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Studies in the Propiothiolactone Series.* III. Preparation of L-α-Acylamino-β-Propiothiolactones *via* Carbodiimide Method

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N-(*p*-Toluenesulfonyl)- and *N*-benzyloxycarbonyl-L-cysteine were converted to the corresponding propiothiolactones by cyclization with diisopropyl- and dicyclohexylcarbodiimide in dioxane at room temperature. The propiothiolactones were condensed with esters of amino acids to the following protected dipeptides: *bis*-(*N*-*p*-toluenesulfonyl) - L - cystinyl - di-β-alanine diethyl ester, *bis*-(*N*-*p*-toluenesulfonyl)-L-cystinyl-diglycine dimethyl ester and *bis*-(*N*-benzyloxycarbonyl)-L-cystinyl-diglycine dimethyl ester.

In the previous communications[1,2] we have described the preparation of *N*-phtaloyl, *N*-succinoyl- and *N*-tosyl-α-amino-β-propiothiolactones by debenzylation and cyclization of corresponding *N*-acyl-*S*-benzyl-cysteinyl chlorides with aluminum halides. The propiothiolactones were successfully used for the preparation of cysteine and cystine containing polypeptides[3]. Although the method for the preparation of propiothiolactones via aluminum chloride seems to be applicable to various *N*-acylderivatives of *S*-benzyl-cysteine, its use for the preparation of thiolactones has been limited to the compounds containing the acyl groups not sensitive to aluminum halides and hydrochloric acid which is formed during the course of debenzylation and cyclization.

In the search for a more general method for the preparation of propiothiolactones derived from cysteine, the cyclization of *N*-acyl-L-cysteine has been performed with substituted carbodiimides.

The reactions were carried out in dioxane at room temperature and *N,N'*-diisopropylcarbodiimide+ and *N,N'*-dicyclohexylcarbodiimide were used for the elimination of water. L-α-benzyloxycarbonyl-amino-β-propiothiolactone was isolated in crystalline form, whereas the crude *N*-tosyl++-derivative was used directly in the peptide synthesis. The dipeptides of glycine and β-alanine were prepared from the corresponding ethyl or methyl esters with thiolactones in dioxane at room temperature following the method described by Fleš *et al.*[2]

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* Paper II. D. Fleš, A. Markovac-Prpić, V. Tomašić, and M. Milohnoja, *Croat. Chem. Acta* **30** (1958) 167.

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\+ The same reagent was used by Sheehan *et al.*[4] for the prearation of α-trityl-amino-β-propiolactone from the corresponding derivative of serine.

++ Tosyl = p-toluenesulfonyl.

The method described in this paper represents a very convenient route for the preparation of α-acylamino-β-propiothiolactones under very mild conditions.

EXPERIMENTAL

N-(p-*Toluenesulfonyl*)-L-*cysteine*

A mixture of 3 g. (0.0055 mole) of *N,N'*-ditosyl-L-cystine, 1.4 g. of powdered zinc, 20 ml. of methanol and 20 ml. of 2*N* sulfuric acid was heated on a water bath for 1.5 hour. Zinc was removed by filtration and the solution evaporated *in vacuo*. The residue was extracted with ether, extract dried over magnesium sulfate and ether evaporated yielding 1.25 g. (41.5%) of sulfonamide derivative which upon recrystallization from a mixture of 5 ml. of benzene and 10 ml. of petroleum ether (b. p. 40—60°C) melted at 157—158°, $[\alpha]_D^{20}$ 31.2° (c, 3.070 in dioxane).

Anal. $C_{10}H_{13}NO_4S_2$ (275.34) calc'd.: C 43.62; H 4.76%
found : C 43.91; H 4.57%

L-α-(p-*Toluenesulfonamido*)-β-*propiothiolactone*

N-Tosyl-L-cysteine (0.75 g., 0.0027 mole) was dissolved in 5 ml. of dry dioxane and 0.40 g. (0.0032 mole) of *N,N'*-diisopropylcarbodiimide[6] added. The reaction mixture was kept for 1.5 hour at room temperature and the crystals removed by suction filtration yielding 0.4 g. of diisopropylurea.

The filtrate was evaporated *in vacuo* at room temperature and a yellow oil (0.75 g.) was obtained. The crude product was used directly in the next step, since it polymerized during an attempt to purify it by aluminum oxide chromatography.

A sample was treated with an aqueous solution of sodium bicarbonate and gave a polymer identical with the one previously described[2].

Bis (N-p-*Toluenesulfonyl*)-L-*cystinyl-di*-β-*alanine-Diethyl Ester*

A solution of 1.5 g. (0.0128 mole) of β-alanine ethyl ester and 0.5 g. (0.0019 mole) of crude L-α-(*p*-toluenesulfonamido)-β-propiothiolactone in 8 ml. of dioxane was kept overnight at room temperature. The solvent was evaporated under reduced pressure and the residue dissolved in 50 ml. of ethyl acetate, washed with two 10 ml. portions of 5% hydrochloric acid, followed by 10 ml. of water, dried over magnesium sulfate and the solvent evaporated *in vacuo* yielding 0.8 g. of a crystalline product. After two recrystallizations from absolute ethanol 0.3 g. (41.7%) of the product was obtained, m. p. 171—173°, $[\alpha]_D^{25}$ 48.2° (c, 0.166 in dioxane). Sodium nitroprusside test was negative.

Anal. $C_{30}H_{42}N_4O_{10}S_4$ (746.93) calc'd.: C 48.23; H 5.67%
found : C 48.10; H 5.57%

Bis (N-p-*Toluenesulfonyl*)-L-*cystinyl-diglycine Dimethyl Ester*

Similarly 0.5 g. (0.002 mole) of L-α-(*p*-toluenesulfonamido)-β-propiothiolactone and 0.49 g. (0.0056 mole) of methyl glycinate were converted to the corresponding dipeptide. The crude oily product (0.7 g.) obtained upon evaporation of dioxane was dissolved in ethyl acetate, poured on a column of 0.7 g. of neutral aluminum oxide, eluted with 25 ml. of ethyl acetate and the solvent evaporated. The crude dipeptide (0.35 g.) was recrystallized from 3 ml. of ethanol, yielding 0.12 g. (17.9%), m. p. 171—172°, $[\alpha]_D^{20}$ 57,2° (c, 0.940 in dioxane) of the analytically pure product. Sodium nitroprusside test was negative. In admixture with an authentic specimen of *bis* (*N-p*-toluenesulfonyl)-L-cystinyl-diglycine dimethyl ester[2] the melting point was undepressed.

Anal. $C_{26}H_{34}N_4O_{10}S_4$ (690.82) calc'd.: C 45.20; H 4.96%
found : C 45.47; H 5.27%

L-α-Benzyloxycarbonylamino-β-propiothiolactone

N-Benzyloxycarbonyl-L-cysteine[5] (1.95 g., 0.0078 mole) was dissolved in 5 ml. of dioxane and 1.6 g. (0.0078 mole) of *N,N'*-dicyclohexylcarbodiimide was added with shaking. White crystals precipitated immediately. The reaction mixture was kept for 1.5 hour at room temperature and the crystals removed by suction filtration yielding 1.60 g. of dicyclohexylurea. The filtrate was evaporated and the semi-crystaline residue (0.75 g.) was crystallized from a mixture of 2 ml. of methylene-dichloride and 3 ml. of petroleum ether (b. p. 40—60°). Yield: 0.2 g. (14%), m. p. 125° (with softening at 115°), $[\alpha]_D^{20}$ —50° (c, 1 in chloroform).

Anal. $C_{11}H_{11}NO_3S$ (236.27) calc'd.: C 55.69; H 4.67%
found : C 56.12; H 5.38%

Bis (N-Benzyloxycarbonyl)-L-cystinyl-diglycine Dimethyl Ester

A solution of 0.25 g. (0.001 mole) of L-α-benzyloxycarbonylamino-β-propiothiolactone and 0.25 g. (0.0028 mole) of glycine methyl ester in 4 ml. of dioxane was kept overnight at room temperature and worked up as previously described. Yield of pure dipeptide was 0.34 g. (86.8%), m. p. 143—145°, $[\alpha]_D^{20}$ —34° (c, 0.672 in dioxane).

Anal. $C_{28}H_{34}N_4O_{10}S_2$ (650.71) calc'd.: C 51.69; H 5.27%
found : C 51.93; H 5.22%

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IZVOD

Studije u redu propiotiolaktona. III. Priprava L-α-acilamino-β-propiotiolaktona primjenom karbodiimidske metode

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N-(*p*-Toluensulfonil)- i *N*-benziloksikarbonil-L-cistein prevedeni su u odgovarajuće propiotiolaktone ciklizacijom pomoću diizopropil- i diciklohekSilkarbodiimida u dioksanu kod sobne temperature. Nastali propiotiolaktoni kondenzirani su sa esterima amino kiselina u slijedeće zaštićene dipeptide: *bis* (*N*-*p*-toluensulfonil)-L-cistinil--di-β-alanin dietil ester, *bis* (*N*-*p*-toluensulfonil)-L-cistinil-diglicin dimetil ester i *bis* (*N*-benziloksikarbonil)-L-cistinil-diglicin dimetil ester.

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