A Study of Unnatural Amino Acids and Their Peptides. I.
The Synthesis of DL-β-(2-Furyl)-α-alanine[*1]

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With the development of the chemistry of antibiotics and other microbial products, or of the constituents of microbes themselves, e. g., the cell wall of bacteria, it has been clarified that there are many sorts of amino acids which have not hitherto been encountered in common proteins. One of them is the D-amino acid, e. g., D-phenylalanine in gramicidin S[1] or tyrocidine,[2] D-serine in cycloserine,[3] and D-glutamic acid in the capsular substance of *Bact. anthracis*.[4] Another group is the amino acid with a hetero-ring, e. g., β-(2-thiazole)-β-alanine in bottromycin[5] or 3-oxy-2-picolic acid and 4-oxopipecolic acid in staphylomycin[6] and ostreogrycin.[7]

We are interested in these " unnatural amino acids " and in their peptides, especially those of the latter group, in connection with their biological properties. Therefore, we intend to begin to study in this field. In this paper the synthesis of DL-β-(2-furyl)-α-alanine (I), an amino acid with a furyl residue in place of the phenyl residue in phenylalanine, will be reported.

This amino acid has not yet been found in nature, but it has been synthesized in many ways, such as through the condensation of furfural with hydantoin,[8] rhodanine,[9] diketopiperazine,[10] or hippuric acid.[11] The condensation of furfurylchloride with acetamidomalonate[12] or acetamidocyanoacetate[12] has also been utilized for this purpose. Of these, the rhodanine method was tested first, but this was found not suitable for large-scale preparations. The acetamidomalonate method was then tried. The condensation of furfurylchloride (III) with ethyl acetamidomalonate proceeded smoothly, but no promising results were obtained in the hydrolysis of the condensation product. Although this difficulty can be avoided, according to Herz,[12] by using acetamidocyanoacetate instead of acetamidomalonate, the total yield is still too low. Thus ethyl formamidomalonate (IV) was chosen because the removal of formyl group is, in general, far easier than that of the acetyl group, and because, moreover, the formylfurylalanine itself may be useful as an intermediate for the further peptide synthesis.

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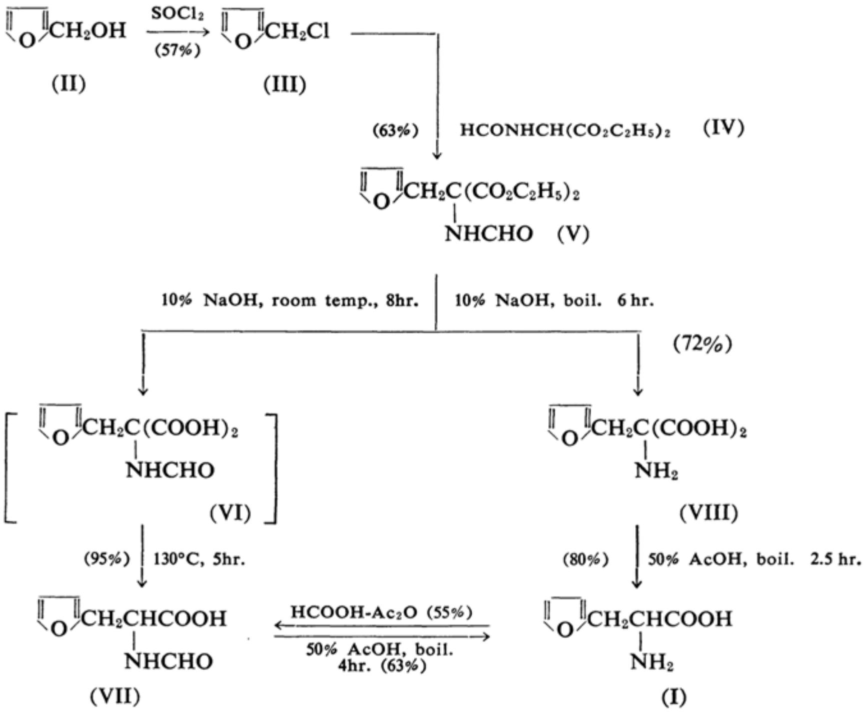

Diethyl formamido-(2-furfuryl)-malonate (V) was obtained as a well-crystallizable compound in a 63% yield by the reaction of III and IV in ethanol using sodium as a condensing agent.

Acylamidomalonates can be hydrolyzed smoothly with mineral acid in one step, but in our case such an acid hydrolysis is inapplicable because of the sensitivity of the furan ring. In fact, when furylalanine was boiled with 6 N hydrochloric acid, it was decomposed very rapidly and the ninhydrin reaction became completely negative.

The alkaline hydrolysis of acylamidomalonate needs a step-by-step treatment, as has been shown in the synthesis of tryptophan[13]; hence, the hydrolysis conditions were investigated. First, V was refluxed with a 10% sodium hydroxide solution for one hour. The extraction of the acidified hydrolysate with ethyl acetate gave a crystalline product, a part of which decomposed at about 120°C under effervescence, though it all melted clearly at 145°C. Comparing these figures with the melting point of formylfurylalanine (VII), 144—146°C, we suspected that the product obtained here might be formylfurylalanine contaminated with a compound decomposing at about 120°C, probably VI, a precursor of formylfurylalanine. In order to verify this assumption, the product was heated at 130°C for 5 hr. Then it was observed that it melted at 146°C without preliminary effervescence and showed a good agreement with an authentic sample of formylfurylalanine prepared by the formylation of furylalanine (as ascertained by a mixed melting point determination and by a comparison of the infrared spectra).

When the refluxing period was elongated to 6 hr., the hydrolysate gave plenty of white precipitates at pH ca. 5.6 (72% yield), which showed a positive ninhydrin reaction and which could be formylated with formic acid and acetic anhydride, the product of which, in turn, afforded formylfurylalanine when heated at 120°C for about 3 hr. From these results we assumed this compound to be amino-(2-furfuryl)-malonic acid (VIII), though, unfortunately, we did not succeed in ascertaining this by analysis. Schlossberger and Kuch[14] have also obtained an intermediate analogous to ours during the synthesis of tryptophan derivatives.

The decarboxylation of the substituted malonic acid (VIII) was attempted by heating it in water. The reaction was found to be very slow, even under prolonged refluxing, but it proceeded smoothly at 190°C in an autoclave. The paper chromatogram of the reaction product showed its almost complete conversion into furylalanine, though a faint spot was still

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[13] N. F. Albertson, S. Archer and C. M. Suter, *J. Am. Chem. Soc.*, **66**, 500 (1944); H. R. Snyder and C. W. Smith, ibid., **60**, 350 (1944).

[14] H. G. Schlossberger and H. Kuch, *Ann.*, **662**, 132 (1963).

observed near the position corresponding to the starting material even after 6 hrs' heating. By the elongation of the reaction time, this spot became more distinct, and refluxing it with hydrochloric acid could not extinguish it. Those facts indicate that this spot does not respond to the original malonic acid derivative (VIII), but to some further degradated product. The comparison of its R_f-value with that of DL-aspartic acid proved the identity of the two compounds. It is probable, as will be seen later, that part of the furylalanine produced by the decarboxylation is oxidized further at the furan ring during the reaction into aspartic acid.

In the investigation of the tryptophan synthesis, Schlossberger14) has used 50% acetic acid and obtained good results in the decarboxylation of aminomalonic acid derivatives with an acid labile indole nucleus. Thus, the decarboxylation was carried out according to his method, that is, by heating it in 50% acetic acid, because furylalanine itself was found, in a preliminary test, to be stable under such conditions. The reaction proceeded very rapidly without any formation of aspartic acid; thus we could obtain furylalanine in an excellent yield (80%).

In order to obtain formylfurylalanine, an intermediate for the peptide synthesis, it will be enough to hydrolyze the ester V into VI, reserving the formyl group, and to decarboxylate the reaction product. Thus V was stirred with a 10% sodium hydroxide solution for 8 hr. at room temperature, and then the product was heated at 130°C for 5 hr.; we thus succeeded in obtaining formylfurylalanine almost quantitatively.

The method of deformylating formylfurylalanine was also investigated. In general, the formyl-protecting group is removed by either oxidative cleavage or acid solvolysis. The former method was found, however, not to be applicable to furylalanine, for furylalanine, when treated with hydrogen peroxide even under mild conditions, is oxidized into DL-aspartic acid very rapidly; this was confirmed by a mixed melting point determination, by a comparison of the infrared spectra, and by paper electrophoresis with an authentic sample. The acid hydrolysis with hydrochloric acid in dioxane at room temperature was attempted, but it was also found to be impractical because of its low yield. With hydrochloric acid in methanol, deformylation and esterification occurred simultaneously and the methyl ester of furylalanine, also an important intermediate in the peptide synthesis, was obtained in one step, but the yield was not as satisfactory. On the other hand, the hydrolysis with alkali or 50% acetic acid gave good results; the yields were 59% and 63% respectively.

An attempt to obtain furylalanine in one step from V by treating it with 50% acetic acid proved unpromising. The reaction was too slow and not complete even after 20 hr.

These results described above are summarized in the scheme.

Experimental

DL-β-(2-Furyl)-α-alanine (I) by the Rhodanine Method.—According to Gränacher,9) rhodanine15) was condensed with furfural to 5-furfulidenerhodanine (m. p. 2282—28.5°C) in a 89% yield, and was then hydrolyzed with 3 N sodium hydroxide to β-(2-furyl)-α-thioketopropionic acid (m. p. 113—116°C) in a 99% yield. The thioketoacid was then converted by the action of hydroxylamine into β-(2-furyl)-α-oximino-propionic acid (m. p. 138—140°C) in a 56% yield. The reduction of the oximino acid with lactic acid and the sodium-amalgam afforded I in a 64% yield; m. p. 251—252°C (from 80% ethanol); lit.9) m. p. 252°C (decomp.). $R_f=0.60—0.64$ (circular paper chromatography, *n*-butanol - acetic acid - water (4 : 2 : 1) system). Furylalanine showed a characteristic color reaction by ninhydrin on paper (greenish or reddish gray at first, but gradually changing to gray).

DL-β-(2-Furyl)-α-alanine (I) by the Formamidomalonate Method.—*α-Furfurylchloride (III).*—The chloride was prepared from furfuryl alcohol and thionyl chloride in a 57% yield; b. p. 49—50°C/27 mmHg; lit.16) b. p. 49.6—50°C/27 mmHg. The chloride was so unstable that it was used at once after distillation.

Diethyl Formamidomalonate (IV).—According to Shaw and Nolan,17) diethyl malonate was converted by the action of sodium nitrite and acetic acid to diethyl oximinomalonate in a 89% yield, then isolated as an addition compound with sodium acetate, and finally reduced with zinc and formic acid to diethyl formamidomalonate in a 79% yield; m. p. 51—53°C; lit.17) m. p. 53—54°C.

Diethyl Formamido-(2-furfuryl)-malonate (V).—IV (20.5 g.) was dissolved in a sodium ethoxide solution (absolute ethanol 100 ml., sodium 2.3 g.), into which III (12 g.) was then added at once. After the mixture had been refluxed for 2 hr., sodium chloride was filtered out and washed with hot ethanol. The combined ethanolic solution was concentrated in vacuo. Water (100 ml.) was added to the residue, and the mixture was left to stand overnight in a refrigerator. Brown needle crystals (22.8 g.) were filtered off and recrystallized from hot ethanol; yield 18 g. (63%). Slightly brown needles; m. p. 98—99.5°C (from aqueous ethanol).

Found: C, 55.20; H, 5.97; N, 4.98. Calcd. for $C_{13}H_{17}O_6N$; C, 55.12; H, 6.05; N, 4.95%.

***N*-Formyl-DL-β-(2-furyl)-α-alanine (VII).**—*a) From Furylalanine (I).*—Acetic anhydride (16.6 ml.)

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16) W. R. Kirner, *J. Am. Chem. Soc.*, **50**, 1955 (1928).

17) K. N. F. Shaw and C. Nolan, *J. Org. Chem.*, **22**, 1668 (1957).

was added drop-by-drop into a solution of I (3.1 g.) in 88% formic acid (5 ml.) at 50—60°C. After it had been stirred at room temperature for 2 hr., the mixture was diluted with ice water (16 ml.) and concentrated in vacuo. The crystalline residue was then dissolved again in water, decolorized and, after a small amount of hydrochloric acid had been added, extracted with ethyl acetate. White crystals were obtained on evaporating the solvent; yield 2 g. (55%); m. p. 143—145°C. Recrystallized from ethyl acetate-petroleum ether; m. p. 144—145°C.

Found; C, 52.29; H, 4.88; N, 7.63. Calcd. for $C_8H_9O_4N$: C, 52.46; H, 4.95; N, 7.65%.

(b) From Diethyl Formamido-(2-furfuryl)-malonate (V).—V (52 g.) was suspended in 10% sodium hydroxide (270 ml.) and stirred at room temperature. After 5 hr. all the crystals were dissolved. Stirring was continued for another period of 3 hr., and then the solution was left to stand overnight. After the unreacted material had been extracted with ethyl acetate, the solution was made strongly acidic with concentrated hydrochloric acid and extracted with ethyl acetate. After the solvent had been evaporated, the residue was heated at 120—130°C for 5 hr. and the resultant product was dissolved in ethanol and decolorized. White crystals were obtained on evaporating off the solvent; yield 32 g. (95%); m. p. 143.5—145.5°C. Recrystallized from ethyl acetate, m. p. 144.5—146°C. The identity of this compound with that obtained by a) was confirmed by a mixed melting point determination and by a comparison of the infrared spectra.

When hydrolysis was carried out by refluxing with 10% sodium hydroxide, the yield was decreased with the elongation of the reaction time as follows; 0.5 hr., 65%; 1 hr., 50%; 2 hr., 39%; 3 hr., 16%.

Amino-(2-furfuryl)-malonic Acid (VIII).—V (358 g.) was refluxed with a 10% sodium hydroxide solution (1900 ml.) for 6 hr. After the unreacted V had been removed by extraction with ethyl acetate, the solution was acidified with hydrochloric acid. A white precipitate was obtained at pH ca. 5.6; yield 180 g. (72%); m. p. 285—288°C (from aqueous ethanol). $R_f=0.48$.

When this compound (10 g.) was formylated by the usual method with 85% formic acid and acetic anhydride, crystals (4 g.) melting at ca. 120—135°C with effervescence were obtained. After they had been heated at 120°C for several hours, the crystals melted at 143—145°C; they showed no depression of melting point when mixed with authentic formylfurylalanine.

DL-β-(2-Furyl)-α-alanine (I).—*a) From Amino-(2-furfuryl)-malonic Acid (VIII).*—i) VIII (3 g.) was dissolved in water and heated at 190°C for 6 hr. in an autoclave. After being decolorized and concentrated to dryness, the product was recrystallized from aqueous ethanol; yield 1.5 g. (64%); m. p. 244—247°C.

ii) VIII (20 g.) was refluxed with 50% acetic acid for 2.5 hr. and then concentrated to dryness after decolorization. The residue was dissolved in water, and a small amount of ethanol was added. On cooling, colorless crystals were separated; yield 12.5 g. (80%).

b) From N-Formyl-DL-β-(2-furyl)-α-alanine (VII).—i) VII (1 g.) was boiled with 25% sodium hydroxide (2 ml.) for 10 hr. After being decolorized and neutralized with hydrochloric acid, the mixture was filtered and the filtrate was made to have a pH value of 5 with hydrochloric acid. When the solution was cooled and a small amount of ethanol added, white crystals were obtained; yield 0.5 g. (59%).

ii) VII (1 g.) was boiled with 50% acetic acid (3 ml.) for 4 hr. and treated as above; yield 0.53 g. (63%); m. p. 248—250°C.

The identity of both the compounds with authentic furylalanine was confirmed by the mixed melting point determination of the purified samples and by a comparison of the R_f values in paper chromatography.

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