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REACTION OF 2-AMINOBENZENETHIOL WITH ACYL-*dl*-ASPARTIC ANHYDRIDES. 2-(2-BENZOTHIAZOLYL)ALANINE

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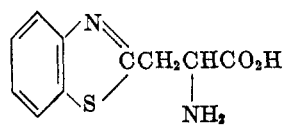
In recent years intensive research has been carried out in the preparation of antimetabolites of amino acids with the eventual goal of finding useful drugs (1, 2, 3, *inter alia*). Our own work in this field stemmed from the related observations that: (a) 2,6-diaminobenzothiazole (4), 5-aminobenzothiazole (5), and 2-mercaptobenzothiazole (6) possessed varying degrees of antitubercular activity; and (b) that analogs of *beta*-phenylalanine were effective in inhibiting the growth of virulent *Mycobacterium tuberculosis* (7). These observations, coupled with our own interest in benzazoles (8), prompted us to prepare *beta*-(2-benzothiazolyl)alanine (I).



I

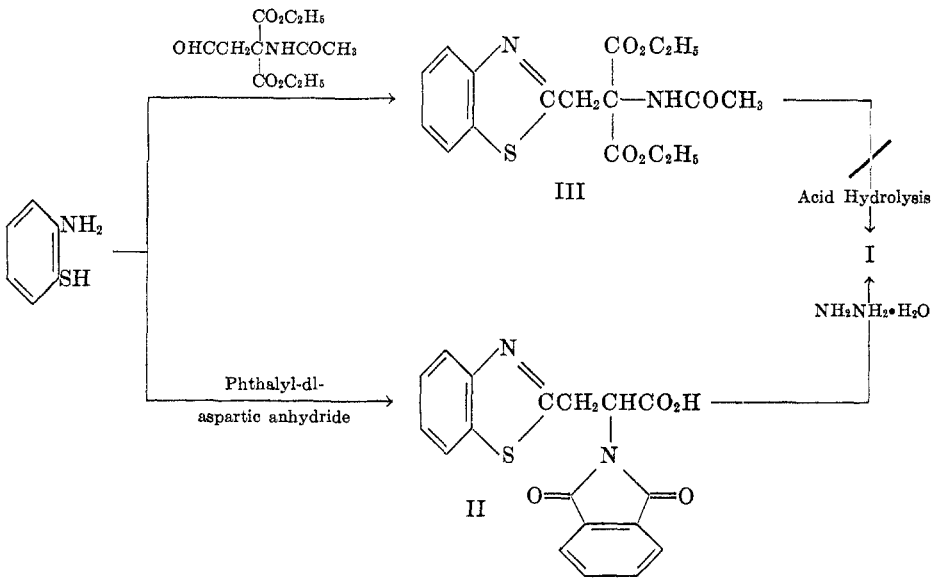
Since none of the standard amino acid syntheses appeared to be adaptable to the ready synthesis of I, two other synthetical routes were explored. The preparative methods shown diagramatically in Flow Sheet A depend upon the fact that 2-aminobenzenethiol reacts facilely with acid anhydrides, aldehydes and the like to form either benzothiazoles or benzothiazolines. The latter derivatives are easily converted to benzothiazoles by mild oxidation.

Considerable research has been carried out dealing with the ring scission of acylaspartic anhydrides by amines and ammonia (8). The inconsistencies which appear in the literature describing the isolation of both *alpha*- and *beta*-amides may well be explained by the excellent germane work of Tanenbaum (9) who demonstrated that either asparagine or isoasparagine could be obtained from phthalyl-*dl*-aspartic anhydride depending on the reaction conditions employed. King and Kidd (10) had earlier described the synthesis of *dl*-asparagine from phthalyl-*dl*-aspartic anhydride under anhydrous conditions and a *beta*-anilide from the same intermediate. Lettré, Fritsch, and Porath (11) employed carbobenzoxy-*dl*-aspartic anhydride in a condensation reaction with *o*-phenylenediamine and upon subsequent hydrogenolysis isolated *beta*-(2-benzimidazolyl)-alanine.

In the present investigation the easily available phthalyl-*dl*-aspartic anhydride was caused to react with 2-aminobenzenethiol under anhydrous conditions and an 86% yield of II was realized. This material was an amorphous, slightly hygroscopic white powder which resisted several recrystallization attempts. Purification was accomplished by dissolution of II in sodium bicarbonate and

subsequent precipitation with dilute acid. Treatment of II with hydrazine hydrate resulted in a 70% yield of I, m.p. 224–225°.

FLOW SHEET A



The alternative synthesis involved the reaction of *beta*-acetamido-*beta*, *beta*-dicarbethoxypropionaldehyde with 2-aminobenzenethiol to give III. Unfortunately the yield of pure III was so small that further work was precluded when it was found that mild hydrolysis of III gave no identifiable product.

Inasmuch as 2-aminobenzenethiol contains both an acidic and a basic group and Tanenbaum postulated that the formation of normal or iso derivatives of aspartic acid depends upon the acidic or basic nature of the reactant, the determination of the structure of I was important.

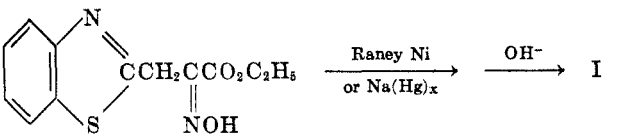
Compound I gave an orange to orange-red color with ninhydrin and was shown to be a single entity by paper chromatography. It was further demonstrated that II was a single substance and that a mixture of I and II was easily separable using the lower layer of a butanol-water-acetic acid (5:5:1.2) system. The *rf* values for I and II are listed in the following table:

	Rf Values *Butanol-Water-Acetic Acid*
I	0.58
II	0.87

In order to demonstrate that I was an *alpha*-amino acid, a variation of the method of Van Slyke (12) utilizing ninhydrin titration was employed. By this method the theoretical amount of *alpha*-amino group was obtained.[1]

After the completion of this work, Ried and Schiller (13) described the synthesis of I by reduction of the oximino ester (IV) but reported a melting point

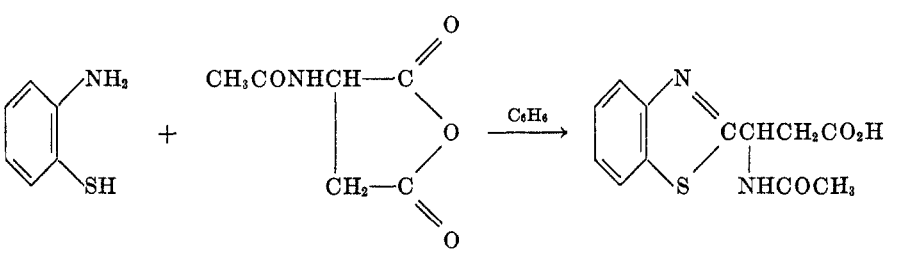
[1] This analysis was standardized by the Clark Microanalytical Laboratory.



IV

of 214°. Since this constituted an unequivocal synthesis of I, this work was repeated employing a zinc and acetic acid reduction in preference to the reduction methods of Ried and Schiller. A 53% yield of crude I was obtained; m.p. 210–212°. It is possible that the crude material is contaminated by benzothiazoline inasmuch as the melting point rises slowly to 224–225° upon recrystallization from water. Comparison of the two different preparations of I established their identity.

Treatment of I with acetic anhydride and sodium carbonate in water resulted in an N-acetyl derivative, m.p. 149–151°. This derivative was not identical with the compound, m.p. 214–215°, isolated in 80% yield from the reaction of 2-aminobenzenethiol with acetyl-*dl*-aspartic anhydride in benzene. It is thus apparent that V even under anhydrous conditions gave rise to at least an 80% yield of the *beta*-acetamido acid and differs markedly in behavior from the phthalyl-*dl*-aspartic anhydride which gave unilateral ring opening to produce the *alpha*-amino acid derivative.



V

Microbiological results. At a concentration of 200 mcg./ml. I was not effective in inhibiting the growth of *E. coli* A.T.C.C. 9662 (tryptophane requiring), *S. aureus* FDA 209, or *Leuconostoc mesenteroides* P-60. At a concentration of 100 mcg./ml. only partial inhibition of *M. tuberculosis*, H37RV was noted.

The author is grateful to Mr. James Burns for the paper chromatographic work and the microbiological results.

EXPERIMENTAL

Phthalyl-2-(2-benzothiazolyl-dl-aspartic acid. Into a 250-ml. flask fitted with a water separator were placed 4.9 g. (0.02 mole) of phthalyl-*dl*-aspartic anhydride, 2.5 g. (0.02 mole) of 2-aminobenzenethiol, and 100 ml. of benzene. This mixture was heated for 18 hours and the resultant yellow solution was cooled and decanted into 500 ml. of cyclohexane. The white amorphous solid was collected, dried, and dissolved in dilute sodium bicarbonate. This solution was treated with charcoal and the filtrate was acidified with dilute hydrochloric acid. The solid was collected, redissolved in 100 ml. of hot benzene, concentrated to 50 ml. and added to 300 ml. of cyclohexane. The white amorphous solid was collected and

dried to yield 6.1 g. (86%). Several attempts to crystallize this material were unsuccessful. The sample was purified by redissolving in benzene and precipitating with cyclohexane.

Anal. Calc'd for $C_{18}H_{12}N_2O_4S$: C, 61.36; H, 3.41.

Found: C, 61.46; H, 3.50.

2-(2-Benzothiazolyl)-dl-alanine. Phthalyl-2-(2-benzothiazolyl)-*dl*-alanine (5 g.) was dissolved in 2.1 ml. of 100% hydrazine hydrate and 20 ml. of water. The solution was cooled to maintain a temperature below 35° and filtered into a 50-ml. flask. After standing at room temperature for 3 hours, a white solid had precipitated. This solid was collected, suspended in water, and acidified to *p*H 6 with dilute glacial acetic acid. The white solid was collected and dried to give 1.28 g., m.p. 223–225°. The original filtrate was allowed to stand overnight at room temperature and was acidified to *p*H 2 with hydrochloric acid. This suspension was filtered and the filtrate was neutralized with solid sodium bicarbonate. After standing 12 hours at 0–5°, the crystalline material was collected to give an additional 0.83 g., m.p. 215–218°. The total yield was 2.11 g. (70%). A sample recrystallized from water separated as white needles, m.p. 224–225°, dec.

Anal. Calc'd for $C_{10}H_{10}N_2O_2S$: C, 54.05; H, 4.50.

Found: C, 54.27; H, 4.50.

An *alpha*-amino nitrogen determination was carried out by the Clark Microanalytical Laboratory and the following results were obtained: Calc'd: N (α), 6.29. Found: N (α), 6.23 and 6.27.

A blank run with *beta*-alanine gave no amino nitrogen using this technique.

For the paper chromatographic work a mixture of 500 ml. of *n*-butanol, 500 ml. of water, and 120 ml. of glacial acetic acid was prepared, shaken, and allowed to separate into two layers. The bottom layer was used in the chromatographic separation.

Diethyl (2-benzothiazolylmethyl)acetamidomalonate. Into a 100-ml. flask equipped for refluxing were placed 5.0 g. (0.02 mole) of *beta*-acetamido-*beta*, *beta*-dicarbethoxypropionaldehyde (15), 2.5 g. (0.02 mole) of 2-aminobenzenethiol, 4.0 g. of ammonium acetate, and 20 ml. of glacial acetic acid. This mixture was heated gently under reflux for 3½ hours, chilled to 10°, and poured into 200 ml. of water containing 10 ml. of hydrochloric acid. The resulting yellow oil was taken up in ether and the solvent was removed to yield 4.4 g. of oil which partially crystallized. This oil did not react with 2,4-dinitrophenylhydrazine. Inasmuch as this material still contained oil, it was triturated with a small amount of methanol and 3.4 g. of crystalline material was obtained. A sample was recrystallized from benzene-cyclohexane four times and then subjected to vacuum sublimation. This material, m.p. 169–170°, was sent for analysis.

Anal. Calc'd for $C_{17}H_{20}N_2O_5S$: C, 56.04; H, 5.49.

Found: C, 56.36; H, 4.84.

dl-2-(2-Benzothiazolyl)-N-acetylalanine. A solution of 1.1 g. (0.005 mole) of *dl*-2-(2-benzothiazolyl)alanine, 4.0 g. (0.04 mole) of sodium carbonate, and 25 ml. of water was prepared by heating gently to accomplish complete solution. The solution was chilled to 0°, clarified, and the filtrate treated with 2.0 g. (0.02 mole) of acetic anhydride. This mixture was shaken until the acetic anhydride had reacted and the resultant solution was acidified to *p*H 4 with concentrated hydrochloric acid. After standing at 0–5° of two hours the precipitate was collected to yield 870 mg. (67%); m.p. 147–149°. A sample recrystallized twice from a small amount of water separated as white needles; m.p. 149–152°.

Anal. Calc'd for $C_{12}H_{12}N_2O_3S$: C, 54.55; H, 4.55.

Found: C, 54.51; H, 4.58.

dl-2-Acetamido-2-(2-benzothiazolyl)propionic acid. A mixture of 4.0 g. (0.025 mole) of acetamido-*dl*-aspartic anhydride, 3.13 g. (0.025 mole) of 2-aminobenzenethiol, and 200 ml. of benzene was heated under reflux, using a water separator, for 5 hours. The solution was cooled and the solid was collected to yield 5.3 g. (80%) of white solid. A sample recrystallized from methanol melted at 214–215°.

Anal. Calc'd for $C_{12}H_{12}N_2O_3S$: C, 54.55; H, 4.55; N, 10.61.

Found: C, 54.55; H, 4.50; N, 10.60.

Zinc and acetic acid reduction of ethyl alpha-oximino-beta-(2-benothiazolyl)propionate. A solution of 2.5 g. of the oximino ester (14) in 25 ml. of acetic acid and 1½ ml. of water was heated to reflux and 3.5 g. of zinc dust was added portionwise over a period of five minutes. Refluxing was continued for an additional 20 minutes and then the hot supernatant solution was decanted from the residual zinc. After adding ice and water, the mixture was made slightly alkaline with sodium hydroxide and then shaken with ethyl acetate. The resulting emulsion was filtered to remove zinc hydroxide and the ethyl acetate was separated, washed with water, and dried over magnesium sulfate. Evaporation of the solvent on the steam-bath left a yellow oil which was treated with a hot solution of one gram of sodium hydroxide in 40 ml. of 50% methanol, and this in turn was evaporated to dryness on the steam-bath to leave crystals of the sodium salt. These crystals were dissolved in 50 ml. of water and treated with charcoal (Darco). Neutralization with acetic acid precipitated the product which was collected, washed with water, and dried. The yield was 1 g. (53%), m.p. 210–212°. Two recrystallizations from water raised the melting point to 222–224°. This material was identical with I prepared by the phthalyl-*dl*-aspartic anhydride method.

SUMMARY

The synthesis of 2-(2′-benzothiazolyl)alanine from 2-aminobenzenethiol and phthalyl-*dl*-aspartic anhydride is described.

Reaction of acetamido-*dl*-aspartic anhydride with 2-aminobenzenethiol gave rise to a derivative of *beta*-alanine.

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