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Synthetic Studies of Bacitracin. III.[1] Synthesis of (S)-2-(2-Methylbutyryl)-thiazole-4-carboxylic Acid and Its Peptide Derivative

Yasuo ARIYOSHI,[*1] Tetsuo SHIBA and Takeo KANEKO

Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka

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An optical active 2-(2-methylbutyryl)thiazole-4-carboxylic acid obtained from bacitracin F by an acid hydrolysis was synthesized starting from L-isoleucine. Applying the usual methods for the peptide synthesis, 2-(2-methylbutyryl)thiazole-4-carbonyl-L-leucyl-D-glutamic acid α-methyl-γ-*t*-butyl ester corresponding to the *N*-terminal tetrapeptide of bacitracin F, was then synthesized from the thiazole carboxylic acid. UV spectrum of the synthetic tetrapeptide was very similar to that of bacitracin F.

In the preceding paper,[1] a total synthesis of the antibiotic bacitracin A was attempted, and the branched ring dodecapeptide with the cysteine residue was synthesized, which was found to have no ability to be cyclized under an acidic condition to the thiazoline derivative, namely becitracin A (Craig's formula). For a purpose of the determination of the structure of bacitracin A, a synthesis of bacitracin F derived from A by its oxidation, could also give a strong criteria, since a difference

*1 Present address: Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Kanagawa.

1) Part II: Y. Ariyoshi, T. Shiba and T. Kaneko, This Bulletin, **40**, 2648 (1967).

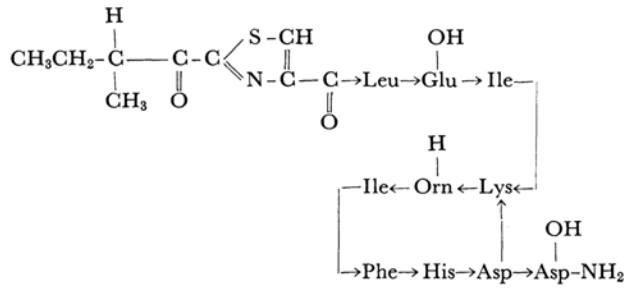
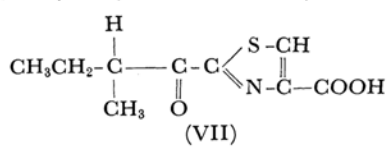


Fig. 1. Bacitracin F.

in the structure of F from that of A is limitted evidently only in the *N*-terminal part, where the isoleucyl thiazoline group in A is oxidized to 2-(2-methylbutyryl)thiazole group in F.[2-4] Furthermore, the thiazole ring in F is more stable than the thiazoline ring in A on the chemical treatments. This would be a favorable character particularly for planning synthetic studies in this field. In the present investigation, therefore, a synthetic approach to bacitracin F was attempted.

Whole structure of bacitracin F proposed by Konigsberg and Craig[4] is shown in Fig. 1. From an acid hydrolyzate of the purified bacitracin F, a ketothiazole acid was obtained by them, and its structure was assumed to be 2-(2-methylbutyryl) thiazole-4-carboxylic acid (VII) as shown in Fig. 2 from the results of UV spectrum, the empirical formula, and the formation of 2, 4-dinitrophenylhydrazone.[5]

Concerning a synthesis of the ketothiazole acid (VII), they only mentioned a synthetic method



(VII)

Fig. 2

similar to that employed by Brookes *et al.* for a synthesis of 2-isobutyrylthiazole-4-carboxylic acid,[6] in the footnote in their paper,[4] but any experimental details have not been published so far. A consideration of the formation of bacitracin F from A leads us that a steric configuration of an asymmetric carbon atom in the ketothiazole acid (VII) should have S-configuration. Therefore, a synthesis of the ketothiazole acid starting from L-isoleucine was first described in the present paper, according to a synthetic route as shown in Fig. 3. Although a few natural peptides containing the thiazole ring have been known,[6-9] none of them have been synthesized so far.

Benzyloxycarbonyl-L-isoleucine amide was dehydrated to give (2S : 3S)-2-benzyloxycarbonylamino-3-methylvaleronitrile,[10] which was then converted to an oily thioamide (I) by an addition of hydrogen sulfide in methanol using ammonia as a catalyst. Refluxing of I with ethyl bromopyruvate[11] in anhydrous ethanol secured an oily ethyl (1S : 2S) - 2 - (1 - benzyloxycarbonylamino-2-methylbutyl)thiazol-4-carboxylate (II). The ester (II) was saponified to a carboxylic acid derivative (III). A crystallization of this product (III) was difficult, while the pure crystal of III was obtained by the benzyloxycarbonylation of IV through Schotten-Baumann reaction. The benzyloxycarbonyl group of III was removed by a treatment with hydrogen bromide in acetic acid to give (1S : 2S)-2-(1-amino-2-methylbutyl)thiazole-4-carboxylic acid dihydrobromide (IV) in a crystalline state, which was then converted to a free amino acid (V) by a neutralization with aqueous ammonia or pyridine. Either an oxidation of IV with 2% potassium permanganate in a weak alkaline medium (pH 8), or an oxidation of a hydroxyacid (VI), obtained from IV by the nitrous acid treatment, with sodium dichromate afforded the same (S)-2-(2-methylbutyryl)thiazole-4-carboxylic acid (VII) with melting point of 89.5—90.0°C. Although a purity of the ketothiazole acid (VII) thus obtained was certified by elementary analysis, NMR spectrum, UV spectrum, IR spectrum and thin-layer chromatography, the synthetic compound

2) L. C. Craig, J. R. Weisiger, W. Hausmann and E. J. Harfenist, *J. Biol. Chem.*, **199**, 259 (1952).
3) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **47**, 257 (1950).
4) W. Konigsberg and L. C. Craig, *J. Org. Chem.*, **27**, 934 (1962).
5) J. R. Weisiger, W. Hausmann and L. C. Craig, *J. Am. Chem. Soc.*, **77**, 3123 (1955).
6) P. Brookes, A. T. Fuller and J. Walker, *J. Chem. Soc.*, **1957**, 689.
7) D. F. W. Cross, G. W. Kenner, R. C. Sheppard and C. E. Stehr, *ibid.*, **1963**, 2143.
8) J. M. Waisvisz, M. G. Van der Hoeven and B. te Nijenhuis, *J. Am. Chem. Soc.*, **79**, 4524 (1957).
9) D. J. Cram, O. Theander, H. Jager and M. K. Stanfield, *ibid.*, **85**, 1430 (1963).
10) This compound was prepared by the dehydration of benzyloxycarbonyl-L-isoleucine amide with *p*-toluenesulfonyl chloride in pyridine according to a direction by Y. Hirotsu *et al.*, mp 27—28°C. Y. Hirotsu, T. Shiba and T. Kaneko, Abstract of the 19th Annual Meeting of the Chemical Society of Japan, April, 1966, IV, p. 64. The details will be published soon in This Bulletin.
11) H. Erlenmeyer and C. J. Morel, *Helv. Chim. Acta*, **25**, 1073 (1942).

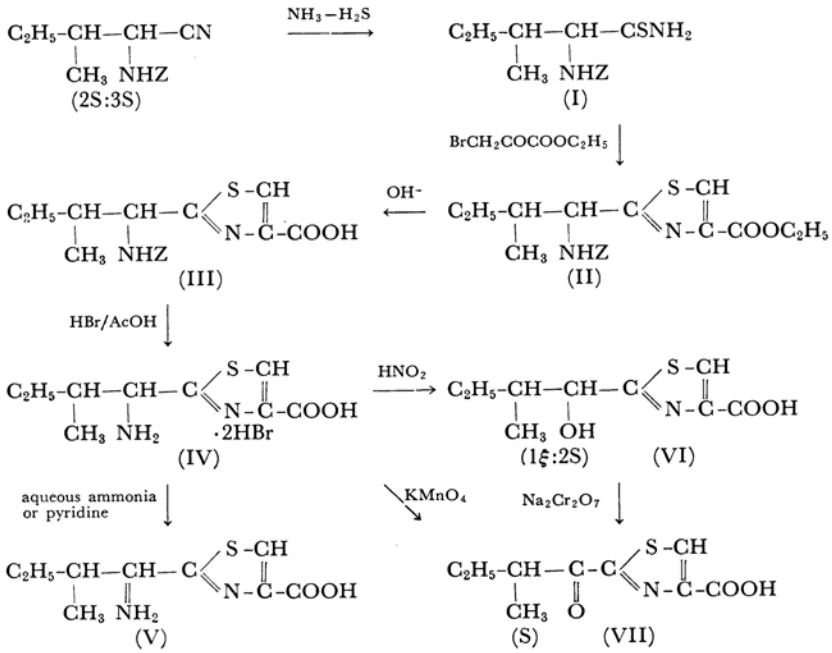


Fig. 3. Z=$C_6H_5CH_2OCO$-

(VII) gave a little different melting point from that of the corresponding one derived from the natural bacitracin F.[12] This may not come from a partial racemization during the synthesis, but from the different measuring method of the melting point.

For an elongation of the ketothiazole acid (VII), a coupling reaction of the benzyloxycarbonyl-amino acid (III) with an amino acid ester using *N, N'*-dicyclohexylcarbodiimide was first tried. In this reaction, a relatively large amount of the acylurea was often formed and an expected product of a peptide derivative could not be obtained at all in some cases. Contrary to this, the ketothiazole acid (VII) itself was found to be easily coupled with an amino acid ester or a peptide ester either by the *p*-nitrophenyl ester method or by the carbodiimide method.[13]

Benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester was condensed with α-methyl-γ-*t*-butyl D-glutamate to give a dipeptide derivative (VIII), which was hydrogenated to a free dipeptide ester (IX) as shown in a synthetic scheme in Fig. 4. A coupling of the ketothiazole acid (VII) with the dipeptide ester (IX) using *N, N'*-dicyclohexylcarbodiimide afforded (S)-2-(2-methylbutyryl)thiazole-4-carbonyl-L-leucyl-D-glutamic acid α-methyl-γ-butyl ester (X), corresponding to the *N*-terminal tetrapeptide part of bacitracin F.

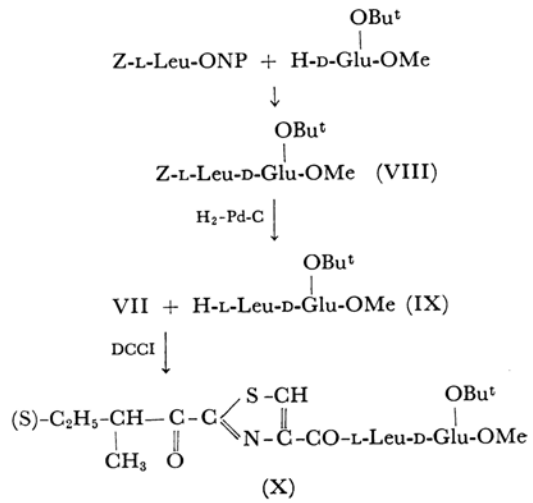


Fig. 4. Z=$C_6H_5CH_2OCO$-, NP=*p*-NO_2-C_6H_4-, DCCI=*N, N'*-Dicyclohexylcarbodiimide

The thiazole ring involved in those peptide derivatives was found to be stable under a condition of a saponification of the ester group or for a treatment with hydrogen bromide in acetic acid, while it was immediately decomposed by sodium in liquid ammonia.[13]

The UV spectrum of the synthetic tetrapeptide (X) was very similar to that of bacitracin F. This finding would support the presence of the ketothiazole structure in the molecule of bacitracin F as proposed by Craig *et al.*[4] In addition, the results of this synthetic approach will give a clue to the total synthesis of bacitracin F.

12) The melting point of 94—95°C for VII derived from the natural peptide was reported in the literature.[5]

13) The unpublished result. Y. Ariyoshi, T. Shiba and T. Kaneko, Abstract of the 20th Annual Meeting of the Chemical Society of Japan, April, 1967, III, p. 665.

Experimental

All melting points are uncorrected. The infrared spectra were obtained in Nujol mull with a Nihon Bunko IR-S and a Hitachi EPI-2 spectrophotometer. The ultraviolet spectra were obtained with a Hitachi EPS-2 spectrophotometer in 2 N hydrochloric acid and in ethanol. The NMR spectrum was obtained with a Varian A-60 spectrometer in deuteriochloroform, tetramethylsilane being used as an internal standard. The chemical shifts are expressed as ppm from tetramethylsilane. Thin-layer chromatography was carried out by the ascending method on silica gel G.

(2S : 3S)-2-Benzyloxycarbonylamino-3-methylvalerothioamide (I). Dry hydrogen sulfide was passed into a solution of 275 g (1.12 mol) of (2S : 3S)-2-benzyloxycarbonylamino-3-methylvaleronitrile[10] and 39 g of ammonia in 1.5 *l* of methanol for 20 hr at room temperature. After filtration, the filtrate was concentrated *in vacuo* to give an oil, which did not crystallize; wt, 311 g (99.4%), ν_{max} 3200, 1633 ($-CSNH_2$) cm^{-1}.

Ethyl (1S : 2S)-2-(1-Benzyloxycarbonylamino-2-methylbutyl)thiazole-4-carboxylate (II). A solution of 210 g (0.75 mol) of I and 146 g (0.75 mol) of ethyl pyruvate[11] in 1.8 *l* of anhydrous ethanol was refluxed for 2 hr. The reaction mixture was concentrated *in vacuo*, and the oily residue thus obtained was taken up in 2.5 *l* of ethyl acetate and washed with a 5% sodium bicarbonate solution (three times), and water (twice), and then dried over anhydrous sodium sulfate. Concentration *in vacuo* gave 275 g (97.8%) of II, which did not crystallize; ν_{max} 3340, 1520 (–OCONH–), 1730 (ester) cm^{-1}.

(1S : 2S)-2-(1-Benzyloxycarbonylamino-2-methylbutyl)thiazole-4-carboxylic Acid (III). To a solution of 275 g of II in 1 *l* of ethanol there was added 400 m*l* of 2 N sodium hydroxide with stirring at room temperature. After standing for 2 hr, the reaction mixture was acidified to pH 3 with 6 N hydrochloric acid. Upon concentration to a small volume, an oily material appeared. This was taken up in 3 *l* of ethyl acetate. The solution was washed with water (three times), and dried over anhydrous sodium sulfate. Concentration *in vacuo* gave 240 g (94.3%) of III, which did not crystallize at this stage.

The pure crystalline III was obtained from IV by Schotten-Baumann reaction as follows. To a solution of 20.0 g (0.052 mol) of IV in 160 m*l* of N sodium hydroxide, 9.8 g of benzyl chloroformate and 58 m*l* of N sodium hydroxide were added simultaneously over a period of 1.5 hr with vigorous stirring on cooling in an ice bath. Stirring was continued for an another 2 hr at room temperature. The reaction mixture was washed with ether, and then acidified to pH 3 with 6 N hydrochloric acid. The solution was extracted with ether. The ether layer was washed with water, and then dried over anhydrous sodium sulfate. Concentration *in vacuo* gave 16.0 g (88.4%) of an oily III, which was crystallized from ethyl acetate - petroleum ether as needles; mp 88—90°C, $[\alpha]_D^{29}$ −6.7° (*c* 3.2, ethyl acetate).

Found: C, 58.61; H, 5.76; N, 8.10; S, 9.25%. Calcd for $C_{17}H_{20}O_4N_2S$: C, 58.60; H, 5.79; N, 8.04; S, 9.20%.

(1S: 2S)-2-(1-Amino-2-methylbutyl)thiazole-4-carboxylic Acid Dihydrobromide (IV). A solution of 240 g of III in 800 m*l* of 31% (w/w) hydrogen bromide in acetic acid was shaken for 3 hr, and then 3*l* of anhydrous ether was added. A semisolid material precipitated was separated by decantation, and triturated with acetone to give a crystalline compound IV, which was collected by filtration. From the filtrate, some of the same product was recovered after a concentration *in vacuo*. Total yield 175 g (66.0%). A purification from acetone gave fine crystals with mp of 159—161°C. ν_{max} 3420 (H_2O) cm^{-1}. R_f=0.69 (Thin - layer chromatography in *n*-propanol 28% aqueous ammonia=67 : 33[14]). With the ninhydrin spraying on a paper or on a thin-layer of silica gel G, the substance gave a yellow spot that slowly turned to purple.

Found: C, 28.26; H, 4.70; N, 7.27%. Calcd for $C_9H_{16}O_2N_2SBr_2 \cdot \frac{1}{2}H_2O$: C, 28.07; H, 4.45; N, 7.00%.

(1S : 2S)-2-(1-Amino-2-methylbutyl) thiazole-4-carboxylic Acid (V). A solution of 20.0 g of IV in 200 m*l* of water was neutralized to pH 7 with 28% aqueous ammonia. The fine prisms which appeared were collected; yield 5.8 g (52.1%), mp 250—252°C (decomp.), $[\alpha]_D^{17}$ +1.3° (*c* 3.5, 3 N hydrochloric acid). λ_{max}^{2NHCl} 238.5 mμ (ε 7380).

Found: C, 50.39; H, 6.67; N, 13.06; S, 14.73%. Calcd for $C_9H_{14}O_2N_2S$: C, 50.44; H, 6.59; N, 13.07; S, 14.96%.

(1ξ : 2S)-2-(1-Hydroxy-2-methylbutyl) thiazole-4-carboxylic Acid (VI). To a solution of 20.0 g (0.052 mol) of IV in 62 m*l* of N hydrochloric acid, a solution of 3.9 g (0.057 mol) of sodium nitrite in 15 m*l* of water was added portionwise with stirring at 0°C over a period of 50 min. After standing for 10 min, the reaction mixture was continuously extracted with ether for 6 hr. The extract was dried over anhydrous sodium sulfate, and the solvent was removed by evaporation. An oily residue thus obtained was crystallized from ether - petroleum ether to give 10.0 g (89.3%) of the crude VI; mp 93—98°C. Repeated recrystallization from the same solvent gave colorless needles; mp 121.0—121.5°C, $[\alpha]_D^{17}$ −0.9° (*c* 3.9, dimethylformamide). $\lambda_{max}^{ethanol}$ 238 mμ (ε 6980). λ_{max} 3360 (–OH), 1695 (–COOH) cm^{-1}.

Found: C, 50.10; H, 6.01; N, 6.52; S, 14.76%. Calcd for $C_9H_{13}O_3NS$: C, 50.21; H, 6.09; N, 6.51; S, 14.90%.

(S)-2-(2-Methylbutyryl)thiazole-4-carboxylic Acid (VII). a) When 20.0 g (0.052 mol) of IV was dissolved in 250 m*l* of 0.5 N sodium hydroxide, the crystals immediately appeared. To this suspension (pH 8), a 2% aqueous potassium permanganate solution was added dropwise with stirring at room temperature during 1 hr, until a pink tinge, being stable for 3 min, was observed (Five hundred millilliters of the potassium permanganate solution were needed.). Manganese dioxide precipitated was just dissolved by a passage of sulfur dioxide gas, and the clear solution (pH 4) thus obtained was continuously extracted with ether for 6 hr. After drying over anhydrous sodium sulfate, the extract was evaporated to give 6.0 g (54.2%) of a solid material, which was crystallized from petroleum ether to secure 4.5 g (40.7%) of colorless plates, mp 86—88°C. Recrystallization from chloroform - petroleum ether raised the melting point to 89.5—

14) M. Brenner and A. Niedrwieser, *Experientia*, **16**, 378 (1960).

90.0°C, lit.,[5] mp 94—95°C. Further recrystallization or sublimation did not raise the melting point. $[\alpha]_D^{17}$ +24.5° (*c* 3.0, chloroform). $\lambda_{max}^{ethanol}$ 284.5 mμ (ε 5600), lit.,[5] λ_{max} 285 mμ (ε 5600). ν_{max} 1695, 1250, 1210, 950, 910, 750 cm^{-1}. The NMR spectrum showed a triplet at 0.93 ppm (3H, J=7.5 cps, $-CH_2\mathbf{CH_3}$), a doublet at 1.28 ppm (3H, J=7.0 cps, $>CH\mathbf{CH_3}$), multiplets at 1.71 ppm (2H, $-\mathbf{CH_2}CH_3$) and 3.87 ppm (1H, $>\mathbf{CH}CH_3$), singlets at 8.66 ppm (1H, ring proton) and 11.68 ppm (1H, –COO**H**). R_f=0.73[14] (detected by iodine vapor on the thin-layer chromatography).

Found: C, 50.70; H, 5.29; N, 6.66; S, 14.96%. Calcd for $C_9H_{11}O_3NS$: C, 50.69; H, 5.20; N, 6.57; S, 15.04%.

b) To a solution of 4.3 g (0.02 mol) of VI in 10 m*l* of hot acetic acid (previously distilled from chromium trioxide), a hot solution of 2.1 g (0.007 mol) of sodium dichromate dihydrate in 5 m*l* of acetic acid was added. The reaction mixture was permitted to stand at 60—65°C for 1.5 hr, and then concentrated *in vacuo*. The residue thus obtained was taken up in 100 m*l* of water and extracted continuously with ether for 4 hr. The colorless plates were obtained in the same way as described in the procedure (a); yield 0.5 g (11.7%), mp 86.0—87.5°C. Recrystallization raised the melting point to 89.5—90.0°C. $[\alpha]_D^{17}$ +24.5° (*c* 3.1, chloroform). Its IR spectrum was identical with that of the product by the method (a).

Benzyloxycarbonyl-L-leucyl-D-glutamic Acid α-Methyl-γ-*t*-butyl Ester (VIII). To a cold solution of 2.54 g (0.01 mol) of α-methyl-γ-*t*-butyl D-glutamate hydrochloride[1] and 1.02 g of triethylamine in 10 m*l* of chloroform there was added 50 m*l* of anhydrous ether. The filtrate from triethylamine hydrochloride was concentrated *in vacuo* and the oily residue thus obtained was dissolved in 10 m*l* of chloroform. To this solution, 3.86 g (0.01 mol) of benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester[15] was added. After the reaction mixture had stood for 3 days at room temperature, an additional 140 m*l* of chloroform was added, and the solution was washed successively with water, aqueous N ammonia (twice), aqueous 0.5 N ammonia (three times), water, a 5% citric acid solution, water, a saturated sodium bicarbonate solution, and water, and finally dried over anhydrous sodium sulfate. Concentration *in vacuo* gave an oily residue, which was crystallized from anhydrous ether - petroleum ether; yield 3.70 g (79.7%), mp 80.0—81.5°C. Recrystallization from the same solvent gave 3.10 g (66.7%) of needles, mp 80.5—81.5°C, $[\alpha]_D^{29}$ −6.3° (*c* 3.2, ethyl acetate).

Found: C, 62.45; H, 7.93; N, 6.00%. Calcd for $C_{24}H_{36}O_7N_2$: C, 62.05; H, 7.81; N, 6.03%.

L-Leucyl-D-glutamic Acid α-Methyl-γ-*t*-butyl Ester (IX). Through a solution of 1.5 g of VIII in a mixture of 20 m*l* of ethanol, 3 m*l* of water and 2 m*l* of acetic acid, hydrogen gas was passed in an open vessel in the presence of 0.5 g of 5% palladium on charcoal for 4.5 hr at room temperature. The filtrate from the catalyst was concentrated *in vacuo*. An oily residue thus obtained was dissolved in water and the concentration to dryness was repeated. When the oily residue obtained was dried *in vacuo* over phosphorus pentoxide and sodium hydroxide, it spontaneously crystallized. Acetate of IX; ν_{max} 3380, 1690 (–CONH–), 1735 (ester) cm^{-1}. A suspension of this material in 100 m*l* of ethyl acetate was washed with a saturated sodium bicarbonate solution until a clear solution was obtained. The organic layer was then washed with water. All washings were combined and extracted with ethyl acetate. The combined ethyl acetate extract was washed with water, and then dried over anhydrous sodium sulfate. Concentration *in vacuo* gave 1.0 g (93.5%) of crystals of IX; ν_{max} 3300, 1670 (–CONH–), 1735 (ester) cm^{-1}.

(S)-2-(2-Methylbutyryl)thiazole-4-carbonyl-L-leucyl-D-glutamic Acid α-Methyl-γ-*t*-butyl Ester (X). To a solution of 0.62 g (0.003 mol) of VII and 1.0 g (0.003 mol) of IX in 10 m*l* of chloroform, 0.62 g *N*, *N'*-dicyclohexylcarbodiimide was added with stirring on cooling. After the reaction mixture had stood overnight at room temperature, a few drops of acetic acid was added, and the urea derivative formed was filtered off. After an additional 40 m*l* of chloroform was added, the reaction mixture was washed successively with a 5% citric acid solution, water, a saturated aqueous sodium bicarbonate solution, and water (twice), and then dried over anhydrous sodium sulfate. Concentration *in vacuo* gave an oily residue, which was crystallized from ether - petroleum ether; yield 1.0 g (65.4%), mp 121—123°C. Recrystallization from the same solvent gave 0.77 g (50.3%) of fine needles; mp 130.5—131.5°C, $[\alpha]_D^{29}$ +20.7° (*c* 3.0, ethyl acetate), ν_{max} 3320, 1660, 1560 (–CONH–), 1760, 1730 (ester), 1695 (–CO–) cm^{-1}, $\lambda_{max}^{ethanol}$ 289 mμ (ε 5560), $FeCl_3$ test (−).

Found: C, 57.14; H, 7.56; N, 8.00; S, 5.91%. Calcd for $C_{25}H_{39}O_7N_3S$: C, 57.12; H, 7.48; N, 7.99; S, 6.10%.

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15) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).