Synthesis of N-Acetyl-O-benzyl-DL-threonine and O-Benzyl-L-threonine

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Difficulties are encountered in the synthesis of the peptides containing serine or threonine owing to the presence of the hydroxyl group that induces an unexpected side reaction during the synthetic procedure.

In order to avoid an undesirable side reaction, one of the authors[1] had already synthesized *O*-benzyl-L-serine in which the free hydroxyl group was protected with the benzyl rest.

In this communication, the authors will describe the synthesis of the *O*-benzyl-L-threonine from *N*-acetyl-DL-threonine, which was prepared by the direct acetylation of the DL-threonine with the acetic anhydride method.

Synthesis of *N*-Acetyl-DL-threonine [II].—DL-Threonine (12 g.) was dissolved in 200 ml. of an aqueous solution containing sodium carbonate (13.5 g.). Into the above solution was added dropwise acetic anhydride (12 g.) under vigorous stirring at +5°C. After the addition was over, the stirring was continued for about 30 min. at room temperature. When the reaction was over,

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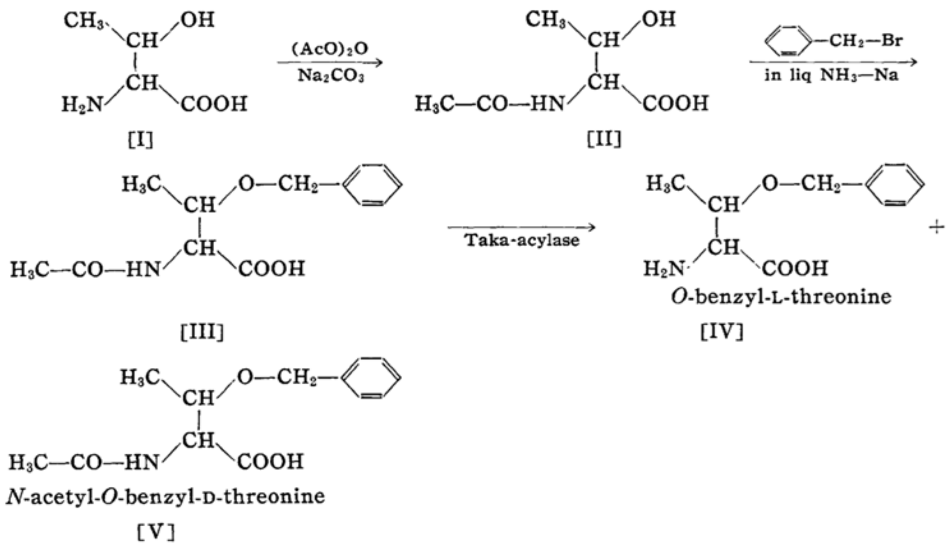
[1] K. Okawa, This Bulletin, **29**, 486 (1956).



about 100 ml. of Dowex 50 (H-form) was added to the reaciton mixture under stirring until the evolution of the carbon dioxide ceased. The resulting mixture was passed through the Dowex 50 (H-form) ion exchange column (7 $cm^2 \times 30$ cm.). By elution with water, the effluent was collected and concentrated in vacuo. The resulting syrupy materials were crystallized with ethylacetate. The crude products weighed 14 g., 87% yield. The recrystallization from ethylacetate containing alcohol gave a pure material (12.9 g.) in 80% yield, m. p. 132∼133°C.

Anal. Found: C, 44.73; H, 6.96; N, 8.78. Calcd. for $C_6H_{11}O_4N$: C, 44.75; H, 6.88; N, 8.70%.

N-Acetyl-O-benzyl-DL-threonine [III]. — *N*-Acetyl-DL-threonine (8.5 g.) was dissolved in liquid ammonia (200 ml.), followed by portionwise addition of methallic sodium (2.3 g.) at −60°C. After benzylbromide (18 g.) was added into the above solution, the reaction vessel was taken out from the cooling Dewar's bottle; then the temperature of the reaction mixture raised to −28°C and ammonia was gradually evolved off at this temperature for 2.5 hr. The resulting mixture was dissolved in an aqueous solution of sodium bicarbonate, the aqueous solution extracted with ether being acidified with 6 N hydrochloric acid. The crystals produced were extracted with ethylacetate, and the organic layer was washed with water and dried. By removing the solvent, the crude product (2.0 g.) was obtained in 16% yield. Recrystallization from hot water gave a pure material (1.3 g.), m. p. 141∼142°C.

Anal. Found: C, 61.69; H, 6.67; N, 541. Calcd. for $C_{13}H_{17}O_4N$: C, 62.13; H. 6.82; N, 5.57%.

Optical Resolution of N-Acetyl-O-benzyl-DL-threonine.—*Preparation of enzyme solution.*—Takadiastase (1 g.) was extracted with 10 ml. of water at 0°C. for 2 hr. and an insoluble part was filtered off using Cellite. The crude enzyme solution was used for the resolution without further purification.

Enzymic resolution of N-acetyl-O-benzyl-DL-threonine. — *N*-Acetyl-*O*-benzyl-derivative (1 g.) was dissolved in an aqueous solution of 2 N sodium hydroxide containing a slight excess of sodium hydroxide which brought the solution to pH 7.4. The above enzyme solution, together with the substrate, was incubated at 36.5°C for 24 hr. After incubation was over, the mixture was passed through the Amberlite IR-120 column (H-form). By the first elution with water, the acidic component was removed off, and then the amino acid component was liberated by the second elution with an aqueous 1 N ammonia (about 150 ml.). The latter eluate collected was concentrated in vacuo. The resulting product was dissolved in a small amount of hot water, boiled for 10 min. to remove the contaminant and filtered. The filtrate was concentrated and dried in vacuo. The residual weighed about 330 mg., 79% yield. Crystallization from hot water gave fine crystals of *O*-benzyl-L-threonine which melted at 197°C with decomposition. $[\alpha]_D^{19^\circ} -3.04$ ($c=1$, 1 N HCl).

Anal. Found: C, 57.61; H, 7.62; N, 6.31. Calcd. for $C_{11}H_{15}O_3N \cdot H_2O$: C, 58.13; H, 7.54; N, 6.16%.

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