Studies of Unusual Amino Acids and Their Peptides. XI. The Resolution of β-(2-Furyl)-β-alanine and β-(2-Thienyl)-β-alanine and Their Absolute Configurations

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(+)-β-(2-Furyl)-β-alanine and (+)-β-(2-thienyl)-β-alanine were obtained by resolving the *N*-benzyloxycarbonyl-DL-amino acids with quinine and by removing the protecting group from them. It was concluded, from the changes in their ORD curves depending on the pH, and also from the sign of the Cotton effects of their DCHA salts of the *N*-ethylthiocarbonothioyl derivatives, that both the compounds belong to the L-series. This conclusion was supported by the fact that the aspartic acid obtained by the oxidation of these amino acids belongs to the D-series.

In connection with β-(2-thiazolyl)-β-alanine,[1] present in a peptide antibiotic bottromycin as a C-terminal amino acid, we had an interest in the β-amino acids bearing a heterocycle in the β-position. Among these amino acids, we attempted to prepare β-(2-furyl)- and β-(2-thienyl)-β-alanine as analogs of the thiazolylalanine. Both the compounds are known,[2,3] but no attempt has been made to resolve them or to determine the absolute configuration of their optically active form.

These β-amino acids were prepared by the reaction of 2-furaldehyde or 2-thiophenecarbaldehyde with malonic acid in the presence of ammonium acetate according to the literature.[2a,3b] The racemates could be successfully resolved by treating the *N*-benzyloxycarbonyl derivatives with quinine in ethyl acetate or ethanol. From the crystalline quinine salts, separated out from the solution, the alkaloid base was removed; the following optically active compounds were thus obtained: *N*-benzyloxycarbonyl-(+)-β-(2-furyl)-β-alanine, mp 123—125 °C, $[\alpha]_D^{20}$ +55.9° (*c* 1, MeOH), *N*-benzyloxycarbonyl-(+)-β-(2-thienyl)-β-alanine, mp 128—129 °C, $[\alpha]_D^{20}$ +45.4° (*c* 1, MeOH). The *N*-protecting group could be removed by hydrobromic acid in acetic acid in the usual way, though the yields were unexpectedly low in both cases. The amino acids obtained were: (+)-β-(2-furyl)-β-alanine, $[\alpha]_D^{20}$ +12.9° (*c* 1, H_2O), (+)-β-(2-thienyl)-β-alanine, $[\alpha]_D^{20}$ +15.3° (*c* 1, H_2O). By hydrogenolytic deprotection, the former amino acid of $[\alpha]_D^{20}$ +13.7° (*c* 1, H_2O) was obtained in a much better yield than in the case of acidolysis.

In previous papers we proposed two empirical rules for the determination of the absolute configuration of the β-amino acid. One is that the value of the molecular rotation of L-β-amino acid[4] in water at various pH decreases in the order of neutral>acidic>alkaline media.[5] The other is that the dicyclohexylamine (DCHA) salt of the *N*-ethylthiocarbonothioyl (ETCT) derivative of the L-β-amino acid shows a negative Cotton effect.[6] In order to apply these rules to the optically active β-amino acids obtained here, their ORD spectra in water at different pH values were measured; it was found that the values decreased in both the β-amino acids in the order of neutral> acidic>alkaline media. On the other hand, the CD spectra of the DCHA salts of the *N*-ETCT-β-amino acids in some solvents showed negative Cotton effects in all the cases, as is shown in Table 1. Both the results denote that these β-amino acids belong to the L-series. It should be pointed out that solvents have a remarkable influence on the Cotton effects of the *N*-ETCT-β-aromatic β-amino acids, depending on the structure of the aromatic ring (Table 1).

Table 1. Cotton effects of *N*-ETCT-L-β-aromatic β-amino acids ($C_2H_5SSC-NHCHCH_2COOH$, X) and their DCHA salts in several solvents

X	Free acid			DCHA salt		
	MeOH	C_6H_6	$CHCl_3$	MeOH	C_6H_6	$CHCl_3$
Phenyl-[6]	−	−	−	−	−	−
2-Thiazolyl-	−	−, +	+	−	−	−
2-Thienyl	−	+	+	−	−	−
2-Furyl-	+	+	+	−	−	−

Terent'ev reported that *N*-benzoyl-DL-β-(2-furyl)-β-alanine or its amide was oxidized with alkaline permanganate to the benzoyl derivative of aspartic acid[7] or asparagine[2c] in a good yield. We also observed that β-(2-furyl)-α-alanine was easily converted into aspartic acid.[8] These facts suggest another route for determining the configuration of β-(2-furyl)- or β-(2-thienyl)-β-alanine by oxidizing it and by examining the chirality of the resulting aspartic acid. The β-amino acids belonging to the L-series should give D-aspartic acid upon the oxidation.

The oxidation of *N*-benzyloxycarbonyl-(+)-β-(2-furyl)-β-alanine with alkaline permanganate proceeded very smoothly and afforded *N*-(benzyloxycarbonyl) aspartic acid almost quantitatively; it was determined to be the D-isomer by a comparison of the optical rotation of the dimethyl ester with that of a sample derived from L-aspartic acid. On the other hand, the oxidation of *N*-benzyloxycarbonyl-(+)-β-(2-

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thienyl)-β-alanine to an aspartic acid derivative did not proceed so smoothly, and it was accompanied by many by-products. From the oxidation products obtained at 40 °C, *N*-(benzyloxycarbonyl)aspartic acid was isolated in a 12% yield after repeated purification by preparative silica gel TLC; benzoic acid was also isolated in a 51% yield. The configuration of the aspartic acid obtained here was confirmed to be the D-form, quite similar to the above case. Consequently, the original (+)-β-(2-furyl)- and (+)-β-(2-thienyl)-β-alanine could be attributed to the L-series.

The identity of the conclusions deduced both from the empirical rules and from the chemical evidences would demonstrate the usefulness of these rules.

Experimental

The melting points are uncorrected. The optical rotations were measured on a JASCO DIP-4 polarimeter. The ORD and CD curves were recorded on a JASCO ORD/UV-5 spectropolarimeter.

DL-β-(2-Furyl)-β-alanine (I). This compound was synthesized in a 28% yield by the condensation of 2-furaldehyde, malonic acid, and ammonium acetate according to the literature;[2a] mp 199—201 °C (dec), lit,[2a] mp 200—201 °C (dec).

N-Benzyloxycarbonyl-DL-β-(2-furyl)-β-alanine (II). I was acylated with benzyloxycarbonyl chloride and sodium hydroxide as usual; 79% yield; mp 143—143.5 °C (from aq EtOH).

Found: C, 62.31; H, 5.29; N, 4.71%. Calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84%.

Optical Resolution of DL-β-(2-Furyl)-β-alanine. *N-Benzyloxycarbonyl-(+)-β-(2-furyl)-β-alanine (III)*: II (8.7 g) and quinine (9 g) were dissolved in ethyl acetate (150 ml) and then allowed to stand for several days in a refrigerator. The crystals thus separated were collected and recrystallized from ethyl acetate: mp 109—111 °C, $[\alpha]_D^{20}$ −86.0° (*c* 1, MeOH).

By the removal of quinine from this salt with hydrochloric acid, optically active *N*-benzyloxycarbonyl-β-(2-furyl)-β-alanine was obtained; mp 123—125 °C (from aq EtOH), $[\alpha]_D^{20}$ +55.9° (*c* 1, MeOH). The overall yield from II was 70%.

(+)-β-(2-Furyl)-β-alanine (IV). Debenzyloxycarbonylation by Acidolysis: A 25% hydrogen bromide solution in acetic acid was added to III (600 mg), which dissolved immediately, forming a deep blue solution. After about ten minutes, the solution was concentrated under reduced pressure, and the resulting residue was washed with dry ether several times. After the remaining viscous, deep blue material had been dissolved in water and decolorized, the aqueous solution was passed through an Amberlite CG-120 (H^+ form) column. The amino acid was then eluted with aq ammonia, and the eluate was, after decolorization, concentrated to dryness under reduced pressure; yield, 44%. Recrystallization from water afforded large plates; mp 205—207 °C (dec), $[\alpha]_D^{20}$ +12.9° (*c* 1, H_2O).

Debenzyloxycarbonylation by Hydrogenolysis:[9] In a quantitative hydrogenation apparatus, a solution of III (723 mg; 2.5 mmol) in a mixture of 1 M NaOH (7 ml) and methanol (4 ml) was hydrogenated in the presence of 5% Pd–C (80 mg) at room temperature. Hydrogenation was stopped when 50 ml of hydrogen (*ca.* 2.2 mmol) had been absorbed. The reaction mixture was then filtered, and the catalyst was washed thoroughly with water. The filtrate and the washings were combined, acidified with hydrochloric acid, and extracted with ethyl acetate. The aqueous layer neutralized with 1 M NaOH to pH 6, was passed through the Amberlite CG-120 (H^+ form) column, which had been thoroughly washed with water. The amino acid was eluted with aq ammonia, and the eluate was concentrated to dryness under reduced pressure: 360 mg (93%). The NMR spectrum of this compound was identical with that of the (+)-β-(2-furyl)-β-alanine obtained by acidolysis and showed no peak attributable to the tetrahydrofuran ring. Recrystallization from water afforded large plates; $[\alpha]_D^{20}$ +13.7° (*c* 1, H_2O).

DL-β-(2-Thienyl)-β-alanine (V). This compound was synthesized in a 43% yield by the condensation of 2-thiophenecarbaldehyde,[10] malonic acid, and ammonium acetate according to the literature;[3b] mp 199—201 °C (dec), lit,[3b] mp 201—203 °C.

N-Benzyloxycarbonyl-DL-β-(2-thienyl)-β-alanine (VI). V was benzyloxycarbonylated as usual in an 86% yield; mp 118—118.5 °C (from aq EtOH).

Found: C, 59.02; H, 4.94; N, 4.34%. Calcd for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59%.

Optical Resolution of DL-β-(2-Thienyl)-β-alanine. The resolution was carried out in a manner similar to that used in the case of the furan derivative.

Easily crystallizable quinine salt of *N*-benzyloxycarbonyl-β-(2-thienyl)-β-alanine was obtained in a 60% yield; mp 155—156 °C (from EtOH), $[\alpha]_D^{20}$ −29.3° (*c* 1, MeOH).

N-Benzyloxycarbonyl-(+)-β-(2-thienyl)-β-alanine (VII). Yield, 95%; mp 128—129 °C (from aq EtOH), $[\alpha]_D^{20}$ +45.4° (*c* 1, MeOH).

(+)-β-(2-Thienyl)-β-alanine (VIII). VII was debenzyloxycarbonylated with hydrogen bromide in acetic acid and worked up similarly to the case of furylalanine. Yield, 54%; mp 206—208 °C (dec), $[\alpha]_D^{20}$ +15.3° (*c* 1, H_2O).

Measurement of CD Spectra of N-ETCT-amino Acids and Their DCHA Salts. Both β-amino acids, IV and VIII, and (+)-β-(2-thiazolyl)-β-alanine[1] were converted to the corresponding ethyl dithiocarbamates as well as their DCHA salts according to the literature.[11] The signs of the Cotton effects of their CD spectra in several solvents are summarized in Table 1.

Oxidation of N-Benzyloxycarbonyl-(+)-β-(2-furyl)-β-alanine. III (289 mg) was dissolved in a sodium hydroxide solution (NaOH 40 mg, H_2O 5 ml), into which an alkaline potassium permanganate solution ($KMnO_4$ 1.6 g, NaOH 0.5 g, H_2O 50 ml) was added, drop by drop, under ice-cooling until the violet color of the reaction mixture had come to persist. The residual permanganate was destroyed with aqueous sodium sulfite, the precipitated manganese dioxide was filtered off, and the filtrate was extracted with ethyl acetate after having been acidified with hydrochloric acid. The organic layer was then washed with water and dried over anhydrous sodium sulfate. The evaporation of the solvent gave white crystals (mp 110—114 °C), the IR spectrum of which agreed well with that of *N*-benzyloxycarbonyl-L-aspartic acid; the yield was 266 mg (quantitative). The optical rotation was measured after converting it to dimethyl ester with diazomethane; $[\alpha]_D^{20}$ +15.3° (*c* 1.7, MeOH), $[\alpha]_D^{20}$ −29.5° (*c* 1.3, $CHCl_3$). (*N*-Benzyloxycarbonyl-L-aspartic acid dimethyl ester:[12] $[\alpha]_D^{20}$ −20.4° (*c* 1, MeOH), $[\alpha]_D^{20}$ +27.5° (*c* 1, $CHCl_3$)).

Oxidation of N-Benzyloxycarbonyl-(+)-β-(2-thienyl)-β-alanine. VII (600 mg) was oxidized with alkaline permanganate similarly to the case of the furan derivative, except that the reaction temperature was kept at about 40 °C. The color change of the reaction mixture was very slow compared

with that of the furan derivative, so a large excess of the oxidizing reagent was added to ensure the oxidation. The separation of the crude oxidation products was carried out by preparative TLC (adsorbent: Merck silica gel GF_{254}; developing solvent, $CHCl_3$:MeOH:AcOH=95:15:3). The zone of the chromatogram containing *N*-(benzyloxycarbonyl)-aspartic acid was scraped off and extracted with methanol. The extract was then concentrated and purified again in the same manner. The methanol extract was then dissolved in ethyl acetate, and the solution was washed with water and dried over sodium sulfate. The subsequent evaporation of the solvent gave white crystals (mp 114—115 °C), the IR spectrum of which agreed well with that of *N*-benzyloxycarbonyl-L-aspartic acid; the yield was 64 mg (12%); dimethyl ester, $[\alpha]_D^{20}$ +17.7° (*c* 1.2, MeOH), $[\alpha]_D^{20}$ −20.3° (*c* 1.2, $CHCl_3$). Another component of the oxidation product, with a larger R_f value than that of the aspartic acid derivative, was also isolated in a similar manner. This compound melted at 119—121 °C after recrystallization from water and showed no depression of the melting point when admixed with benzoic acid. The yield was 122 mg (51%).

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