general, a 4-aminopteridine may be readily hydrolyzed by mineral acid but is resistant to the action of nitrous acid. A 2-aminopteridine requires considerably more strenuous treatment for hydrolysis to take place but reacts readily with nitrous acid to form the corresponding hydroxy compound unless an amino group is also present in the 4-position. Several explanations for this behavior are suggested.

The synthesis and absorption spectra of two new pterins, 4-amino-2-hydroxypteridine and 4-amino-2-hydroxy-6,7-diphenylpteridine, are reported.

ITHACA, N. Y. RECEIVED FEBRUARY 21, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK & CO., INC., RAHWAY, N. J.]

A New Method of Resolution of DL-Threonine[1]

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In 1937 West and Carter[2] reported a synthesis of DL-threonine in which one of the intermediates is N-formyl-O-methyl-DL-threonine. This synthesis not only represents the first practical method for the preparation of the racemic amino acid, but also affords a satisfactory method for the preparation of its optically active isomers. These same investigators found that this intermediate could be resolved with brucine and that D- and L-threonine subsequently resulted upon hydrolysis of the optically active derivatives.

Recently a new and improved synthesis of DL-threonine has been developed.[3] However, since neither N-formyl-O-methyl-DL-threonine, nor any other such suitable derivative which might readily lend itself to resolution is an intermediate in this synthesis, it became desirable to investigate other methods which might lead to the direct resolution of DL-threonine. Since threonine is not readily converted to N-formyl-O-methyl-DL-threonine, Fischer's[4] method for the resolution of DL-serine was first studied. In this method N-*p*-nitrobenzoyl-D-serine is precipitated as the quinine salt from an alcoholic solution. After decomposing the more soluble quinine salt of the L-derivative with alkali, the L-isomer is precipitated with brucine. When the method was applied to DL-threonine, however, it was found to be generally unsatisfactory.

We wish to report a simple and direct method of resolving DL-threonine in which only brucine is used, and in which, in contrast to most resolutions of this type, the physiologically active L-form separates first. The separation of the optically active isomers is based upon the difference in solubility of the brucine salts of the N-*p*-nitrobenzoyl derivatives of D- and L-threonine in methanol. In an extended study of the solubility of these derivatives under varying conditions, an interesting phenomenon was observed. Whereas the brucine salt of N-*p*-nitrobenzoyl-L-threonine is an almost colorless, highly insoluble compound, the brucine salt of the D-isomer exists in two forms. When first prepared in methanol a soluble, low-melting, deep orange-colored salt is obtained which slowly changes to an insoluble, high-melting, light yellow product. Under the proper conditions of time and temperature this change is kept at a minimum thus affording an excellent separation of the more insoluble L-isomer.

Experimental

N-*p*-Nitrobenzoyl-DL-threonine.—To a solution of 32 g. (0.269 mole) of DL-threonine in 1200 cc. of water and 270 cc. of normal sodium hydroxide at 0°, was added with vigorous agitation over a period of one hour, a total of 50 g. (0.269 mole) of *p*-nitrobenzoyl chloride (freshly distilled, m. p. 72–74°) in equal portions at three-minute intervals. Simultaneously, over this period of time, 135 cc. of 2 *N* sodium hydroxide solution were added dropwise. The solution was stirred for an additional twenty minutes and then acidified to congo red with 40 cc. of concentrated hydrochloric acid. After cooling in an ice-bath for one hour, the crude *p*-nitrobenzoyl-DL-threonine was filtered, and without drying was extracted with 300 cc. of boiling water. The insoluble portion, *p*-nitrobenzoic acid, weighed 4.1 g. The product began to crystallize from the hot solution immediately, and after chilling at 0–5° for one hour was filtered, washed with two 75-cc. portions of ice-cold water and dried at 60°; m. p. 159–162°. For further purification, the 56.5 g. of crude threonine derivative was extracted twice with hot ether to remove additional quantities of *p*-nitrobenzoic acid. The yield of pure *p*-nitrobenzoyl-DL-threonine was 50.7 g. (70.5%); m. p. 166–167°.

Brucine Salt of *p*-Nitrobenzoyl-DL-threonine.—To a warm solution (50–55°) of 64 g. (0.149 mole) of brucine Merck dissolved in 160 cc. of methanol was added 40 g. (0.149 mole) of *p*-nitrobenzoyl-DL-threonine, and the mixture was heated until solution was complete. With rapid stirring and scratching, the solution was cooled in an ice-bath, whereupon crystallization took place within a short time. The cooling and stirring were continued for a total of five minutes, during which time a heavy, yellowish precipitate separated out, and the temperature dropped to 25°. The flask was reheated to 50° with stirring, and then cooled to 25° over a five-minute period as in the previous case. The brucine salt of *p*-nitrobenzoyl-L-threonine was filtered, and the small amount of product adhering to the flask was transferred with the mother liquors. After washing the cake thoroughly in a mortar with 60 cc. of cold methanol, the slurry was transferred back to the funnel and washed, again using approximately 60 cc. of methanol for this operation. As much of the wash liquor as possible was removed by suction, after which the product was finally washed with two 50-cc. portions of ether, and

(1) Throughout this paper the nomenclature is used in the amino acid sense. For the sake of brevity however the subscript s has been deleted: Vickery, *J. Biol. Chem.*, **169**, 237 (1947).

(2) West and Carter, *J. Biol. Chem.*, **119**, 109 (1937).

(3) Pfister, Robinson, Shabica and Tishler, THIS JOURNAL, **70**, 2297–2298 (1948); Attenburrow, Elliot and Penny, *J. Chem. Soc.*, 310 (1948).

(4) Fischer and Jacobs, *Ber.*, **39**, 2942 (1906).

these washes were collected in a separate receiver. The dried L-threonine salt, which was almost white in color, weighed 46 g. (88% yield) and melted at 145–150°.[5]

The combined filtrate and methanol washes (volume about 260 cc.) were allowed to stand at room temperature overnight, and then cooled in an ice-bath for two hours. The yellow brucine salt of *p*-nitrobenzoyl-D-threonine was filtered and dried without washing; weight 41 g. (78.5% yield); m. p. 190–192°.

By concentrating the mother liquors to a volume of 90 cc. *in vacuo*, scratching to induce crystallization, and cooling in an ice-bath for one hour, an additional 2 g. of the brucine salt of *p*-nitrobenzoyl-L-threonine (m. p. 147–150°) was obtained.

The resulting mother liquors and washes were again concentrated to a volume of 25 cc., whereupon on standing in the refrigerator for two hours an orange mass separated. After filtering, washing with a small amount of cold methanol and drying, 5 g. of the brucine salt of *p*-nitrobenzoyl-D-threonine was obtained.

The combined total yield of the brucine salt of *p*-nitrobenzoyl-L-threonine was 48 g. or 92%. The combined total yield of the brucine salt of *p*-nitrobenzoyl-D-threonine was 46 g. or 88.5%.

The brucine salts of the L-isomer were found to crystallize with two molecules of water.

Anal. Calcd. for $C_{34}H_{38}O_{10}N_4 \cdot 2H_2O$: C, 58.49; H, 6.07; N, 8.05. Found: C, 58.11; H, 6.44; N, 8.24.

L-Threonine.—To a mixture of forty-eight grams of the brucine salt of *p*-nitrobenzoyl-L-threonine in 500 cc. of water at 40–50° was added slowly with vigorous stirring 80 cc. of *N* sodium hydroxide solution, and the solution was stirred for an additional hour under the same conditions. After chilling in an ice-bath for thirty minutes, the brucine was collected by filtration and washed thoroughly with two 100-cc. portions of ice water. The recovered dried brucine weighed 26.5 g. Traces of brucine remaining in the mother liquors and washes were removed by extracting the combined solutions with 150 cc. of chloroform and 150 cc. of ether. The aqueous solution containing the sodium salt of *p*-nitrobenzoyl-L-threonine was concentrated *in vacuo* to a volume of 80 cc., and then refluxed for four hours with 56 cc. of constant boiling hydrobromic acid. After storing the mixture in the refrigerator overnight, the *p*-nitrobenzoic acid was filtered, washed with two 50-cc. portions of cold water and dried. The calculated amount of acid (11.7 g.) was recovered. The mother liquors, combined with the washings, were concentrated to a sirup *in vacuo*, redissolved in 50 cc. of water, and again concentrated *in vacuo*. The resulting sirup was warmed on the steam-bath with 50 cc. of absolute ethanol until all particles of oil were dissolved, and only the white, insoluble sodium bromide remained. To insure as complete removal of water and hydrobromic acid as possible, the solution was again concentrated and the resultant oil was finally dissolved in 100 cc. of absolute ethanol, after which the solution was treated with 0.2 g. of Darco G-60 and filtered. The residue was washed with a total of 40 cc. of cold absolute ethanol. To the filtrate and washings, warmed to 50°, were added 20 cc. of concentrated ammonium hydroxide, whereupon the crude L-threonine precipitated immediately. After chilling for fifteen minutes in an ice-bath, the product was filtered and washed with ethanol. The crude material (weight 7.0 g.) gave a slight test for bromide ion. It was dissolved in 30 cc. of hot water and treated with 0.2 g. of charcoal, and after filtration the charcoal cake was washed with 5 cc. of hot water. To the warm filtrate (50–55°) a total of 140 cc. of absolute ethanol was added slowly with stirring. The solution was allowed to stand at room temperature for fifteen minutes and then placed in an ice-bath for the same length of time. After filtering, washing with ethanol, and drying, pure L-threonine was obtained as brilliantly white leaflets. The yield was 6.25 g. This represents an over-all yield of 49.5% based on the DL-threonine used: $[\alpha]^{26}D$ −27.9° (5% aqueous solution); purity by solubility data, 99.7 ± 0.1%.

D-Threonine.—Using the same procedure as described above, pure D-threonine was obtained in an over-all yield of 43.6%. From 46 g. of the brucine salt of *p*-nitrobenzoyl-D-threonine, 5.88 g. of crude product was obtained. The recrystallized material weighed 5.5 g., $[\alpha]^{26}D$ +27.8°; purity by solubility data, 99.7 ± 0.1%.

Acknowledgment.—We wish to thank Dr. Max Tishler for invaluable advice and suggestions throughout the course of this study and Mr. F. A. Bacher for the phase solubility analyses.

Summary

A resolution of DL-threonine in satisfactory yield is reported. Because of the high solubility of an unstable form of the brucine salt of *p*-nitrobenzoyl-D-threonine, the physiologically active L-form is obtained first.

RAHWAY, N. J. RECEIVED JANUARY 25, 1949

(5) Two factors, time and temperature, are very critical in this method of resolution. Advantage is taken of the difference in solubility in methanol of the brucine salts of L- and D-*p*-nitrobenzoyl derivatives. This difference in solubility becomes smaller as the soluble orange form of the *p*-nitrobenzoyl-D-threonine salt slowly changes over to an insoluble light yellow form. This change is affected by temperature and length of time of standing. The volume should be kept to a minimum so that the L-salt will precipitate rapidly. It may then be removed before a significant quantity of the soluble form of the D-salt has had an opportunity to be converted to the insoluble form.