Notes

Chem. Pharm. Bull. 26(8)2562—2564(1978)

UDC 547.587.42.04 : 547.562-261.04

Suppressing Effect of Thioanisole on a Side Reaction during the Acidolytic Cleavage of Protecting Groups of Tyrosine[1]

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(Received December 19, 1977)

The use of thioanisole as a scavenger was found to suppress a side reaction when the O-protecting groups of tyrosine were deblocked under the acidic conditions. This good result gives a promising outlook for the synthesis of tyrosine-containing peptides by solid-phase method.

Keywords——peptide synthesis; thioanisole as a scavenger; deblocking reagents; O-benzylated tyrosine; 3-benzylated tyrosine; high-speed liquid chromatography

The phenolic hydroxyl group of tyrosine has been generally protected by the benzyl group,[3] and by the more acid stable 2,6-dichlorobenzyl group,[4] especially in solid-phase peptide synthesis.[5] These protecting groups are known to yield a side product,[4b,6] 3-benzylated tyrosine[4b] upon removal under acidic conditions. Erickson and Merrifield[4b] reported that 15% of the Tyr(Bzl) residues rearrange intramolecularly to form 3-benzyltyrosine during acidolysis with 50% HF-anisole, and 5% of the Tyr(2,6-Cl_2Bzl) residues rearrange to form 3-(2,6-dichlorobenzyl)tyrosine. With HF alone, the intramolecular rearrangement products occur to the extent of 40% in both cases.

This side reaction is a serious problem in solid-phase peptide synthesis. In order to suppress this O-to-C rearrangement, a scavenger in acidolysis is needed that is a more electron-rich cation acceptor than anisole. Since Yajima, *et al.*[7] found that the use of thioanisole as a scavenger suppressed the formation of a by-product, Lys(Bzl), in the cleavage of Lys(Z) with trifluoromethanesulfonic acid (TFMSA)–trifluoroacetic acid (TFA), we have examined the effect of thioanisole as a potential scavenger for a side reaction in the acidolytic cleavage of O-protecting groups of tyrosine. Thioanisole was employed as a scavenger in the synthesis of somatostatin with the use of boron tris(trifluoroacetate) by Bauer and Pless.[8]

The extent of rearrangement of Tyr(Bzl) was at first examined in TFMSA–TFA,[6d] methanesulfonic acid (MSA)–TFA[6e] and HF.[6b] Progress of the reaction was followed by

1) Abbreviations: Boc=*t*-butoxycarbonyl, Bzl=benzyl, 2,6-Cl_2Bzl=2,6-dichlorobenzyl, Lys(Z)=ε-benzyloxycarbonyl-L-lysine, TFA=trifluoroacetic acid, MSA=methanesulfonic acid, TFMSA=trifluoromethanesulfonic acid, Tyr=L-tyrosine.

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thin-layer chromatography, and quantitative analysis was performed by high-speed liquid chromatography.

In the case of TFMSA–TFA, Tyr(Bzl) was completely deprotected for 30 min at 0°, and the molar ratio of tyrosine to 3-benzyltyrosine (3-BzlTyr) was 58: 42 (Table I). Treatment with anisole–TFMSA–TFA formed 13 mol% of 3-BzlTyr, while thioanisole–TFMSA–TFA reduced the formation of 3-BzlTyr to only 0.8 mol%. Treatment with anisole–MSA–TFA for 60 min at 0° formed 21 mol% of 3-BzlTyr, while the use of thioanisole as a scavenger reduced the formation of 3-BzlTyr to 1.1 mol%. Also, in the case of HF, the use of thioanisole reduced the formation of 3-BzlTyr to 4 mol%. Tyr(Bzl) in TFA at 25° for 48 hr was not completely deprotected and the molar ratio of Tyr to 3-BzlTyr was 57: 43. Thioanisole was better than anisole as a scavenger when the O-benzyl group of tyrosine was removed under acidic conditions.

Table I. Molar Ratio of Tyrosine to 3-Benzylated Tyrosines during Acidolysis of O-Benzylated Tyrosines

Deblocking reagent[a]	Run	Tyr: 3-BzlTyr	Run	Tyr: 3-(2,6-Cl_2Bzl)Tyr
Anisole–TFMSA–TFA	1	86.7 : 13.3	1′	95.5 : 0.5
Thioanisole–TFMSA–TFA	2	99.2 : 0.8	2′	>99.7 : 0.3>
Anisole–MSA–TFA	3	78.8 : 21.1	3′	>99.7 : 0.3>
Thioanisole–MSA–TFA	4	98.9 : 1.1	4′	>99.7 : 0.3>
Anisole–HF	5	86.2 : 13.8[b]	5′	95.3 : 4.7[c]
Thioanisole–HF	6	96.0 : 4.0	6′	>99.7 : 0.3>
TFMSA–TFA	7	57.8 : 42.2	7′	59.4 : 40.6
TFA	8	57.0 : 43.0		——

a) Details of deblocking conditions are given in experimental part.
b) lit.[4b] 85: 15.
c) lit.[4b] 95: 5.

The extent of rearrangement of Tyr(2,6-Cl_2Bzl) was next examined in the acidic conditions. Treatment with anisole–TFMSA–TFA for 3 hr at 0° formed 0.5 mol% of 3-(2,6-Cl_2Bzl)-Tyr while treatment with thioanisole–TFMSA–TFA for 30 min at 0° suppressed the formation of 3-(2,6-Cl_2Bzl)Tyr to less than detectable levels (0.3%). The molar ratio of tyrosine to 3-(2,6-Cl_2Bzl)Tyr was 59: 41 in TFMSA–TFA without a scavenger.

In thioanisole–TFMSA–TFA, Tyr(2,6-Cl_2Bzl) disappeared within 30 min at 0°, while in anisole–TFMSA–TFA, Tyr(2,6-Cl_2Bzl) still remained even after a period of 2 hr at 0°. This phenomenon suggests that a powerful scavenger such as thioanisole has not only a suppressing effect on a side reaction, but also a promoting effect on an acidolytic cleavage. Details of this catalytic effect of thioanisole on an acidolysis will be published in a separate paper.

Tyr(2,6-Cl_2Bzl) in anisole–MSA–TFA for 15 hr at 0° was not completely deprotected, while in the case of thioanisole–MSA–TFA completely deprotected. In both cases the formation of 3-(2,6-Cl_2Bzl)Tyr was less than detectable levels (0.3%). In the case of HF, the use of thioanisole suppressed the formation of a side product to less than detectable levels (0.3%).

Thus an improvement was obtained by the use of thioanisole as a scavenger when the O-protecting groups of tyrosine were removed under the acidic conditions. This good result gives a promising outlook for the synthesis of tyrosine-containing peptides by solid-phase method.

Experimental

The solvent used for thin-layer chromatography (TLC) (silica gel G, Merck) was chloroform–methanol–water (8: 3: 1, lower layer) and spots were visualized with ninhydrin. *Rf* values of reference samples: Tyr, 0.15; 3-BzlTyr, 0.21; Tyr(Bzl), 0.42; 3-(2,6-Cl_2Bzl)Tyr, 0.24; Tyr(2,6-Cl_2Bzl), 0.44. High-speed liquid chromatography was performed with a Du Pont 830 Liquid Chromatograph using a strong cation exchange

Zipax® SCX column (sulfonated fluorocarbon polymer, 1 m × 2.1 mm i.d.). Operating conditions were as follows: mobil phase, pH 2.9 phosphate buffer (was prepared by adjusting the pH to 2.9 with addition of 0.1 M phosphoric acid (160 ml) to 1 M KH_2PO_4 (1000 ml) and used after filtration); column pressure, 1000 psig; flow rate, 0.5 ml/min; column temperature, 43°; detector, UV photometer at 254 nm. Retention time of reference samples: Tyr, 1.75 min; 3-BzlTyr, 3.5 min; 3-(2,6-Cl_2Bzl)Tyr, 7.4 min.

Reaction of Tyr(Bzl) with Deblocking Reagents (I)——Run 1. Reaction with Anisole–TFMSA–TFA: Tyr(Bzl) (27 mg) was treated with anisole(0.6 ml)–TFMSA(0.05 ml)–TFA(2 ml) for 30 min at 0°. TLC of the reaction mixture showed *Rf* 0.15 and 0.21. The solvent was evaporated *in vacuo*, the resulting residue was dissolved in pH 2.9 phosphate buffer (20 ml) and after washing with ether, subjected to high-speed liquid chromatography. Analysis is given in Table I.

Run 2. Reaction with Thioanisole–TFMSA–TFA: The above procedure was repeated with the use of thioanisole (0.6 ml) in place of anisole.

Run 3. Reaction with Anisole–MSA–TFA: Tyr(Bzl)(27 mg) was treated with anisole(0.6 ml)–MSA(0.5 ml)–TFA(2 ml) for 60 min at 0° and worked up as described above.

Run 4. Reaction with Thioanisole–MSA–TFA: The above procedure was repeated with the use of thioanisole(0.6 ml) in place of anisole.

Run 5. Reaction with Anisole–HF: HF(about 2 ml) was distilled into a fluorocarbon vessel containing a mixture of Tyr(Bzl) (27 mg) and anisole(2 ml) precooled to −70°. The vessel was surrounded with an ice-water bath, and the reaction mixture was stirred magnetically for 20 min. The HF was evaporated under water aspiration, the resulting residue was dissolved in pH 2.9 phosphate buffer(20 ml), and after washing with ether, subjected to high-speed liquid chromatography.

Run 6. Reaction with Thioanisole–HF: The above procedure was repeated with the use of thioanisole-(2 ml) in place of anisole.

Run 7. Reaction with TFMSA–TFA: Tyr(Bzl)(27 mg) was treated with TFMSA(0.05 ml)–TFA(2 ml) for 30 min at 0°. TLC of the reaction mixture showed another unknown spot (*Rf* 0.32) (presumably dibenzyl derivative) besides *Rf* 0.15 and 0.21, and was worked up as described in Run 1.

Run 8. Reaction with TFA: Tyr(Bzl)(27 mg) was treated with TFA(2 ml) for 48 hr at 25°. TLC of the reaction mixture showed that starting material still remained, and was worked up as described above.

Reaction of Tyr(2,6-Cl_2Bzl) with Deblocking Reagents (II)——Run 1′. Reaction with Anisole–TFMSA–TFA: Boc-Tyr(2,6-Cl_2Bzl)(44 mg) was treated with anisole(0.6 ml)–TFMSA(0.05 ml)–TFA(2 ml) for 3 hr at 0° and worked up as described in (I).

Run 2′. Reaction with Thioanisole–TFMSA–TFA: Boc-Tyr(2,6-Cl_2Bzl)(44 mg) was treated with thioanisole(0.6 ml)–TFMSA(0.05 ml)–TFA(2 ml) for 30 min at 0° and worked up as described above.

Run 3′. Reaction with Anisole–MSA–TFA: Boc-Tyr(2,6-Cl_2Bzl) (44 mg) was treated with anisole(0.6 ml)–MSA(0.5 ml)–TFA(2 ml) for 15 hr at 0° and worked up as described above.

Run 4′. Reaction with Thioanisole–MSA–TFA: The above procedure was repeated with the use of thioanisole(0.6 ml) in place of anisole.

Run 5′. Reaction with Anisole–HF: Boc-Tyr(2,6-Cl_2Bzl)(44 mg) was treated by the same procedure as described in (I).

Run 6′. Reaction with Thioanisole–HF: The above procedure was repeated with the use of thioanisole (2 ml) in place of anisole.

Run 7′. Reaction with TFMSA–TFA: Boc-Tyr (2,6-Cl_2Bzl) (44 mg) was treated with TFMSA (0.05 ml)–TFA (2 ml) for 3 hr at 0° and worked up as described in (I).

Acknowledgements The authors wish to express their sincere appreciation to Prof. Haruaki Yajima for his encouragement and advice during the course of this investigation. Thanks also extend to Miss Rosemarie Kelly for her help in the preparation of this manuscript.