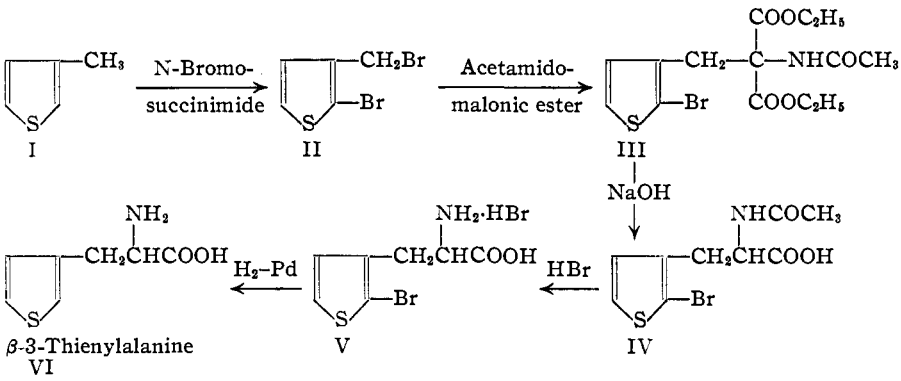
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

The Synthesis and Microbiological Properties of β-3-Thienylalanine, a New *anti*-Phenylalanine[1,2]

BY KARL DITTMER

That β-2-thienylalanine is an antagonist of phenylalanine has been demonstrated by du Vigneaud and co-workers.[3] This inhibitory physiological action is brought about by the interchange of a vinylene group for an aromatic sulfide, a structural change which has also produced antagonists of nicotinic acid[4] and thiamine.[5]

As part of a large research program designed to further establish what structural changes consistently yield metabolite antagonists and what changes do not, the preparations of other heterocyclic amino acids related to phenylalanine have been completed in this Laboratory. Clark and Dittmer[6] found that β-2-furylalanine is about one-third as good a phenylalanine antagonist as β-2-thienylalanine. Herz, Dittmer and Cristol[7] prepared β-2-pyrrylalanine and, although not obtained in pure form, found that it is an antiphenylalanine.



Since all of these β-2-heterocyclic alanines were active as antagonists of phenylalanine it seemed important to determine what influence the position of the alanine side chain would have on the antiphenylalanine activity of the thiophene analog. To determine which of the two possible isomers of thienylalanine is the better antagonist, the β-3-thienylalanine was prepared by the reactions.

During a study of the bromination of 3-methylthiophene with N-bromosuccinimide, 2-bromo-3-(bromomethyl)-thiophene (II) was obtained in good yield.[8,9] The condensation with diethyl acetamidomalonate resulted in a 73% yield of diethyl (2-bromo-3-thenyl)-acetamidomalonate (III). Pure samples of β-(2-bromo-3-thienyl)-alanine hydrobromide (V) were most easily obtained if the condensation product (III) was converted to the water insoluble N-acetyl intermediate (IV) which was then hydrolyzed with hydrobromic acid to yield V. The removal of the bromine with hydrogen in the presence of palladium catalyst on charcoal according to the method of Mozingo and co-workers[11] was tried on compounds III, IV and V. Best results were obtained when the bromine was removed from β-(2-bromo-3-thienyl)-alanine hydrobromide (V).

The structure of β-3-thienylalanine (VI) could be inferred by its synthesis from 3-methylthiophene (I), it was strengthened by analysis, and established by oxidizing it with alkaline permanganate to 3-thiophenecarboxylic acid which showed no depression of the melting point with an authentic sample of 3-thiophenecarboxylic acid.

β-3-Thienylalanine was tested for its inhibitory action on the growth of *Saccharomyces cerevisiae*, strain 139, and an unidentified strain of *Escherichia coli*.

Experimental

N-Acetyl-β-(2-bromo-3-thienyl)-alanine (IV).—Partial hydrolysis of 8.66 g. (0.022 mole) of diethyl (2-bromo-3-thenyl)-acetamidomalonate (III), prepared as previously described,[8] was accomplished by heating under reflux for four hours with 50 ml. of 10% sodium hydroxide.[12] The reaction mixture was acidified to *p*H 3 with hydrochloric acid and heated for one hour. It was treated with Darco, filtered and cooled overnight. The yield of fairly pure N-acetyl-β-(2-bromo-3-thienyl)-alanine (IV) was 2.72 g. (45%). Recrystallization from water yielded a pure crystalline product, m. p. 148–149°.

(1) This work was supported in part by a research contract with the Office of Naval Research.

(2) Presented in part at the 113th meeting of the American Chemical Society at Chicago, April, 1948.

(3) du Vigneaud, McKennis, Simmonds, Dittmer and Brown, *J. Biol. Chem.*, **159**, 385 (1945).

(4) Erlenmeyer, Block and Kiefer, *Helv. Chim. Acta*, **25**, 1066 (1942).

(5) Woolley and White, *J. Exp. Med.*, **78**, 489 (1943).

(6) Clark and Dittmer, *J. Biol. Chem.*, **173**, 313 (1948).

(7) Herz, Dittmer and Cristol, THIS JOURNAL, **70**, 504 (1948).

(8) Dittmer, Martin, Herz and Cristol, *ibid.*, **71**, 1201 (1949).

(9) Early in the bromination studies we obtained primarily 2-bromo-3-(bromomethyl)-thiophene which was employed in the synthesis of β-3-thienylalanine here described. Through a personal communication we learned that Campaigne synthesized β-3-thienylalanine from 3-thenyl bromide. We have likewise obtained β-3-thienylalanine by that more convenient method.[10]

(10) Campaigne, Bourgeois, Garst, McCarthy, Patrick and Day, THIS JOURNAL, **70**, 2611 (1948).

(11) Mozingo, Harris, Wolf, Hoffhine, Easton and Folkers, *ibid.*, **67**, 2092 (1945).

(12) Snyder, Shekleton and Lewis, *ibid.*, **67**, 310 (1945).

Anal. Calcd. for $C_9H_{10}O_3BrNS$: N, 4.79; neut. equiv., 292. Found: N, 4.59; neut. equiv., 289.

β-(2-Bromo-3-thienyl)-alanine Hydrobromide (V).—With vigorous stirring 3.6 g. (0.012 mole) of IV and 150 ml. of 10% hydrobromic acid solution were heated for four hours. The hydrobromic acid and water were removed *in vacuo*. The residue was dissolved in ethyl alcohol; the solution was treated with Darco and filtered; and ethyl acetate was added to induce crystallization. Upon cooling and the addition of more ethyl acetate, 2.42 g. (61% yield) of white crystalline hydrobromide (V) was obtained.

Anal. Calcd. for $C_7H_9Br_2O_2NS$: N, 4.23; neut. equiv., 331. Found: N, 4.18; neut. equiv., 330.

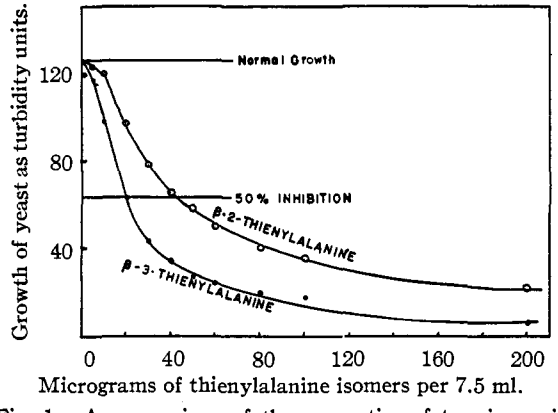
β-3-Thienyl-DL-alanine (VI).—To 2 g. of reduced palladium on charcoal catalyst in 30 ml. of 50% aqueous methanol was added 1.05 g. (0.0032 mole) of β-(2-bromo-3-thienyl)-alanine hydrobromide (V). The catalyst was prepared and reduced at 60 cm. of mercury pressure according to Mozingo and co-workers.[11] During the first ten minutes of shaking at room temperature 64 ml. (STP) of hydrogen at 1.6 atmospheres pressure was absorbed which represents 90% of the theoretical amount required to debrominate V. After shaking another hour the theoretical amount of hydrogen was used. The catalyst was removed by filtration. The filtrate was concentrated to dryness *in vacuo*, dissolved in 5 ml. of water and again taken to dryness. The β-3-thienylalanine hydrobromide was dissolved in 10 ml. of absolute ethanol; the solution was treated with Darco, filtered, and the filtrate was exactly neutralized with 10 *N* ammonium hydroxide. Crystals began to form almost immediately and after cooling, 330 mg. (61% yield) of β-3-thienylalanine (VI) was collected. This sample gave a negative Beilstein test and a positive ninhydrin reaction. β-3-Thienylalanine was recrystallized by dissolving in a small amount of absolute ethanol containing a slight excess of hydrobromic acid. After the solution had been treated with Darco the hydrobromic acid was exactly neutralized with 10 *N* aqueous ammonium hydroxide. The crystals were washed with absolute alcohol and dried, melting point 244–248° in a capillary when placed into the bath at 240°.

Anal. Calcd. for $C_7H_9O_2NS$: C, 49.10; H, 5.30; N, 8.18. Found: C, 48.98; H, 5.65; N, 8.34.

The position of the side chain was established by oxidizing the β-3-thienylalanine to 3-thiophenecarboxylic acid. To 40 ml. of water containing 1.8 g. of sodium hydroxide was added 256 mg. (0.0015 mole) of β-3-thenylalanine and 1.0 g. of potassium permanganate. The reaction mixture was heated to boiling and kept warm for two hours. After cooling, the manganese dioxide was removed by filtration and the filtrate was acidified and extracted with ether. The ether solution was dried with sodium sulfate and then concentrated to a small amount of oil which soon crystallized. The solid was dissolved in 1 ml. of hot water; the hot solution was treated with Darco, filtered and cooled overnight. The yield of 3-thiophenecarboxylic acid was 110 mg. (57%), m. p. 137–138°. Mixed melting point with an authentic sample of 3-thiophenecarboxylic acid, was 137–138°, whereas, when mixed with 2-thiophenecarboxylic acid the melting point was 120–124°.

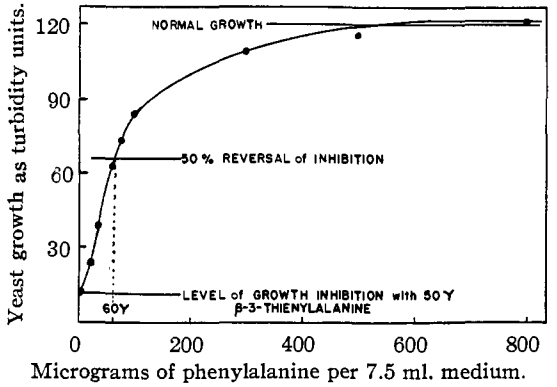
Microbiological Tests.—The methods of comparing the inhibitory properties of β-3-thienylalanine with the antiphenylalanine properties of β-2-thienylalanine on the growth of *Saccharomyces cerevisiae*, strain 139, and on an unidentified strain of *Escherichia coli* were the same as previously described.[6]

The curve in Fig. 1 illustrates the relative potency of the two thienylalanines as growth inhibitors of *S. cerevisiae*. It will be observed that 20.0 micrograms of β-3-thienyl-DL-alanine per 7.5 ml. of medium inhibited growth to 50% of normal, whereas for the same degree of inhibition 42.5 micrograms of β-2-thienyl-DL-alanine were required. Figure 2 illustrates that phenylalanine completely reversed the toxicity of β-3-thienylalanine. In addition to phenylalanine, tryptophan, leucine, isoleucine and valine also reversed the toxicity of β-3-thienylalanine. These are the same amino acids which nullified the toxicity of β-2-thienylalanine.[13] β-(2-Bromo-3-thienyl)-alanine (V) did not inhibit the growth of yeast when tested up to a concentration of 1 mg. per 7.5 ml.



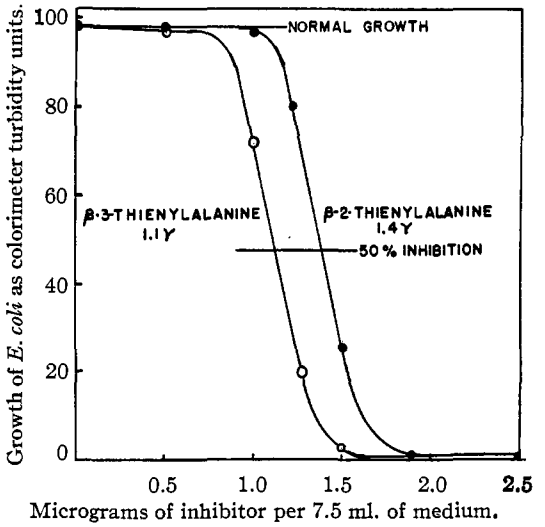
Micrograms of thienylalanine isomers per 7.5 ml.

Fig. 1.—A comparison of the properties of two isomeric thienylalanines as yeast growth inhibitors.



Micrograms of phenylalanine per 7.5 ml. medium.

Fig. 2.—Nullification of the β-3-thienylalanine inhibition of the growth of *S. cerevisiae*.



Micrograms of inhibitor per 7.5 ml. of medium.

Fig. 3.—A comparison of the properties of two isomeric thienylalanines as inhibitors of *E. coli*.

(13) Dittmer, Ellis, McKennis and du Vigneaud, *J. Biol. Chem.*, **164**, 761 (1946).

When increasing amounts of β-3-thienyl-DL-alanine were added to the medium in which *E. coli* grew well, it completely inhibited the growth and was about 30% more effective than β-2-thienylalanine. This is illustrated by the data of Fig. 3. The toxicity on the growth of *E. coli* was also prevented by phenylalanine.

Discussion

The results of previous work[3,6,7,13] show that the replacement of the benzene ring in phenylalanine by the thiophene, furan or pyrrole ring results in the formation of antagonists of phenylalanine. Of these three antagonists the thiophene analog was probably the most active. The results described in this paper indicate that when the alanine side chain is in the 3-position the antagonist is more potent than if it is in the 2-position. These results suggest that if the best antagonist of a compound which contains a phenyl ring is to be designed it would be most likely obtained by replacing the phenyl group by a thiophene ring with the side chain in the 3-position.

Acknowledgment.—The author wishes to acknowledge the very excellent assistance of Mr. Robert P. Martin in the chemical phase of this work, and of Mrs. Charmion McMillan and Mrs. Virginia Janda for the microbiological tests. The analyses were made by Mrs. Philippa Szerwo of this department. The author also wishes to thank Mr. G. A. Harrington of the Socony-Vacuum Oil Company for a generous gift of 3-methylthiophene.

Summary

β-3-Thienyl-DL-alanine was synthesized by alkaline hydrolysis of diethyl (2-bromo-3-thenyl)-acetamidomalonate, yielding N-acetyl-β-(2-bromo-3-thienyl)-alanine. The N-acetyl derivative was converted to the β-(2-bromo-3-thienyl)-alanine hydrobromide by acid hydrolysis. The bromine was removed by hydrogenation in the presence of palladium on charcoal catalyst, yielding pure β-3-thienylalanine.

This new isostere of phenylalanine was tested for its inhibitory properties on the growth of *Saccharomyces cerevisiae*, strain 139, and on an unidentified strain of *Escherichia coli*. For yeast, the β-3-thienylalanine was about twice as active as β-2-thienylalanine. It was about 30% more effective against *E. coli*. In each instance the toxicity was reversed by phenylalanine.

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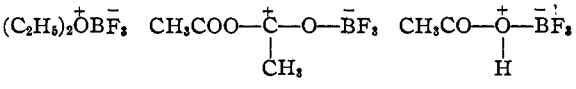
Further Studies in the Acylation of Thiophene and Furan in the Presence of Boron Fluoride Complexes[1]

BY ROBERT LEVINE, JOHN V. HEID[2] AND MARTIN W. FARRAR

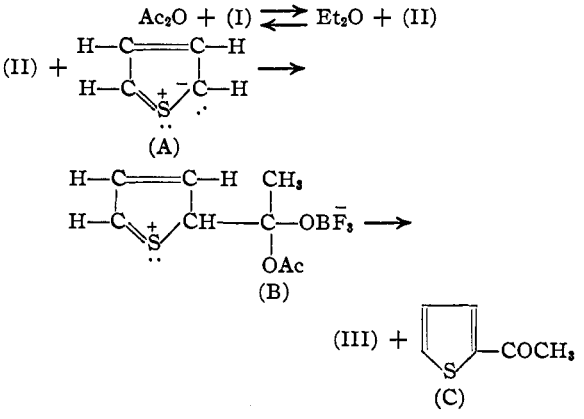
In an earlier paper[3] from this Laboratory it was reported that high yields of 2-thienyl and 2-furyl ketones are obtained when thiophene and furan are acylated with anhydrides in the presence of catalytic amounts of boron fluoride etherate as the condensing agent.

In the present work, we were interested in investigating the course of these acylations. In this discussion, the boron fluoride complexes will be referred to as follows



(I)	(II)	(III)
Etherate	Complex with acetic anhydride	Complex with acetic acid

The following scheme indicates a possible reaction mechanism whereby catalytic amounts of (I) could result in high yields of the heterocyclic ketones. The acylation of thiophene with acetic anhydride is taken as an example.



The first step in the reaction probably involves the interaction between acetic anhydride and (I) to give (II). This reactive intermediate then condenses with thiophene (A), to give the complex of (II) and thiophene (B), which then decomposes to produce (III) and 2-thienyl methyl ketone (C). We postulate that the acylating agent is (II) and the actual condensing agent (the catalyst) is (III). As this complex of acetic acid and boron fluoride (III) is formed it functions as (I) did in the first step of the reaction and is con-

(1) Presented before the Organic Division of the American Chemical Society, St. Louis Meeting, September, 1948.

(2) Present address: Mellon Institute, Pittsburgh, Pa.

(3) Heid and Levine, *J. Org. Chem.*, **13**, 409 (1948).