Studies of Unusual Amino Acids and Their Peptides.[1] V. The Synthesis and the Absolute Configuration of β-(2-Thiazolyl)-β-alanine Present in Bottromycin

Yasuhiko SETO, Kazuhira TORII, Kōichi BORI,* Kenichiro INABATA,**
Shigeru KUWATA, and Hiroshi WATANABE

Laboratory of Chemistry, Faculty of Science, Kōnan University, Okamoto, Higashinada, Kobe 658

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In order to obtain an optically-active β-(2-thiazolyl)-β-alanine, *N*-acyl-L-aspartic α-thionamide-β-methyl ester was condensed with diethyl bromoacetal, but the amino acid thus obtained showed no perceptible optical activity. The racemic amino acid, however, could be resolved into its antipodes by treating its phthalyl derivative with brucine. Thus, the (+)-amino acid, a constituent of bottromycin, was isolated in a pure state; it was determined to belong to the L-series by examining its optical behavior. This amino acid was proved to racemize easily under the conditions of the acid hydrolysis of bottromycin.

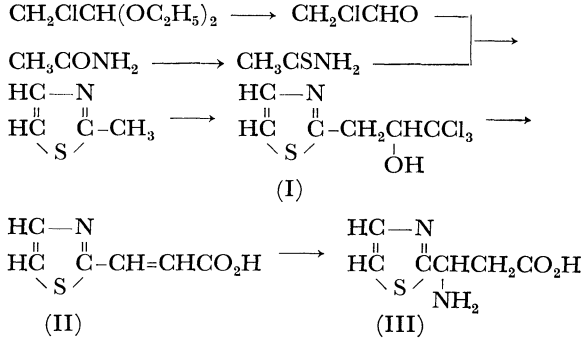
Bottromycin is a peptidic antibiotic first obtained by Waisvisz and his co-workers[2] from the fermentation broths of *Streptomyces bottropensis*. The chemical structure of this antibiotic was partly determined by the same authors,[3] and later the formula shown in Fig. 1 was proposed by Umezawa and his school.[4]

It differs from other peptidic antibiotics in several respects. In the first place, it is a linear oligopeptide ester, unlike usual peptidic antibiotics such as gramicidin S, tyrocidine or polymixin, which are cyclic oligopeptides containing D-amino acids. Secondly, it contains too many unusual amino acids. Bottromycin A, for instance, contains a total of 6 amino acids, 4 of which are unusual. Thirdly, it contains an iminopeptide bond in the middle of the molecule.

Interest in these differences, especially the relationship between these characteristics and its biological activities,[5] led us to make a synthetic study of this antibiotic.

In the proposed formula, the absolute configurations of the carboxyl-terminal two amino acids had been left undetermined. One of them, β-methylphenylalanine, was recently determined by Arold[6] to belong to the *erythro*-L-series. The other and last amino acid, β-(2-thiazolyl)-β-alanine, has had ambiguities from the beginning: by hydrolyzing the antibiotic with concentrated hydrochloric acid, Waisvisz[3] obtained a sample of this amino acid showing no optical activity. On the other hand, Umezawa and others[7] obtained an optically-active amino acid $[\alpha]_D^{18}$ +9° (*c* 1, water), by hydrolyzing the product produced by treating the antibiotic with acetic anhydride.

As the first step in our work concerning the study of bottromycin, we prepared β-(2-thiazolyl)-β-alanine and resolved it into two antipodes. The racemic form of this amino acid has already been synthesized by Waisvisz; here the same scheme was used. Though an effort was made to improve the yield, and though in effect the step giving (II) from (I) was improved



Scheme 1. The scheme of the synthesis of β-(2-thiazolyl)-β-alanine by the method of Waisvisz.

pretty well, on the whole there were too many steps and the overall yield was far from satisfactory. It should be mentioned that, in this series of experiments, we found a noticeable fact. Though the amination of β-(2-thiazolyl)-acrylic acid with hydroxylamine proceeded, the yield (30%) was so poor that in an experiment the hydroxylamine was replaced with ammonia. The yield of the addition product was remarkably enhanced, but it consisted cheifly of α-amino acid (yield, 70%); the desired β-amino acid was obtained only in a trace.

In order to resolve the racemate obtained here,

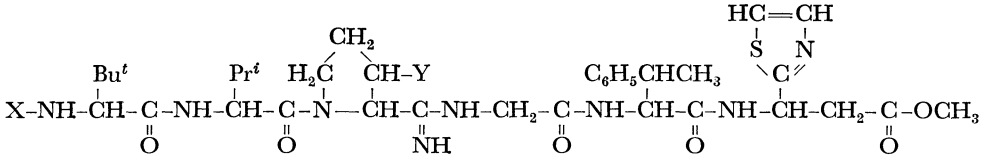


Fig. 1. The structure of bottromycin proposed by Umezawa *et al.* X=pivaloyl or Δ^1-isocaproyl; Y=H or CH_3; Bu^t=*tert*-butyl; Pr^i=isopropyl

* Present address: Daicel, Ltd., Central Research Laboratory, Tsurugaoka 171, Oimachi, Iruma-gun, Saitama-ken.

** Present address: Inabata Perfumery Co., Ltd., Fragrance Laboratory, Tagawadori 6-16, Higashiyodogawa-ku, Osaka.

Table 1. Salt formation of acyl derivatives of β-(2-thiazolyl)-β-alanine with alkaloids in some solvents

Acyl-group	Quinine		Brucine		Ephedrine
	Acetone	Ethanol	Acetone	Ethanol	Ethanol
Z-	—	—	—	oil	—
Bz-	±	—	—	+	—
Pht-	—	±	+	oil	—
Tos-	—	+	—	—	

Z=benzyloxycarbonyl, Bz=benzoyl, Pht=phthalyl, and Tos=tosyl. +, crystalline salt; ±, amorphous solid; —, no precipitate

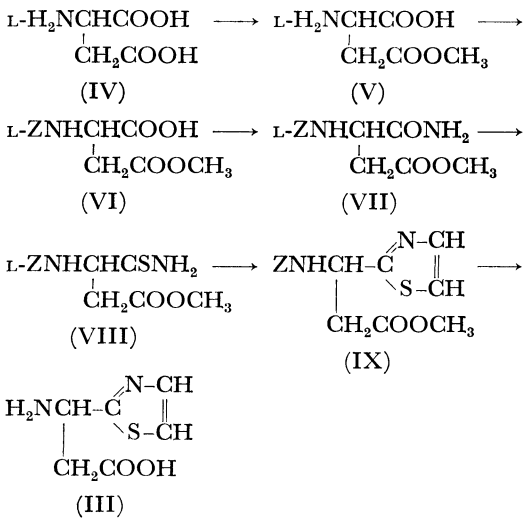
benzoyl, tosyl, benzyloxycarbonyl, and phthalyl derivatives were prepared and treated with quinine, brucine, and ephedrine in various solvents; the results are shown in Table 1. As may be seen there, the brucine salt of the phthalyl derivative was proved to be useful for the resolution when fractionally crystallized from a mixture of chloroform and acetone. By this treatment, a less soluble salt, $[\alpha]_D^{25}$ +10.6° (*c* 1, chloroform), and an easily soluble one, $[\alpha]_D^{25}$ −39° (*c* 1, chloroform), were obtained; from the former, the free acid of $[\alpha]_D^{25}$ +33.4° (*c* 1, methanol) was liberated and from the latter, that of $[\alpha]_D^{25}$ −35.4° (*c* 1, methanol). The hydrazinolysis of the (+)-phthalyl amino acid gave β-(2-thiazolyl)-β-alanine with $[\alpha]_D^{17}$ +22.4° (*c* 1, water).

The value of $[\alpha]_D$ of the amino acid isolated here is markedly larger than the one reported by Umezawa and his co-workers,[7] though the signs themselves are equal. This amino acid showed no detectable change in its optical activity when it was left to stand in 6 M hydrochloric acid at room temperature, but after the solution had been heated to reflux for 8 hr no activity was detected. This fact suggests that the optically-inactive β-(2-thiazolyl)-β-alanine obtained by Waisvisz[3] by a prolonged hydrolysis of bottromycin and the amino acid of $[\alpha]_D^{18}$ +9° obtained by Umezawa by a milder treatment from the same source were entirely or partly racemized products.

The susceptibility of this amino acid to racemization is also retained in its derivatives, *i.e.*, the *N*-phthalyl amino acid and its methyl ester, both of which lost their optical activity upon being heated with 6 M hydrochloric acid more easily than the amino acid itself, though no change was observed when they were treated with a sodium bicarbonate solution, when they were passed through a silica gel column, when the amino acid was allowed to stand in 0.1 M sodium hydroxide for 12 days, or when it was refluxed with a large excess of hydrazine hydrate in methanol. That other amino acids containing a thiazole nucleus, found in some antibiotics, also show the same tendency to racemize upon acid treatment has been described in some reports.[8]

Trying to determine the absolute configuration of the optically-active amino acids separated here, we attempted to prepare one of them starting from L-aspartic acid according to Scheme 2.

β-Methyl aspartate was prepared from L-aspartic acid by the thionyl chloride method and was then



Scheme 2. The scheme of a synthesis of β-(2-thiazolyl)-β-alanine from aspartic acid (a-series). In b-series phthalyl group was used as an amino protecting group in place of benzyloxycarbonyl group (Z).

derived to the benzyloxycarbonyl or phthalyl derivative in the usual way. The amides of these *N*-protected L-aspartic acid β-methyl esters were prepared by the mixed acid anhydride method. The thionamide (VIII) was obtained from the amide (VII) in a good yield of 70—80% by only stirring the amide with phosphorus pentasulfide in dioxane at room temperature. This simple method was also applicable to other *N*-protected amino acid amides, *e.g.*, the amides of benzyloxycarbonylated glycine and L-phenylalanine.

The thionamides thus obtained from the aspartic acid derivatives were proved unstable when they were in contact with silica gel. When these compounds were chromatographed on a thin-layer of Merck silica gel G (ethyl acetate–cyclohexane (1:1)), the sample gave two spots; a major one with a low R_f value, which was identical with that of the desired compound, and a minor one with a high R_f value, which was the same as that of the compound obtained as a by-product in the course of the preparation of the thionamide. The amount of the latter compound increased with the lapse of time after the spotting of the sample. With the benzyloxycarbonyl derivative, after 2 hr, while with the phthalyl derivative, after 15 min, the two spots of the low and high R_f values occupied about the same area; the spot of the low R_f value of the phthalyl derivative disappeared after 2 hr. It is apparent that the compound responsible for the spot of the high R_f value is identical with the by-product of thionamide-synthesis mentioned above, because both the isolated compounds showed no depression of the melting point when mixed. We may conclude that this compound is phthalylamino thionsuccinimide produced by the intramolecular condensation of the thionamide (VIII-b).

Thiazole-ring formation was performed by condensing the thionamide (VIII) with bromoacetaldehyde or, more conveniently, though in a somewhat lower yield, with bromoacetal.

The *N*-phthalyl derivative of β-(2-thiazolyl)-β-alanine methyl ester (IX-b) thus obtained unexpectedly showed

a melting point of 113—115 °C, identical with that of the racemic methyl ester, but quite different from that of the optically-active one (mp 100 °C). The corresponding *N*-benzyloxycarbonyl acid was also identical with the racemic one in melting point, IR spectrum, and thin-layer chromatogram. The optical activities of both the *N*-phthalyl (IX-b) and *N*-benzyloxycarbonyl esters (IX-a) were so obscure that it was difficult to deduce any conclusion concerning their configurations. On the other hand, the methyl ester obtained from the optically-active *N*-phthalyl amino acid by treating it with diazomethane was apparently optically active. From these facts, we could not but conclude that, during the step of cyclization ((VIII)→(IX)), a substantial racemization did occur.

We could not attain the initial aim of determining the absolute configuration of β-(2-thiazolyl)-β-alanine by synthesis, but the synthetic route itself is valuable for a larger scale preparation of this amino acid because the steps are fewer and the total yield is better than those in Waisvisz's method.

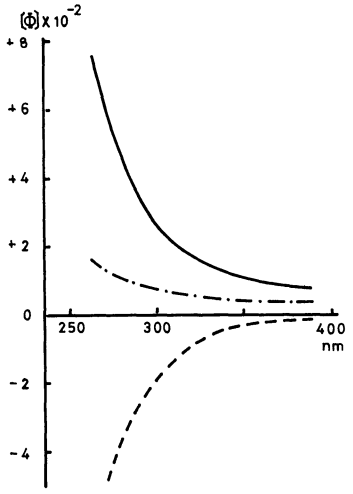


Fig. 2. Optical rotatory dispersion curves of (+)-β-(2-thiazolyl)-β-alanine; *c* 0.377, water, 26 °C.
——: neutral, —•—: acidic, ----: alkaline.

In order to determine the absolute configuration of this β-amino acid, we studied the optical properties of some optically-active β-amino acids, which have been reported previously.[9] When the optical rotatory dispersion (ORD) of (+)-β-(2-thiazolyl)-β-alanine was measured in acidic, neutral, and alkaline media, the value of the optical rotatory power decreased in the order of; neutral>acidic>alkaline (Fig. 2). This suggests that the amino acid belongs to the L-series.[9] The fact that the *N*-dithiocarbamate of this amino acid shows a negative Cotton effect in methanol, especially markedly in the presence of triethylamine, may also support this deduction.[9] Further evidence may be supplied by a comparison of the optical behavior of this amino acid derivative with that of the corresponding one of β-phenyl-β-alanine. (+)-β-(2-Thiazolyl)-β-alanine and L-β-phenyl-β-alanine gave positive ORD curves in water; the *N*-phthalyl derivatives of both the amino acids also gave positive Cotton effects in methanol. According to Freudenberg,[10] this means that both the amino acids have the same configuration. For these reasons, we conclude that the β-(2-thiazolyl)-β-alanine present in bottromycin belongs to the L-series.

Experimental[11]

2-(3',3',3'-Trichloro-2'-hydroxypropyl)-thiazole (I). (I) was synthesized from 2-methylthiazole and chloral; yield, 30%; mp 124—127 °C, lit,[12] mp 126—128 °C.

β-(2-Thiazolyl)-acrylic Acid (II). (II) was obtained by hydrolyzing 43 g of (I) with 87.5 g of potassium hydroxide in 500 ml of methanol at 40 °C for 1 hr and then at 70 °C for 30 min; yield, 26 g (64%); mp 184.5—185.5 °C, lit,[12] mp 187—190 °C.

DL-β-(2-Thiazolyl)-β-alanine (DL-III). This compound was obtained from (II) and hydroxylamine according to the method of Waisvisz; mp 198—199 °C, lit,[3] mp 199—202 °C. Circular paper chromatographies; R_f 0.30 (Solvent A) and 0.29 (Solvent B).

Acyl Derivatives of (DL-III). The following acyl derivatives were prepared by the usual method.

Phthalyl Derivative: Obtained by melting (DL-III) with phthalic anhydride at 150 °C for 40 min; yield, 75%; mp 161—162 °C.

Found: C, 55.58; H, 3.33; N, 9.21%. Calcd for $C_{14}H_{10}N_2O_4S$: C, 55.62; H, 3.33; N, 9.27%.

Benzyloxycarbonyl Derivative: Yield, 63%; mp 122.5—123.5 °C.

Found: C, 55.02; H, 4.56; N, 9.07%. Calcd for $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14%.

Benzoyl Derivative: Yield, 50%; mp 169—170.5 °C.

Found: C, 56.32; H, 4.42; N, 10.10%. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14%.

Tosyl Derivative: Yield, 36%; mp 164—165 °C.

Found: C, 47.84; H, 4.16; N, 8.62%. Calcd for $C_{13}H_{14}N_2O_4S_2$: C, 47.84; H, 4.32; N, 8.58%.

An Optical Resolution of DL-Phthalyl-β-(2-thiazolyl)-β-alanine. To a solution of 10.7 g of DL-phthalyl-β-(2-thiazolyl)-β-alanine and 14.0 g of brucine in 100 ml of chloroform, we added 100 ml of acetone; after the mixture had been allowed to stand at room temperature overnight, the separated crystals were collected; yield, 5.4 g; $[\alpha]_D^{22}$ −3.2° (*c* 1, chloroform). After repeated recrystallizations from chloroform–acetone, the optical rotation settled upon $[\alpha]_D^{22}$ +10.6° (*c* 1, chloroform); yield, 4 g. From the mother liquor, the more soluble salt of $[\alpha]_D^{22}$ −39° (*c* 1, chloroform) was obtained; yield, 7 g.

To remove the brucine, the salt was dissolved in hot water; the solution was ice-cooled quickly and then after a small excess of aqueous sodium hydroxide had been added, the freed base was extracted with chloroform. The aqueous layer was acidified to pH 2 with 6 M hydrochloric acid and then concentrated. After ice-cooling, the separated phthalyl derivative was collected. No change in the optical rotation was observed after recrystallization from methanol–water. The properties of the phthalyl-β-(2-thiazolyl)-β-alanine thus obtained were as follows:

From the less soluble brucine salt; $[\alpha]_D^{22}$ +33.4° (*c* 1, methanol), mp 138—140 °C (solidified immediately and melted again at 162—163 °C).

From the easily soluble brucine salt; $[\alpha]_D^{22}$ −35.4° (*c* 1, methanol); the melting point is completely identical with that of the above sample.

Phthalyl-(+)-β-(2-thiazolyl)-β-alanine Methyl Ester. This compound was obtained from the phthalyl-(+)-acid by treating it with diazomethane. Recrystallization from methanol–water gave a sample with a mp of 99—100 °C, $[\alpha]_D^{15}$

+23.5° (*c* 1.065, methanol).

(+)-β-(2-Thiazolyl)-β-alanine. ((+)-III). To a solution of the phthalyl derivative with $[\alpha]_D^{22}$ +33.4° in a small amount of methanol, we added 3 equivalents of hydrazine hydrate and then heated the mixture to reflux for 30 min. After the solvent had been distilled off, the residue was dissolved in water, acidified with acetic acid, and refluxed for 1 hr. The ice-cooled mixture was filtered, the filtrate was evaporated to dryness, and the remaining hydrazine hydrate was thoroughly removed under a high vacuum. By recrystallizing the residue from alcohol, (+)-β-(2-thiazolyl)-β-alanine was obtained as crystals; yield, 55%, mp 203.5—204.5 °C, $[\alpha]_D^{17}$ +22.4° (*c* 1, water). Further recrystallization from water–ethanol had no influence on the value of the specific rotatory power. Circular paper chromatographies; R_f 0.30 (Solvent A) and 0.29 (Solvent B).

Found: C, 41.62; H, 4.69; N, 16.26%. Calcd for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27%.

Lit,[7] mp 200—201 °C, $[\alpha]_D^{18}$ +9° (*c* 1, water).

((+)-III) in 6 M hydrochloric acid showed no perceptible change in optical rotation in the range of 270—700 nm after 24 hr at room temperature, but after 50 hr a little change was observed. When the solution was refluxed, however, for 1, 2.5, and 8 hr, the optical activity decreased to about 50%, less than 10%, and 0% respectively.

Addition of Ammonia to β-(2-Thiazolyl)-acrylic Acid (II). A solution of 1 g of (II) in 7 ml of 25% aqueous ammonia was heated in a sealed tube at 90 °C for 1 hr and then at 120 °C for a further 40 hr. After the reaction mixture had been treated with charcoal, it was evaporated to dryness and the residue was washed with ethanol. The yield of the crude product (mp 180—184 °C) was 0.79 g (70%). This product consisted of two components, because it gave two ninhydrin positive spots on circular paper chromatography. The major one was brown (R_f 0.26 (Solvent A) and 0.33 (Solvent B)), and the minor one, faint purple (R_f 0.30 (Solvent A) and 0.29 (Solvent B)). The latter was confirmed to be β-(2-thiazolyl)-β-alanine by comparing it with the authentic sample. By recrystallizing the crude product from methanol–water, the major component was obtained in a chromatographically pure state in a good yield, and it coincided with β-(2-thiazolyl)-α-alanine in all respects: mp 202—202.5 °C. Reported value for β-(2-thiazolyl)-α-alanine,[12] mp 197—198 °C. 2,4-Dinitrophenyl derivative, mp 209—210 °C. Lit,[3] mp 203—210 °C. Phthalyl derivative, mp 188.5—189 °C.

Found: C, 55.87; H, 3.42; N, 9.25%. Calcd for $C_{14}H_{10}N_2O_4S$: C, 55.62; H, 3.33; N, 9.27%.

L-Aspartic Acid β-Methyl Ester Hydrochloride (V). This compound was prepared by the method of Brenner;[13] yield, 97%, mp 186—187 °C, $[\alpha]_D^{19}$ +14.4° (*c* 1, ethanol–water (1:3)). Lit,[14] mp 199—200 °C, $[\alpha]_D^{25}$ +14.4° (*c* 1, ethanol–water (1:3)).

Benzyloxycarbonyl-L-aspartic Acid β-Methyl Ester (VI-a). This compound was prepared from (V) and benzyloxycarbonylchloride by the usual method; yield, 82%, mp 91—92 °C, $[\alpha]_D^{26}$ −18.2° (*c* 2.5, pyridine). Lit,[15] mp 97—98 °C, $[\alpha]_D^{25}$ −18.5° (*c* 2.5, pyridine). This product was used for the next reaction without further purification.

Benzyloxycarbonyl-L-aspartic Acid β-Methyl Ester α-Amide (VII-a). To a cold solution of 7.88 g of (VI-a) and 2.83 g of triethylamine in 60 ml of tetrahydrofuran, we added 3.05 g of ethyl chloroformate with ice-cooling over a 7 min period. After stirring for 20 min at −8 °C, 4.20 g (2.5 equivalents) of 28% aqueous ammonia were added; the stirring was continued at −4 °C for a further 40 min and then at room temperature overnight. The mixture was subsequently evaporated under reduced pressure, and the residue was dissolved in ethyl acetate. The extract was washed with water, dried with anhydrous sodium sulfate, and evaporated to yield needle crystals; yield, 5.16 g (66%); mp 118.5—120 °C. After recrystallization from benzene, mp 120—121 °C, $[\alpha]_D^{28}$ +5.3° (*c* 1, acetic acid). ORD; $[\phi]_{270}$ −560°, $[\phi]_{299}$ −280°, $[\phi]_{552}$ 0°, $[\phi]_{650}$ +15° (*c* 2, methanol). Lit,[16] mp 121 °C, $[\alpha]_D^{20}$ +9.0° (acetic acid).

Benzyloxycarbonyl-L-aspartic Acid β-Methyl Ester α-Thionamide (VIII-a). To a solution of 2 g of the amide (VII-a) in 10 ml of dry dioxane, we added 1.74 g of finely powdered phosphorus pentasulfide; stirring was continued for 8 hr at room temperature until the mixture became almost homogeneous. A little residual phosphorus pentasulfide was filtered off, and the filtrate was vigorously stirred into 100 ml of ice–water. The separated pale yellow oil was extracted with ether, and the ether extract was washed with water, dried with anhydrous sodium sulfate, and evaporated to the crude thionamide; yield, 1.81 g (86%); mp 67—87 °C. Recrystallization from tetrahydrofuran–petroleum ether gave crystals, mp 78.5—79.5 °C, $[\alpha]_D^{28}$ −22° (*c* 1, methanol). ORD; $[\phi]_{364}$ −680°, $[\phi]_{378}$ −950°, $[\phi]_{410}$ −470°, $[\phi]_{650}$ −44° (*c* 1, ethanol).

Found: C, 52.97; H, 5.45; N, 9.45%. Calcd for $C_{13}H_{16}N_2O_4S$: C, 52.69; H, 5.44; N, 9.45%.

From the mother liquor of the recrystallization we obtained fine long needles (mp 87—88 °C) which gave a spot with the same R_f value as the thionamide in thin-layer chromatography, but no details were investigated.

Thionamides of Benzyloxycarbonyl-glycine and -L-Phenylalanine. By the same method, both the thionamides were also prepared in good yields.

Benzyloxycarbonyl-glycine Thionamide: mp 146.5—148 °C.

Found: C, 54.18; H, 5.43; N, 12.49%. Calcd for $C_{10}H_{12}N_2O_2S$: C, 53.55; H, 5.39; N, 12.49%.

Benzyloxycarbonyl-L-phenylalanine Thionamide: mp 145—145.5 °C, $[\alpha]_D^{17}$ +13.0° (*c* 0.766, methanol).

Found: C, 64.95; H, 5.97; N, 8.87%. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91%.

Benzyloxycarbonyl-β-(2-thiazolyl)-β-alanine Methyl Ester (IX-a). A mixture of 20.8 g of (VIII-a) and 80.5 g of diethyl bromoacetal in a vessel equipped with a condenser was kept at 60—65 °C in a water bath under reduced pressure (35 mmHg) for 1.5 hr with occasional shaking. After 20 min, the pale yellow solution began to separate into two layers. The upper layer, consisting of an excess of acetal, was decanted off, after which the orange-coloured lower oil was washed several times with ethyl acetate. After being made alkaline with aqueous sodium bicarbonate, the oil was extracted with ethyl acetate, and the extract was washed with water, dried with anhydrous sodium sulfate, and evaporated to dryness. The yield of the crude ester (mp 62—67 °C (sinter from 59 °C)) was 17.4 g (77%). After recrystallization from ethyl acetate–petroleum ether, the ester melted at 70.5—71.5 °C.

Found: C, 56.20; H, 5.10; N, 8.60%. Calcd for $C_{15}H_{16}N_2O_4S$: C, 56.24; H, 5.03; N, 8.74%.

Benzyloxycarbonyl-β-(2-thiazolyl)-β-alanine (X). This compound was obtained by hydrolyzing the ester (IX-a) with 6 M hydrochloric acid at 70 °C for 1 hr; mp 122—123.5 °C, $[\phi]_{280-700}$ 0° (*c* 2, ethanol). The thin-layer chromatograms, melting points (including mixed melting point), and IR spectrum of this compound were identical with those of the authentic sample prepared according to the Waisvisz method.

Phthalyl-L-aspartic Acid β-Methyl Ester (VI-b). This compound was prepared from (V) using the Nefkens reagent;[17] yield, 80%; mp 130—134 °C. After recrystalliza-

tion from benzene, mp 133.5—134.5 °C, $[\alpha]_D^{16}$ −75.8° (*c* 1, methanol).

Found: C, 56.32; H, 4.04; N, 5.00%. Calcd for $C_{13}H_{11}NO_6$: C, 56.32; H, 4.00; N, 5.05%.

Phthalyl-L-aspartic Acid β-Methyl Ester α-Amide (VII-b). Into a cold solution of 15 g of (VI-b) and 5.4 g of triethylamine in 75 ml of tetrahydrofuran, we vigorously stirred 5.90 g of ethyl chloroformate at −10 °C for 7 min. After additional stirring for 10 min at −10 °C, 5 g (1.5 equivalents) of 28% aqueous ammonia were added; the stirring was then continued at 0 °C for 2 hr and at room temperature for 3 hr. The reaction mixture was evaporated, and the residue was washed with water to give the crude amide; mp 163—166 °C. Recrystallization from ethanol gave 11.5 g (77%) of the amide; mp 167—168 °C. $[\alpha]_D^{19}$ −81° (*c* 1, dimethylformamide). ORD; $[\phi]_{326}$ −2150°, $[\phi]_{330}$ −2430°, $[\phi]_{350}$ −1200°, $[\phi]_{650}$ −80° (*c* 1, dimethylformamide).

Found: C, 56.56; H, 4.50; N, 10.05%. Calcd for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14%.

In this reaction a small amount of a crystalline by-product was obtained from the mother liquor. After recrystallization from methanol, it melted at 227—228 °C and yielded aspartic acid upon hydrolysis. From this fact and the elemental analysis of this product, the by-product may be concluded to be the phthalylamino succinimide derived from (VII-b) by the removal of the methanol.

Found: C, 58.79; H, 3.34; N, 11.48%. Calcd for $C_{12}H_8N_2O_4$: C, 59.02; H, 3.30; N, 11.47%.

Phthalyl-L-aspartic Acid β-Methyl Ester α-Thionamide (VIII-b). To a solution of 162 g of the amide (VII-b) in 800 ml of dry dioxane, we added 162 g of finely powdered phosphorus pentasulfide. The mixture was stirred at room temperature for 9 hr. Any insoluble material was filtered off, and the residue was washed with dioxane. The combined filtrate and washings were concentrated to about 1/3 in volume and vigorously stirred into ice water. The oil thus separated, which soon solidified, was isolated by filtration, washed with water, dried over calcium chloride under a vacuum, and recrystallized from methanol; yield, 120 g (70%); mp 158—159.5 °C. Prisms (colourless, but once coloured it is difficult to remove the colour). Analytical sample; mp 163.5—164.5 °C, $[\alpha]_D^{17}$ −6.5° (*c* 1, tetrahydrofuran). ORD; $[\phi]_{358}$ −230°, $[\phi]_{383}$ −550°, $[\phi]_{409}$ −260°, $[\phi]_{650}$ −9° (*c* 1, tetrahydrofuran).

Found: C, 53.79; H, 4.19; N, 9.37%. Calcd for $C_{13}H_{12}N_2O_4S$: C, 53.42; H, 4.14; N, 9.58%.

From the mother liquor, a small amount of crystals was isolated as a by-product. It melted at 220—223 °C after recrystallization from methanol. This compound was found to be produced by treating the thionamide (VIII-b) with Merck silica gel G, *e.g.*, by passing the thionamide through the silica gel column. The identity of the two compounds obtained by the two different methods was proved by a comparison of their melting points, IR spectra, and analytical data; from this identity we conclude that the compound is 2-phthalylamino-L-thionsuccinimide. This compound showed a characteristic Cotton effect with a peak at 426 nm and a trough at 382 nm. ORD; $[\phi]_{360}$ −2500°, $[\phi]_{382}$ −2900°, $[\phi]_{406}$ −1500°, $[\phi]_{426}$ −150°, $[\phi]_{470}$ −360°, $[\phi]_{650}$ −100° (*c* 0.5, tetrahydrofuran).

Found: C, 55.06; H, 3.01; N, 10.79%. Calcd for $C_{12}H_8N_2O_3S$: C, 55.38; H, 3.10; N, 10.76%.

Phthalyl-β-(2-thiazolyl)-β-alanine. A solution of 8.9 g of thionamide (VIII-b) and 8.9 g of diethyl bromoacetal in 8 ml of tetrahydrofuran was heated to reflux over a 7 hr period. After the reaction mixture had then been evaporated, the residual oil was washed with petroleum ether to remove the excess of acetal, made alkaline with aqueous sodium bicarbonate, and extracted with ethyl acetate. The extract was washed with water, dried with anhydrous sodium sulfate, and evaporated to the crude ester (IX-b); yield, 7.4 g (77%); mp 106—110 °C. After recrystallization from methanol, the ester melted at 115 °C.

By the hydrolysis of this ester with 6 M hydrochloric acid at 60 °C for 2.5 hr, phthalyl-β-(2-thiazolyl)-β-alanine was obtained; mp 160—161.5 °C. No optical activity was detected in the range of 380—700 nm. A mixed melting point determination with the phthalyl derivative obtained from (DL-III) showed no depression, and the IR spectra of the two compounds were completely identical.

References

1) The previous heading of this series, "Studies of Unnatural Amino Acids and Their Peptides," is altered as above. Previous reports: This Bulletin, **38**, 1461 (1965); **39**, 2473 (1966); **41**, 1634 (1968); **43**, 2874 (1970).

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11) All the melting points are uncorrected. The optical rotations were measured by means of a Yanagimoto Polarimeter OR-20. ORD curves were recorded on a JASCO ORD/UV-5 spectropolarimeter. Thin-layer chromatographies were performed on Merck silica gel G using *n*-butanol–acetic acid–water (4:1:2 v/v, upper phase) (Solvent A) or pyridine–acetic acid–water (3:12:5 v/v, upper phase) (Solvent B) as the developing solvent. Circular paper chromatographies were performed on Toyo Roshi No. 2.

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