

## A Nonenzymatic Approach to the Selective Cleavage of Threonine Peptides

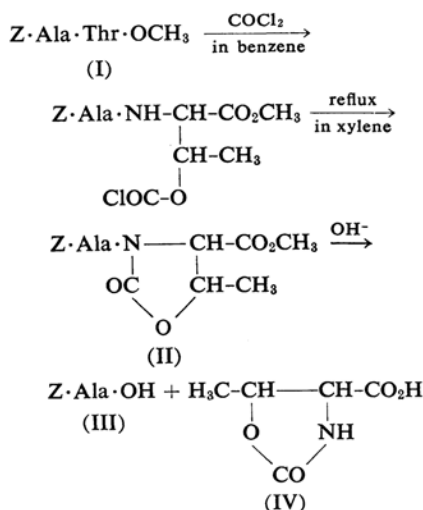
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It has previously been reported that threonine was easily cyclized with phosgene in an alkaline solution to an oxazolidone carboxylic acid.<sup>1)</sup> It has also been found that the cyclization of *N*-benzoyl-DL-threonine methyl ester with phosgene to an *N*-benzoyl oxazolidone derivative through a chlorocarbonyl intermediate proceeded in a similar way when the methods of Bergel<sup>2)</sup> and Ben-Ishai<sup>3)</sup> were applied. In this case, the *N*-benzoyl oxazolidone derivative produced could be hydrolyzed to the oxazolidone carboxylic acid in one step.\* In this hydrolysis, only the benzoyl group could be removed in safety from the oxazolidone ring. In connection with this finding, it seemed that it would be of great interest to investigate whether threonine dipeptide, which involves a peptide linkage between the amino group of threonine and the carboxyl group of another amino acid, can be cyclized to an oxazolidone derivative and then cleft to an oxazolidone carboxylic acid and free amino acid.

From a reaction of *N*-benzyloxycarbonyl-(Z)-DL-alanyl-DL-threonine methyl ester<sup>4)</sup> (DL-DL-I; m.p. 103.5~105°C) with phosgene, an *O*-chlorocarbonyl compound (m.p. 103.5~104.5°C; Found: C, 51.12; H, 5.26; N, 6.91. Calcd. for C<sub>17</sub>H<sub>21</sub>N<sub>2</sub>O<sub>7</sub>Cl: C, 50.94; H, 5.28; N, 6.99%) was obtained. It was cyclized to DL-*trans*-3-(Z-DL-alanyl)-4-methoxycarbonyl-5-methyl-2-oxo-oxazolidine (DL-DL-II; m.p. 129.5

~130°C; Found: C, 56.16; H, 5.52; N, 7.60. Calcd. for C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>7</sub>: C, 56.40; H, 5.53; N, 7.69%). To a solution of 2.46 g. of the oxazolidone derivative (DL-DL-II) in 38 ml. of methanol, 15.1 ml. (2 eq.) of 0.90 N potassium hydroxide were added. The reaction mixture was kept at room temperature for 1.5 hr. After evaporating the methanol in vacuo, the aqueous solution which remained was acidified with hydrochloric acid to pH 3 and then extracted with ethyl acetate. From the extract, Z-DL-alanine<sup>5)</sup> (DL-DL-III; m.p., 113.5~114.5°C; yield: 1.32 g. (88%)) was isolated. An additional amount of hydrochloric acid was added to the remaining acidic aqueous solution (pH 1), which was then evaporated to dryness in vacuo. From an extract of the



1) T. Kaneko and T. Inui, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zasshi)*, **82**, 1075 (1961).

2) F. Bergel and R. Wade, *J. Chem. Soc.*, **1959**, 941.

3) D. Ben-Ishai, *J. Am. Chem. Soc.*, **78**, 4962 (1956).

\* This study will be reported on elsewhere.

4) Th. Wieland, K. Freter and E. Goss, *Ann.*, **626**, 154 (1959).

5) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

residue with ethyl acetate, DL-*trans*-oxazolidone-carboxylic acid<sup>17</sup> (DL-IV; m. p. 126.5~127.0°C; yield: 0.89 g. (91%)) was obtained. In the case of the reaction of an optically-active peptide (L-L-I; m. p. 131.7~132.2°C;  $[\alpha]_D^{16.5} -23.6^\circ$  (MeOH)), the oxazolidone peptide (L-L-II) obtained was hydrolyzed in a way similar to that used for DL-DL-II to L-III<sup>6</sup>) (m. p., 86~87°C;  $[\alpha]_D^{13} -13.8^\circ$  (AcOH)) and L-IV<sup>7</sup>), (m. p. 139.0~139.8°C;  $[\alpha]_D^{11} +40.7^\circ$  (H<sub>2</sub>O)) in yields of 71% and 60% respectively.

Z-Glycyl-DL-threonine methyl ester (m. p., 110.5~111.8°C) was also cyclized to the corresponding oily oxazolidone peptide by the action of phosgene; this was followed by hydrolysis to give Z-glycine<sup>57</sup> and DL-IV in yields

of 71% and 76% respectively.

Similarly, a tripeptide, Z-L-alanyl-L-alanyl-L-threonine methyl ester (m. p. 181.0~181.5°C;  $[\alpha]_D^{11} -56.5^\circ$  (MeOH)) was also cyclized to the corresponding oily oxazolidone peptide ester, which afforded Z-L-alanyl-L-alanine<sup>87</sup>) (m. p., 149.5~151.0°C;  $[\alpha]_D^{13.5} -32.5^\circ$  (MeOH)) and L-IV by a mild alkaline hydrolysis.

These results indicated that this selective cleavage of threonine peptide occurred with no recemization at the peptide linkage in which an amino group of the threonine residue participated.

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6) M. Hund and V. duVigneaud, *J. Biol. Chem.*, **124**, 699 (1938).

7) T. Inui and T. Kaneko, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zasshi)*, **82**, 1078 (1961).

8) W. H. Stein, S. Moore and M. Bergmann, *J. Biol. Chem.*, **154**, 191 (1944).