2 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel. Elution with hexane–AcOEt (5: 1) and recrystallization of the eluate from MeOH gave **2b** (424 mg) as colorless leaflets. mp 218—219°.

6α-d_1-5α-Hydroxycholestan-3-one (3b)——Oxidation of **2b** (120 mg) with CrO_3-pyridine complex (1: 10 w/v) was carried out in the manner as described with **2a**. Recrystallization of the crude product gave **3b** (92 mg) as colorless leaflets. mp 227—228°.

6α-d_1-Cholest-4-en-3-one (4b)——Dehydration of **3b** (93 mg) with anhydrous *p*-toluenesulfonic acid (50 mg) in benzene (15 ml) was carried out in the manner as described with **3a**. The crude product was purified by preparative TLC using hexane–AcOEt (6: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **4b** (58 mg) as colorless plates. IR ν_{max}^{KBr} cm^{-1}: 2150 (C–D). mp 80.5—81°. NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, $J=6$ Hz, 26- and 27-CH_3), 0.90 (3H, d, $J=6$ Hz, 21-CH_3), 1.18 (3H, s, 19-CH_3), 5.68 (1H, d, $J=1.5$ Hz, C_4-H). MS *m/e*: 385 (M^+) (98% d_1).

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Studies on Peptides. LXXII.[1,2] Examination of the N^ε-Alkylation of Lysine in the Methanesulphonic Acid Procedure for Peptide Synthesis

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Methanesulphonic acid cleaved the Z group from H–Lys(Z)–OH without concomitant formation of H–Lys(Bzl)–OH in the presence of the cation scavenger, anisole.

Keywords——N^ε-benzyllysine; alkylation of N^ε–Lys in the trifluoromethanesulphonic acid–trifluoroacetic acid–anisole system; deprotection in the trifluoromethanesulphonic acid–trifluoroacetic acid–thioanisole system; deprotection in the methanesulphonic acid–anisole system; examination of cation scavengers; intramolecular alkylation

In 1975, we reported that methanesulphonic acid (MSA) cleaved the most of protecting groups currently employed in peptide synthesis.[4] However, regeneration of Arg from Arg (NO_2) or Arg (Tos) with this reagent was not effective enough compared to the results of trifluoromethanesulphonic acid (TFMSA).[5] We had therefore to apply the latter stronger acid as a deprotecting reagent at the final step of the synthesis of peptides containing Arg,

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[2] Amino acids mentioned here are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Bzl=benzyl, TFA=trifluoroacetic acid.

[3] Location: *Sakyo-ku, Kyoto, 606, Japan.*

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such as tuftsin⁶⁾ and neurotensin⁷⁾ selected as relatively small model peptides. Recently, Nishimura and Fujino⁸⁾ introduced a new Arg derivative, N^G-*p*-methoxybenzene-sulfonyl-Arg, Arg (MBS), the protecting group of which can be smoothly removed with MSA. This finding made thus feasible to synthesize Arg-containing peptides by the MSA procedure.

More recently, Mitchell and Merrifield⁹⁾ pointed out a side reaction which takes place during the TFMSA–TFA treatment of H-Lys (Z)-OH or Z-Gly-OH. The benzyl cation generated with this reagent attacked the amino group and conseqently H-Lys (Bzl)-OH or Bzl-Gly-OH formed in 3.1 or 1.2% yield respectively, even in the presence of a cation scavenger, such as anisole.

Prior to apply the MSA procedure to the synthesis of complex peptides, we now examined whether such type of alkylation reaction, presumably the intramolecular alkylation, would proceed in this much milder acid system.¹⁰⁾ In any cases, when urethane type protecting groups are cleaved acidolytically, alkyl cations formed have to be trapped, otherwise side reactions take place. Thus cation scavengers play a key important role to suppress this type of side reaction. In the presence of various scavengers, H-Lys (Z)-OH was exposed to MSA or a 50% solution in TFA. The TFMSA–TFA system was also examined as a reference.

When monitered reaction mixtures by Shimadzu dual wavelength thin layer chromatography scanner, this side reaction product, H-Lys (Bzl)-OH, was indeed detectable in the TFMSA–TFA-anisole system as listed in Table 1. Replacement of anisole by *o*-cresol or resorcinol¹¹⁾ suppressed this side reaction in a certain degree. The former seems much better, but not satisfactory enough. *dl*-α-Tocopherol, an antioxidant, was found to have little effect. Among sulfur compound tested, no formation of H-Lys (Bzl)-OH was detected, when thioanisole was employed. Thus the TFMSA–TFA-thioanisole system seems to be a suitable deblocking reagent, if Arg (Tos) and Lys (Z) are applied to peptide synthesis.

Table I. Effect of Various Cation Scavengers during the Treatment of H-Lys(Z)-OH with MSA and TFMSA

Scavenger	10% TFMSA–TFA 60 min, 20°		MSA 60 min, 20°		50% MSA–TFA 3 hr, 20°		ESA 10 hr, 20°	
	Lys	Lys(Bzl)	Lys	Lys(Bzl)	Lys	Lys(Bzl)	Lys	Lys(Bzl)
Anisole	97.1	2.9	100	0	100	0	100	0
o-Cresol	99.6	0.4	100	0	100	0	100	0
Resorcinol	98.3	1.7	100	0	100	0	100	0
dl-α-Tocopherol	93.0	7.0	100	0	100	0	100	0
Anisol+phenol(1:1)	97.0	2.8	100	0	100	0	100	0
Thioanisole	100.0	0	100	0	100	0	100	0
Anisole–2% ethanedithiol	97.2	2.8	100	0	100	0	100	0
Anisole–2% dithiothreitol	97.6	2.4	100	0	100	0	100	0

In contrust to the TFMSA system, formation of H-Lys (Bzl)-OH was not observed in the MSA system regardless of variation of scavengers. Situations were the same in the 50%

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MSA–TFA system, though at least 3 hours were required to remove the Z group completely. At this convenience, H-Lys (Z)-OH was exposed to ethanesulphonic acid (ESA). Although complete removal of the Z group required more than 10 hours, no alkylation was observed. Summarizing the above informations and experimental results herein, MSA seems to have much superior properties than TFMSA or ESA in respect of its acidity and free from the side reaction mentioned above.

Experimental

Rf values on thin-layer chromatography (Silica gel Kiesel gel G, Merck) refer to the solvent system of *n*-BuOH–AcOEt–AcOH–H_2O (1 : 1 : 1 : 1).

Treatment of H-Lys(Z)-OH with TFMSA-TFA or MSA——Treatment of H-Lys(Z)-OH (28 mg, 0.1 mmol) with 10% TFMSA-TFA (0.3 ml, 3.4 equiv.) or MSA (0.3 ml, 46 equiv.) was performed in the presence of various cation scavengers (5 equiv.) at 20° for 60 min. A part of the solution was examined by thin-layer chromatography. The ninhydrin color intensity of respective spots [*Rf* H-Lys(Z)-OH 0.54, H-Lys-(Bzl)-OH 0.47, H-Lys-OH 0.29] was determined quantitatively by Shimadzu dual wavelength thin-layer chromatography scanner, Model CS-900 and the results were listed in Table I. In the 50% MSA–TFA and ESA systems, each solution was examined, after treatment for 3 hr and 10 hr respectively.

Isolation of H-Lys(Bzl)-OH——H-Lys(Z)-OH (5.0 g) was treated with 10% TFMSA-TFA (20 ml) in the presence of anisole (8 ml) at 20° for 60 min and a mixture of ether and H_2O was added. The aqueous layer was washed three times with ether, neutralized with Et_3N and then condensed *in vacuo*. The residue was dissolved in a small amount of the solvent consisting of $CHCl_3$–MeOH–H_2O (8 : 3 : 1) and the solution was applied to a column of silica (3 × 15 cm), which was eluted with the same solvent. Fractions containing the substance of *Rf* 0.47 (contaminated with *Rf* 0.29) were combined. After evaporation of the solvent, the residue was again applied to a column of silica (3 × 5 cm), which was eluted with the same solvent system. The desired fractions free from the contaminant were combined and the solvent was evaporated. The residue was then applied to a column of Sephadex G-10 (3 × 140 cm), which was eluted with 3% AcOH for desalting. Fractions containing a single component (*Rf* 0.47, tube 51—66, ninhydrin stein) were combined and the solvent was removed by lyophilization; yield 85 mg (2.0%). Identity of the product with the authentic sample of H-Lys(Bzl)-OH prepared according to Benoiton[12] was established by comparison with their *Rf* values (both *Rf* 0.47) and NMR spectra (in D_2O); 1.20—2.52 (6H, m, $-(CH_2)_3-$), 3.04 (2H, t, $-NH-CH_2-$), 3.97 (1H, t, α-H), 4.31 (2H, s, benzyl), 7.53 (5H, s, aromatic H).

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