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AN EXPEDIENT TO SOLID PHASE EDMAN DEGRADATION

Synthesis and Properties of 4-Isothiocyanato Benzoyl-DL-Homoserine Lactone

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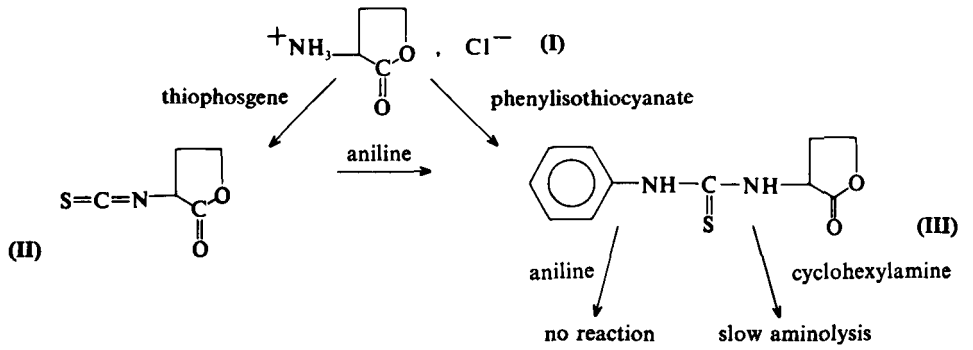
The synthesis of p-isothiocyanatobenzoylhomoserine lactone from DL-homoserine as well as from L-methionine is described. The compound constitutes a good tool in the solid phase Edman degradation of tryptic protein fragments terminated by lysine.

In a separate communication (1) we described the use of p-isothiocyanatobenzoyl-DL-homoserine lactone as an expedient for solid phase Edman degradation. The method consists of the reaction of each amino group in a peptide with the isothiocyanate, to give a substituted thiocarbamide, which on treatment with trifluoroacetic acid loses the N-terminal aminoacyl residue. The remaining part of the molecule is a weakly activated compound bearing its activation at the side chains of the constituent lysyl residues. The derived peptide can be anchored through these groups to an aminated solid support, and subjected to the sequence determination. The advantages of two holding techniques—coupling of a protein-fragment using a diisothiocyanate (2) and coupling through the C-terminal homoserine lactone residue resulting from a bromocyanogen degradation (3)—are combined in this adaptation. Tryptic peptides, terminated by lysine are particularly suited for coupling by this new method. Since it has been shown recently that tryptic hydrolysis of peptide bonds involving arginyl residues can be suppressed (4), the approach thus seems to offer a fairly general solution to peptide sequencing, when the original elegant method of Horn & Laursen (3) cannot be applied because of the absence of methionine. The highly crystalline character of the reagent, the great difference in reactivity of its functional groups and the regenerability of the lactone moiety after its introduction in a peptide, contribute to make it an attractive tool; regenerability may be of importance should the lactone ring become hydrolyzed. Since the method has been used very successfully (de Jong & Herbrink, personal communication) a detailed description of the synthesis of the key-compound seemed desirable.

Synthesis and properties of the reagent

Preliminary evaluation of the relative reactivity of the isothiocyanate versus the homoserine lactone moiety in one compound was performed by preparation of the known (5) phenylthiocarbamyl-DL-homoserine lactone by two methods. DL-homoserine lactone monohydrochloride (I, scheme 1) was prepared according to Fischer (6) and converted to α-isothiocyanato-γ-butyrolactone (II) with thiophosgene. Compound II gave phenylthiocarbamyl homoserine lactone (III) on treatment with aniline. The same compound resulted from the reaction between phenylisothiocyanate and I in the presence of a tert.base (5). The compound was not attacked by aniline (molar ratio 1:1) in alcohols at room temperature. Slow aminolysis occurred with the more nucleophilic cyclohexylamine but the reaction was not complete within 16 h at room temperature. The rate was speeded up considerably at a higher

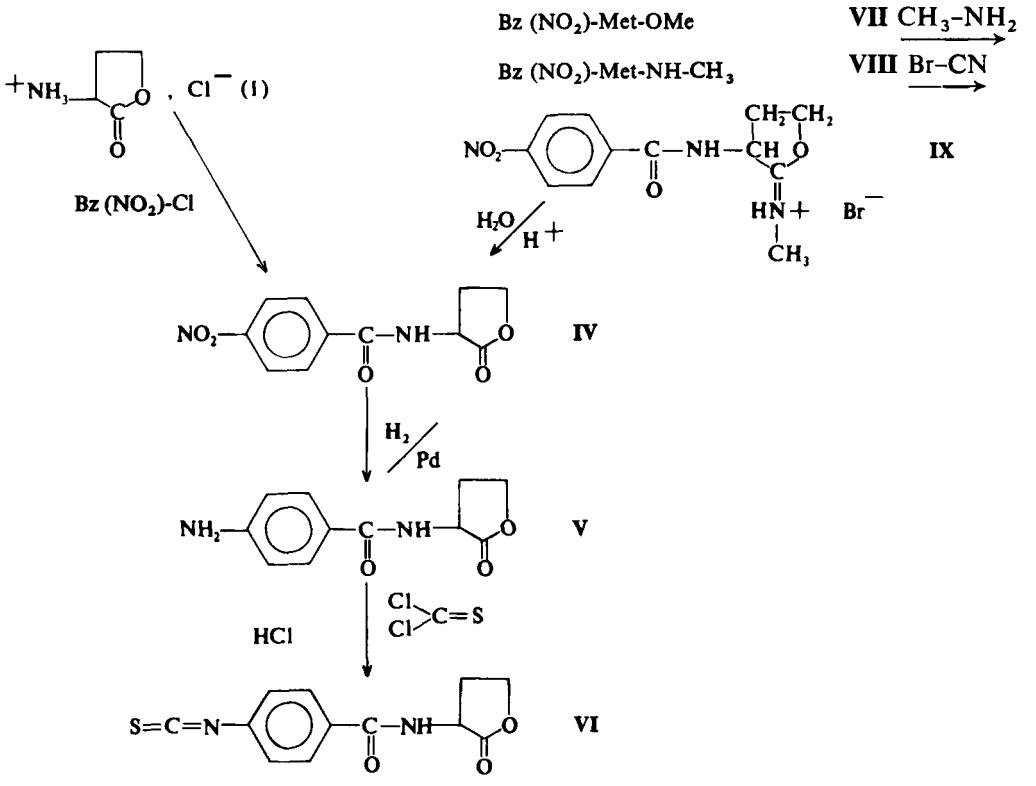


SCHEME 1

Relative reactivity of the two functions in α-isothiocyanato-γ-butyrolactone (II) towards primary amines.

temperature (50°C). The existence of a sufficiently distinct reactivity between both functions was concluded from these experiments.

The synthesis of 4-isothiocyanatobenzoyl-DL-homoserine lactone was initiated with an acylation of I with p-nitrobenzoylchloride in dimethylformamide in the presence of tri-n-butylamine, which resulted in the formation of the crystalline IV (scheme 2). The nitro group was hydrogenated (in methanolic suspension) using palla-



SCHEME 2

Preparation of 4-isothiocyanatobenzoylhomoserine lactone (VI).

dium on charcoal as the catalyst. The resulting aniline derivative (V) was a stable crystalline compound, which dissolved easily in aqueous hydrochloric acid (2.5 N). Compound V was converted into VI by thiophosgene and without the formation of a symmetrical thiourea. The protonation of the amino group acts as an effective protection mechanism (7).

To avoid using the expensive homoserine, reagent IV can be prepared from a suitable p-nitrobenzoyl-methionine derivative using bromocyanogen (8). Therefore, the methyl ester and the methylamide were subjected to the degradation conditions. The ester was not attacked during 5 days (room temperature), but the methylamide reacted overnight. The chromatogram revealed the presence of two new compounds, however, but on treatment with hydrochloric acid (1 N) at increased temperature (70°C) the more polar compound vanished leaving a precipitate of the expected lactone in a solution of methylammonium hydrochloride. The lactone IV in this case was optically active, but partial racemization is not excluded.

EXPERIMENTAL PROCEDURES

α-Isothiocyanato-γ-butyrolactone (II). Homoserine lactone monohydrochloride was obtained in quantitative yield according to Fischer (6). The lactone (0.69 g, 5 mmoles) was dissolved in 1.5 ml of water and added to a stirred suspension of 1 g of finely divided calcium carbonate in 2.5 ml of water and 2.5 ml of chloroform, 0.7 g (0.467 ml) of liquid thiophosgene was then added, also with stirring. The orange color of the reaction mixture gradually faded until a pale yellow suspension remained after 2 h. The mixture was filtered and the lower layer was separated, washed with 0.1 N hydrochloric acid (3 × 5 ml) and water (3 × 5 ml). The solvent was removed *in vacuo* giving a brownish oil (520 mg, 72.5%). The crude product was used without further purification.

N$^{\alpha}$-Phenylthiocarbamyl-DL-homoserine lactone (III). (a) From isothiocyanatobutyrolactone and aniline. The crude lactone (0.44 g, 3.1 mmoles) was dissolved in 5 ml of ethanol and 0.28 ml (3.1 mmoles) of aniline were added. After 4 h the precipitated product (III) was collected by filtration. The product was recrystallized from isopropyl alcohol. Yield 647 mg (55%) m.p. 141–142°C; IR (cm^{-1}): 3160 (NH); 1758 (COO); 1710 (amide I); 1515 (amide II) and 1265 (C=S). *Anal.* calc. for $C_{11}H_{12}N_2O_2S$ (236.29): C, 55.91; H, 5.12; N, 11.85; S, 13.57. Found: C, 56.05; H, 5.15; N, 11.70; S, 13.55.

(b) From DL-homoserine lactone hydrochloride.—A solution of 687.9 mg (5 mmoles) of the lactone in 5 ml of pyridine was mixed with 0.7 ml of phenylisothiocyanate (5 mmoles) and triethylamine (0.7 ml, 5 mmoles). After 3 h the mixture was poured out into water and the precipitate was collected and recrystallized from isopropyl alcohol. Yield 647 mg (55%); m.p. 141–142°, (lit. (5): 141–142°C). *Anal.* calc. for $C_{11}H_{12}N_2O_2S$ (236.29): C, 55.91; H, 5.12; N, 11.85; S, 13.57. Found: C, 56.25; H, 5.15; N, 11.75; S, 13.60. The IR spectrum of this compound is identical with that from product (a).

The sensitivity of the lactone ring towards amines: Thin layer chromatography indicated no alteration of the composition of a solution of III in methanol—ethanol (1:1) containing an equimolar amount of aniline during 2 days at room temperature (nBuOH—AcOH—W = 4:1:1). Exposure to cyclohexylamine under identical conditions resulted in the formation of a new compound, after 14 h at room temperature or after 2.5 h at 50°C. (R_F = 0.39 cyclohexylamine, 0.54 new compound and 0.74 starting material, III.)

N$^{\alpha}$-p-Nitrobenzoyl-DL-homoserine lactone IV. Homoserine lactone monohydrochloride (1.1 g, 8 mmoles) was suspended in 8 ml of dimethylformamide. To the suspension, 1.36 g (7.2 mmoles) of p-nitrobenzoylchloride were added, followed by 3.62 ml (15.2 mmoles) of tributylamine, giving a perfectly clear solution. After 2 h at room temperature, the mixture was poured into water. The white crystals were filtered and recrystallized from ethanol. Yield 1.64 g, (91%); m.p. 191–192.5°C. IR (cm^{-1}): 3320 (NH), 1753 (COO), 1655 (NH-CO), 1545 and 1357 (NO_2). *Anal.* calc. for $C_{11}H_{10}N_2O_5$ (250.21): C, 52.80; H, 4.03; N, 11.20. Found: C, 52.35; H, 4.05; N, 11.15.

N$^{\alpha}$-p-Aminobenzoyl-DL-homoserine lactone V. To a suspension of 1 g (4 mmoles) of the foregoing product in 30 ml of methanol 100 mg of palla-

dized carbon (10%) were added. The suspension was hydrogenated at atmospheric pressure. During the uptake of hydrogen (12 mmoles being absorbed) the compound dissolved. Removal of the catalyst by filtration, followed by concentration of the filtrate *in vacuo* gave a residue which on treatment with ether precipitated the product, (772 mg, 88%) m.p. 178–180°C. IR (cm^{-1}): 3463 (NH amine), 3365 (NH amide), 1775 (COO), 1645 (CONH); the peaks at 1545 and 1357 as occurring in the IR spectrum of IV (NO_2), were absent. *Anal.* calc. for $C_{11}H_{12}N_2O_3$ (220.22): C, 59.99; H, 5.49; N, 12.72. Found: C, 59.95; H, 5.60; N, 12.65.

N^{α} - p - Isothiocyanatobenzoyl - DL - homoserine lactone VI. To a solution of 220 mg (1 mmole) of V, in 10 ml of 2.5 N hydrochloric acid, 0.12 g (1.1 mmoles) of thiophosgene were added dropwise with stirring. A precipitate was formed gradually. The suspension was filtered after being allowed to stand for 16 h in the refrigerator and the residue was dried *in vacuo*. Yield 230 mg (88%); m.p. 162.5–164°C. IR (cm^{-1}): 3410 (NH), 2120 (NCS), 1775 (COO), 1670 (NHCO), 1550 (NHCO, amide II), 1600 and 1500 (C = C). *Anal.* calc. for $C_{12}H_{10}N_2O_3S$ (262.28): C, 54.95; H, 3.86; N, 10.68; S, 12.23. Found: C, 54.60; H, 3.85; N, 10.60; S, 12. 19.

N^{α}-p-Nitrobenzoyl-L-methionine methyl ester (VII). To a solution of 6 g (30 mmoles) of methionine methyl ester hydrochloride in 50 ml of dimethylformamide, 5 g (27 mmoles) of p-nitrobenzoylchloride and 13.57 ml (57 mmoles) of n-tributylamine were added. The reaction mixture was poured into water (200 ml) after 2 h, to yield the acylated ester (6.75 g, 80%). The product was purified by recrystallization from ethanol. M.p. 78–79.5°C $[\alpha]_D^{22} = -38.2°$ (*c* 1.1 in MeOH), chromatographically homogeneous, $R_F = 0.82$ ($CHCl_3$ — MeOH = 4:1). *Anal.* calc. for $C_{13}H_{16}N_2O_5S$ (312.34): C, 49.99; H, 5.16; N, 8.97; S, 10.27. Found: C, 49.90; H, 5.20; N, 9.00; S, 10.20.

N^{α} - p - Nitrobenzoyl - L - methionine methylamide (VIII). A 10% soln. of Bz(NO_2)-Met-OMe (VII) in ethanolic methylamine (11·3 M) was prepared and kept for 3 days in a tightly stoppered bottle. The product separated partially during this time and evaporation *in vacuo* gave a quantitative yield of product, which was recrystallized from acetonitrile (25 ml per g), m.p. 170°C; $[\alpha]_D^{25} = -13.7°$ (*c* 1.12 in $CHCl_3$ – MeOH = 9:1). *Anal.* calc. for $C_{13}H_{17}N_3O_4S$ (311.36): C, 50.15; H, 5.50; N, 13.50; S, 10.30. Found: C, 50.25; H, 5.70; N, 13.45; S, 10.45.

N^{α} - p - Nitrobenzoyl - L - homoserine lactone (IV). Bz(NO_2)-Met-NH-CH_3 (311 mg, 1 mmole) was added to a solution of 318 mg (3 mmoles) of bromocyanogen in 5 ml of 70% formic acid. After 16 h, the solution was evaporated *in vacuo*. The residue contained no starting material ($R_F = 0·71$), but contained two new compounds as shown by t.l.c. (silica; $CHCl_3$—MeOH = 3:1). The more apolar compound ($R_F = 0.65$) migrated the same distance as the racemic lactone IV, the second compound ($R_F = 0.17$) was supposed to be the intermediate N-methylimmonium salt IX. The crude mixture was heated with 1 N hydrochloric acid for a few minutes to 70°C. The presumed immonium compound disappeared during this treatment to give the lactone. 220 mg (88%); $[\alpha]_D^{24} = -20.5°$ (*c* 1.5 in $CHCl_3$—MeOH = 1:1) $= -28.9°$ (*c* 1, DMF); m.p. 169–170°C. *Anal.* calc. for $C_{11}H_{10}N_2O_5$ (250.21): C, 52.80; H, 4.03; N, 11.20. Found: C, 52.60; H, 4.05; N, 11.10.

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