*—Dibenzoyl-L-cystathionine (2·0 g.), suspended in ethyl acetate (20 ml.), was treated with excess of ethereal diazomethane. The solid dissolved completely before the solution gelled. The gel was diluted with ether and the solid collected. Crystallisation from 95% ethanol gave the *dimethyl ester* (1·7 g.) as needles, m. p. 137—138° (Found: C, 60·1; H, 5·8; N, 5·8. $C_{21}H_{26}O_4N_6S$ requires C, 60·2; H, 5·7; N, 6·1%).

Dibenzoyl-L-cystathionine Dihydrazide.—The dimethyl ester (1·1 g.) was suspended in methanol (10 ml.) and treated with hydrazine hydrate (1 ml.). The resulting solution yielded a gelatinous solid which was collected. Crystallisation of the solid from aqueous methanol gave the *dihydrazide hydrate* (1·1 g.), m. p. 193—194° (Found: C, 52·9; H, 5·9; N, 17·7. $C_{21}H_{26}O_4N_6S,H_2O$ requires C, 52·5; H, 5·8; N, 17·7%).

Dibenzoyl-L-cystathionine Dianilide.—The dihydrazide (1·1 g.), dissolved in N-hydrochloric acid (20 ml.) and ethyl acetate (10 ml.), was cooled to 5° and treated dropwise with sodium nitrite (0·5 g.) in water (2 ml.). The ethyl acetate was separated and the aqueous layer extracted with ethyl acetate (2 × 10 ml.). The combined organic layers were treated with aniline (3 ml.) in ethyl acetate (15 ml.) and left to crystallise. Recrystallisation from dimethylformamide–ethanol gave the dianilide, m. p. 281° (undepressed on admixture with dianilide obtained as above), $[\alpha]_D^{23}$ +29° (*c* 1 in dimethylformamide) (Found: C, 67·3; H, 5·1; N, 10·0%).

Hydrolysis of Dibenzoyl-L-cystathionine Dianilide.—The dianilide (2·3 g.) (from the papain route) was refluxed for 6 hr. with 5N-hydrochloric acid (40 ml.) and acetic acid (20 ml.). The gum obtained on evaporation was taken up in water and extracted with ether. Evaporation of the aqueous layer gave a gum which was re-evaporated with ethanol and then taken up in ethanol and treated with excess of triethylamine. The precipitated solid (0·6 g.) was washed with ethanol and dissolved in dilute ammonia and brought to pH 5 with acetic acid. This solution was treated with an equal volume of ethanol and on storage gave L-cystathionine, $[\alpha]_D^{23}$ +18° (*c* 1 in N-HCl) (lit.,[12] $[\alpha]_D^{20}$ +23·7°).

Hydrolysis of Dibenzoyl-D-cystathionine.—Dibenzoyl-D-cystathionine (1·7 g.), hydrolysed as above, gave D-cystathionine (0·6 g.), $[\alpha]_D^{23}$ −21° (*c* 1 in N-HCl) (lit.,[13] $[\alpha]_D^{20}$ −23·5°).

Attempted Resolution of Dibenzoyl-DL-allocystathionine.—Dibenzoyl-DL-allocystathionine (5 g.) was incubated, as described above, with aniline in the presence of papain. Dibenzoyl-DL-allocystathionine monoanilide (7·5 g.) separated and, after crystallisation from ethanol, had m. p. 176—178° (Found: C, 63·7; H, 5·9; N, 8·0; S, 6·1. Calc. for $C_{27}H_{27}O_5N_3S$: C, 64·1; H, 5·4; N, 8·3; S, 6·3%). Fractional crystallisation failed to separate this isomeric mixture.

S-*Benzyl-DL-cysteine.*—A mixture of α-acetamidoacrylic acid (12 g.) and toluene-ω-thiol (12 g.) in water (75 ml.) was brought to pH 8 with N-sodium hydroxide and heated at 80° for 1 hr. under nitrogen. After cooling, the solution was filtered and extracted with ether. Acidification of the aqueous layer gave *N*-acetyl-*S*-benzyl-DL-cysteine (17 g.), m. p. 156—157° (lit.,[14] m. p. 158°), which was hydrolysed for 5 hr. by refluxing 5N-hydrochloric acid (50 ml.)

[12] Du Vigneaud, Brown, and Chandler, *J. Biol. Chem.*, 1942, **143**, 59.
[13] Anslow, Simmond, and du Vigneaud, *J. Biol. Chem.*, 1946, **166**, 35.
[14] Süs, *Annalen*, 1948, **559**, 92.

and acetic acid (25 ml.). Evaporation gave a solid which when dissolved in hot water and brought to pH 6 with ammonia (*d* 0·88) gave *S*-benzyl-DL-cysteine (14·1 g.), m. p. 211° (lit.,[15] m. p. 216°).

Dibenzoyl-lanthionines.—*S*-Benzyl-DL-cysteine (26·2 g.), when treated as described above for DL-methionine, gave a mixture (13·1 g.), m. p. 153—155°, of dibenzoyl-DL- and dibenzoyl-*meso*-lanthionine.

Separation of Dibenzoyl-lanthionines.—The mixed dibenzoyl derivatives (8 g.) were incubated with aniline and papain as described above and the precipitated solid A (3·2 g.) collected. The filtrate was boiled with charcoal and filtered. Acidification of the filtrate with concentrated hydrochloric acid gave a mixture (3·5 g.), m. p. 184—185°, $[\alpha]_D^{21}$ +15·3°, of dibenzoyl-D- and -*meso*-lanthionine. A sample twice crystallised from aqueous ethanol (3 : 1) gave dibenzoyl-*meso*-lanthionine, m. p. 200—201° (Found: C, 57·6; H, 4·9; N, 6·7. Calc. for $C_{20}H_{20}O_6N_2S$: C, 57·7; H, 4·8; N, 6·7%). Solid A was extracted with boiling ethanol, and the insoluble portion dissolved in hot dimethylformamide and treated with ethanol. On cooling, *dibenzoyl-L-lanthionine dianilide* (2·6 g.) separated, having m. p. 277—278° (Found: C, 67·3; H, 5·3; N, 9·7. $C_{32}H_{30}O_4N_4S$ requires C, 67·8; H, 5·3; N, 9·9%). Evaporation of the alcoholic extract and crystallisation of the residue from aqueous ethanol gave *dibenzoyl*-meso-*lanthionine monoanilide* (0·3 g.), m. p. 174—176° (Found: C, 62·7; H, 5·2; N, 8·3. $C_{26}H_{25}O_5N_3S$ requires C, 63·4; H, 5·1; N, 8·5%).

Re-treatment of the mixture (3·3 g.) of dibenzoyl-D- and -*meso*-lanthionine with papain and aniline gave dibenzoyl-*meso*-lanthionine monoanilide (1·1 g.), m. p. 174°, and a mixture (1·5 g.), m. p. 186°, $[\alpha]_D^{24}$ +33°, of dibenzoyl-D-lanthionine and dibenzoyl-*meso*-lanthionine. Further enzymic treatment failed to improve the optical rotatory power of this mixture.

A mixture (0·9 g.) of dibenzoyl-D- and -*meso*-lanthionine, $[\alpha]_D^{23}$ +15°, was suspended in water (5 ml.), and sufficient ammonia (*d* 0·88) was added to bring about dissolution. Acidification with acetic acid gave *dibenzoyl*-meso-*lanthionine monohydrate* (0·45 g.), m. p. 120° and 200° (decomp.), which, after crystallisation from water, had m. p. 127—128° and 200—201° (decomp.), $[\alpha]_D^{22}$ 0° (Found: C, 55·3; H, 4·8; N, 6·7. $C_{20}H_{20}O_6N_2S,H_2O$ requires C, 55·3; H, 5·2; N, 6·5%). Acidification of the filtrate with concentrated hydrochloric acid gave dibenzoyl-D-lanthionine (0·3 g.), m. p. 183—184°, $[\alpha]_D^{22}$ +45°.

Hydrolysis of Dibenzoyl-L-lanthionine Dianilide.—Hydrolysis of the dianilide (1·8 g.), in the usual way, gave L-lanthionine (0·2 g.) (from dilute ammonia), $[\alpha]_D^{20}$ +4° (*c* 2 in N-NaOH) (lit.,[16] $[\alpha]_D^{22}$ +8°).

Hydrolysis of Dibenzoyl-D-lanthionine.—Dibenzoyl-D-lanthionine (0·85 g.), $[\alpha]_D^{23}$ +45°, was hydrolysed as above. The product was taken up in N-sodium hydroxide and adjusted to pH 6 with acetic acid; D-lanthionine (0·25 g.) separated with $[\alpha]_D^{23}$ −5·5° (lit.,[16] $[\alpha]_D^{22}$ −8°). Benzoylation of this material gave dibenzoyl-D-lanthionine, m. p. 176—178°, $[\alpha]_D^{22}$ +31·6°.

Dibenzoyl-L-lanthionine.—Benzoylation of a mixture [17] of L-lanthionine and *meso*-lanthionine gave a product (1 g.), m. p. 187—189°, $[\alpha]_D^{22}$ −18°, which was separated, as described above, by the differential acidification of a solution in ammonia, into dibenzoyl-*meso*- (0·5 g.) and -L-lanthionine (0·35 g.), m. p. 187—189°, $[\alpha]_D^{23}$ −42°.

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[15] Baltazzi and Davis, *Compt. rend.*, 1955, **240**, 208.
[16] Brown and du Vigneaud, *J. Biol. Chem.*, 1941, **140**, 767.
[17] Schöberl and Wagner, *Chem. Ber.*, 1947, **80**, 379.