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PERMANGANATE-OXIDATION OF PEPTIDES. I. THE OXIDATION OF PROLINE RESIDUE IN N-PROTECTED PROLINE TO PYROGLUTAMYL RESIDUE

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The oxidation of N-protected proline and related compounds with permanganate was investigated. The proline residue in N-acyl-L-proline was readily oxidized in the presence of strong acid to pyroglutamyl residue, in a manner different from that of free proline. In the oxidation of N^{δ}-benzyloxycarbonyl-L-ornithine under the same conditions, the methylene group adjacent to the protected δ-amino nitrogen was also attacked.

The oxidative degradation of threonine or serine residue in peptides with chromic acid has often been adopted in order to point out the presence or position of O-acyl aminohydroxycarboxylic acid.[1, 2] Testing the oxidation with permanganate solution for the same purpose, we found that a proline residue was easily degraded by this reagent in addition to the above residues in a model peptide. After hydrolysis of the oxidation product with acid, glutamic acid was detected in an amount nearly corresponding to the loss of proline. Thereupon, we examined the oxidation of benzyloxycarbonyl(Z)-L-proline with potassium permanganate in acid solution, and obtained Z-L-pyroglutamic acid as the main product. The reaction is different from that in the case of free amino acids,[3] which are generally oxidized to aldehydes and ammonia. Besides proline, the amount of glycine residue in peptides also decreased in the oxidation, but more gradually. The present paper reports the permanganate-oxidation of N-protected proline and related compounds.

Oxidation of Z-L-proline (1). The reaction conditions and results are shown in Table 1. After the oxidation, the excess of the reagent was destroyed with formic acid, and the mixture was evaporated. The residue was treated with constant-boiling HCl in an evacuated sealed tube at 110 °C for 16 h. The resulting amino

Table 1. Oxidation of Z-L-proline[a)]

Concn of Additional Acid M	Reaction Time h	Destruction of Proline %	Formation of Amino Acid[b)] Glutamic Acid %	4-Aminobutyric Acid %
-	1	23	37	29
	2	39	40	40
	4	65	38	50
0.10, H_2SO_4	1	76	54	21
	2	87	62	25
0.30, H_2SO_4	1	91	72	11
0.60, CF_3COOH	0.25	90	78	19
	1	94	80	16

a) Reaction conditions: 1, 6.7×10^{-3} M and $KMnO_4$, 7.3×10^{-2} M in 50% CH_3COOH; at room temp.

b) Amino acids were analyzed after hydrolysis of the reaction product. The values are based on the amount of consumed proline.

acids were analyzed with an automatic amino acid analyzer, JEOL JLC-6AS.

The oxidation was accelerated by the addition of a strong acid such as H_2SO_4 or CF_3COOH. The resulting amino acids would indicate that, in the presence of strong acid, the oxidizing agent seems to attack mainly the δ-carbon atom of proline to yield Z-L-pyroglutamic acid(2); however, in the absence of strong acid, the agent seems to act on the α- and δ-carbon atoms at a comparable rate to afford N-Z-2-pyrrolidone(3) and 2.

For the purpose of confirmation of the oxidation products, 1 (2.03 g, 8.15 mmol) was oxidized with $KMnO_4$ (2.74 g, 17.3 mmol) in 100 ml of 1.8 M H_2SO_4/50% CH_3COOH below 10 °C for 20 min. The excess of $KMnO_4$ was destroyed with 2-propanol. Solid $NaHCO_3$ (27 g) was added in the solution. The mixture was filtered and concentrated in vacuo. The residue was extracted with ethyl acetate and the extract was again evaporated. Finally 2 was obtained as a solid by triturating the oily residue with diethyl ether. Yield, 1.01 g (47%). Mp 134-137 °C (lit.[4)] mp 134-135 °C); $[\alpha]_D^{22}$ -34.2° (c 1.0, CH_3OH) (lit.[4)] $[\alpha]_D^{25}$ -29.1° (c 1.01, CH_3OH); NMR ($CDCl_3$) δ=2.00-2.78 (4H, m), 4.67 (1H, m), 5.28 (2H, s), 7.36 (5H, s), and 10.72 (1H, s). The IR spectrum and chromatographic behavior were identical to those of the authentic sample[4)] derived from Z-L-glutamic anhydride.[5)] After the removal of 2, the mother liquor was evaporated. The oily residue was purified by column

chromatography (silicagel, $CHCl_3$-CH_3OH (100:1)) and preparative TLC (Wakogel B-5, $CHCl_3$-CH_3OH (95:5)) to afford 3 (oil, 0.20 g); NMR($CDCl_3$) δ=1.98 (2H, m), 2.51 (2H, m), 3.76 (2H, t), 5.26 (2H, s), and 7.36 (5H, s); IR 1790, 1750, and 1720 cm^{-1}. The acid-hydrolysis of 3 resulted in 4-aminobutyric acid.

In another run, a mixture of CF_3COOH (10 ml) and 50% CH_3COOH (100 ml) was used instead of H_2SO_4/50% CH_3COOH as solvent. The oxidation was carried out for 30 min below 5 °C and 30 min at room temperature. The mixture was treated in a similar manner to that described above, except that the neutralization with $NaHCO_3$ was omitted. The product 2 was obtained in a 44% yield (0.46 g from 1.0 g of 1).

Oxidation of Acetyl-L-proline and L-Proline. Acetyl-L-proline was oxidized in the same manner as Z-L-proline. The hydrolysis of the product yielded mainly glutamic acid also in this case. Next, L-proline was treated in 20% CH_3COOH at room temperature. Proline disappeared much slower than Z- or acetyl-L-proline: decrease in proline (initial concn, 2.0×10^{-2} M), 6% for 1 h in 0.20 M $KMnO_4$ (in the absence of H_2SO_4), 16% for 1 h in 0.13 M $KMnO_4$ and 0.19 M H_2SO_4. No glutamic acid was detected in the hydrolysates prepared by the method described above.

Oxidation of L-Glutamic Acid and Z-L-glutamic acid. L-Glutamic acid was readily oxidized in the absence of H_2SO_4 in contrast with L-proline, the decrease in glutamic acid (initial concn, 1.9×10^{-2} M) being 68% (0.5 h) and 100% (1 h) in 0.20 M $KMnO_4$/19% CH_3COOH at room temperature. However, in the presence of H_2SO_4 (0.17 M), glutamic acid remained almost unaltered over a 2 h period.

Z-L-glutamic acid was fairly stable as was L-proline under similar conditions in both the presence and absence of H_2SO_4.

Oxidation of N^{δ}-Z-L-ornithine(4). The results are shown in Table 2. In the absence of H_2SO_4, 4 was gradually oxidized at the position of its α-methine to afford mainly a 4-aminobutyric acid derivative. The manner of oxidation resembled that of free proline or glutamic acid. On addition of H_2SO_4, 4 was rapidly oxidized at its δ-methylene group to give glutamine derivative, which was detected as glutamic acid after hydrolysis. This mode is similar to that of 1, but the rate of oxidation of 4 is not as fast as that of 1. The reaction of 4 to glutamine derivative was also promoted with CF_3COOH.

From the above results, it can be seen that: (I) the δ-methylene adjacent to the acylated nitrogen atom, such as that in 1 and acetyl proline, is easily oxidized

Table 2. Oxidation of N^{δ}-Z-L-ornithine[a)]

Concn of H_2SO_4 M	Reaction Time h	Destructuion of Ornithine %	Formation of Amino Acid[b)] Glutamic Acid %	4-Aminobutyric Acid %
-[c)]	2	49	27	48
	4	97	6	44
0.054[d)]	2	21	57	30
	4	42	45	22
1.0[e)]	0.5	68	94	trace
	1	77	85	trace

a) Reactions were carried out in CH_3COOH (40-50%)[6)] with $KMnO_4$ in 10 times the molarity of 4 at room temperature.
b) Analyzed in a similar way to that in Table 1.
c-e) Initial concn of N^{δ}-Z-L-ornithine: c), 6.7×10^{-3} M; d), 3.6×10^{-3} M; e), 5.1×10^{-3} M.

with $KMnO_4$ in a strong-acid solution but not in the absence of a strong acid; (II) the δ-methylene of 4 has a similar susceptibility; (III) the α-methine of the non-acylated α-amino acid (i.e., α-position of free glutamic acid and 4) is appreciably resistant to the oxidation in a strong-acid solution in comparison with the δ-methylene group, but is not so stable in the absence of a strong acid; (IV) on the contrary, the oxidation of the α-position of free proline is much slower in both the presence and absence of a strong acid.

In summary, the methylene group adjacent to a protonated imide- or amide-nitrogen seems to be remarkably susceptible to $KMnO_4$ as compared with the methylene or methine group adjacent to a protonated imino- or amino-nitrogen, or to a non-protonated imide- or amide-nitrogen.

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6) N^{δ}-Z-L-ornithine which had been dissolved in a H_2SO_4 or CF_3COOH solution without addition of CH_3COOH was oxidized much more slowly than that in a solution containing CH_3COOH. For the elucidation of the role of CH_3COOH, further studies are needed.

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