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Studies on Peptides. LXXX.[1,2] N^G-Mesitylene-2-sulfonylarginine

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N^G-mesitylene-2-sulfonylarginine was prepared. This protecting group was found to be quantitatively cleaved by hydrogen fluoride, methanesulfonic acid or trifluoromethanesulfonic acid and partially by hydrogen bromide in acetic acid. As a side reaction, partial mesitylenesulfonylation of the phenolic group of tyrosine was noted. However this side reaction could be suppressed by cation scavengers, anisole–thioanisole–cresol (*o*) (1 : 1 : 1), in less than 4%.

Keywords——N^G-mesitylenesulfonylarginine; N^G-*p*-methoxybenzenesulfonylarginine; removal of N^G-mesitylenesulfonyl group; stability of N^G-mesitylenesulfonyl group; side reaction of N^G-mesitylenesulfonyl group; anisole-thioanisole-cresol (*o*) as cation scavengers

Device of a new guanidino protecting group removable by methanesulfonic acid (MSA)[4] or trifluoromethanesulfonic acid (TFMSA)[5] is our present demand to generalize these deprotecting reagents for the synthesis of peptides containing arginine. Among various protecting groups currently employed, arginine derivatives offered considerable limitation for the use of these acids. For example, the nitro group[6] was partially cleaved, even by TFMSA. Complete removal of the Tos group[7] by TFMSA could be achieved only at somewhat elevated temperature, such as at 40° for 60 minutes. Though this condition was tried to the synthesis of a relatively small peptide, neurotensin,[8,9] MSA, a weaker acid than TFMSA, could not be applied, because of incompleteness of its deprotection. Through this model experiment, more acid labile protecting groups were thought to be examined in order to gain further wide applicability of these acids, possibly MSA, as deprotecting reagents at the final step of peptide synthesis containing arginine.

In 1976, Nishimura and Fujino[10] introduced the *p*-methoxybenzenesulfonyl (MBS) group, as an acidolytically removable protecting group for arginine. This group is more easily removable by these acids, as well as hydrogen fluoride,[11] compared to the Tos group. We could applied the MSA deprotecting procedure for the syntheses of granuliberin-R,[12] mamma-

1) Part LXXIX: H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 3144 (1978).
2) Amino acids and peptides mentioned here are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=*p*-toluenesulfonyl, Bzl=benzyl.
3) Location: *a*) *Sakyo-ku, Kyoto, 606, Japan*; *b*) *Yodogawa-ku, Osaka, 532, Japan.*
4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 ((1975).
5) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc. Chem. Commun.*, **1974**, 107.
6) M. Bergmann, L. Zervas, and H. Rinke, *Z. physiol. Chem.*, **224**, 40 (1934).
7) E. Schnabel and C.H. Li, *J. Am. Chem. Soc.*, **82**, 4576 (1960).
8) R. Carraway and S.E. Leeman, *J. Biol. Chem.*, **248**, 6854 (1973).
9) H. Yajima, K. Kitagawa, T. Segawa, M. Nakano, and K. Kataoka, *Chem. Pharm. Bull.* (Tokyo), **23**, 3299 (1975).
10) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **24**, 1568 (1976).
11) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
12) T. Nakajima, T. Yasuhara, Y. Hirai, C. Kitada, M. Fujino, M. Takeyama, K. Koyama, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **25**, 2473 (1977).

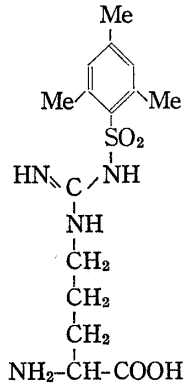
lian glucagon (by M.F. *et al.*)[13] and duck glucagon[14] with an aid of this newly deviced arginine derivative.

In this paper, we wish to introduce the mesitylene-2-sulfonyl (Mts) group, as a more acid labile protecting group for arginine. N^{α}-Protected arginine derivatives were allowed to react with mesitylene-2-sulfonylchloride[15] according to the procedure described in the preparation of Z-Arg(Tos)–OH.[7] Thus three compounds, Z–Arg(Mts)–OH, Boc–Arg(Mts)–OH and Z(OMe)–Arg(Mts)–OH, were prepared and characterized as the respective cyclohexylamine (CHA) salts. H–Arg(Mts)–OH was obtained as a crystalline compound from water, after deprotection of the former by hydrogenolysis and the latter two compounds by trifluoroacetic acid (TFA).

Next, chemical properties of the Mts group was examined by using a Shimadzu dual wavelength thin-layer chromatography (TLC) scanner and the results were listed in Table I.

Table I. Stability of Arg (Mts)

Reagent		Temp	Time	Arg regenerated %
TFA	6 equiv	20	60 min	0
1 N NaOH	2 equiv	20	60 min	0
80% NH_2NH_2	10 equiv	20	24 hr	0
HOBT	2 equiv	20	24 hr	0
Na-liq.NH_3		−15	1 min	60.0
25% HBr–AcOH	6 equiv	20	60 min	71.5
HF		0	60 min	100



The Mts group survived intactly under various conditions required in the practical peptide synthesis, such as treatment of TFA, or 1 N sodium hydroxide or hydrazine hydrate and was removed by hydrogen fluoride, as like as the Tos group.[16] However differently from the Tos group, it could be quantitatively cleaved by TFMSA and MSA at room temperature, while the Tos group was partially cleaved as reported previously.[4,5] It seems worthwhile to note that the Mts group resisted rather to the action of sodium in liquid ammonia, under which the Tos group was quantitatively cleaved. It was found further that more than 70% of the Mts group was cleaved by 25% hydrogen bromide in acetic acid within 60 minutes at

Table II. Removal of the Mts Group by MSA or TFMSA

Arg deriv.		30 min	60 min
H-Arg(NO_2)-OH	MSA	19.3%	25.3%
H-Arg(Tos)-OH	MSA	40.7	43.1
H-Arg(MBS)-OH	MSA	71.4	88.3[a]
	MSA–TFA (9:1)	60.1	84.7[b]
	TFMS–TFA (1:1)	100	100
H-Arg(Mts)-OH	MSA	93.4	98.2
	MSA–TFA (9:1)	92.6	93.7
	TFMSA–TFA (1:1)	100	100

Reaction was performed in the presence of anisole.
a) lit.[10] 99.1%, *b*) lit.[10] 92.4% at 21° for 40 min.

13) M. Fujino, M. Wakimasu, S. Shinagawa, C. Kitada, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 539 (1978).
14) H. Yajima, H. Ogawa, and H. Sakurai, *J. Chem. Soc. Chem. Commun.*, **1977**, 909.
15) C.H. Wang and S.G. Cohen, *J. Am. Chem. Soc.*, **79**, 1924 (1957).
16) R.H. Mazur and G. Plume, *Exp.*, **24**, 661 (1968).

room temperature, while the MBS group was remained unchanged. From these results, it can be concluded that the Mts group possesses properties not inferior to those of the MBS group and as far as the acid susceptibility is concerned, the Mts groups was judged as a more acid labile protecting group than the latter. This tendency could also be seen by careful comparison of the arginine recovery from Arg (MBS) and Arg (Mts) in an amino acid analyzer, after treatment with MSA under identical conditions (Table II).

Despite of such easiness in the acidolytical deprotection of the MBS and the Mts groups, we have to mention one side reaction observable at the tyrosine residue. This side reaction was noticed at the first time during the synthesis of granuliberin-R mentioned above.[12] The protected granuliberin-R, Z(OMe)–Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg(MBS)–Arg(MBS)–Pro–Ala–Ser(Bzl)–NH_2, was treated with MSA in the presence of anisole and when the deprotected peptide was purified by column chromatography on carboxymethyl (CM)-cellulose, a single peak with a small shoulder was observed. This shoulder part was now investigated enzymatically. An aminopeptidase (AP–M)[17] digest contained every amino acid in ratios predicted by theory, except for tyrosine. Tyrosine was completely missed in a chart of an amino acid analyser. Situation was the same, when the peptide was hydrolyzed by 4 N MSA.[18] However, by 6 N HCl hydrolysis, tyrosine was fully recovered. Its elemental analysis revealed the presence of the sulfur content in 1.74%. MSA itself has no ability to sulfonate tyrosine. These results excluded the possibility of the O-(benzyl or *p*-methoxybenzyl) tyrosine formation and rather supported the view that this side product is the O-(*p*-methoxybenzene sulfonyl)-tyrosine derivative, named as [7-Tyr(MBS)]-granuliberin-R. The most plausible explanation of this phenomenon is that the MBS cation liberated from arginine is so stable in a MSA medium that sulfonated the phenolic group of tyrosine, despite of the presence of a cation scavenger, anisole.

Confirmatively, a model peptide, Z(OMe)–Ile–Tyr–Arg(MBS)–OH, was treated with MSA-anisole. Besides H–Ile–Tyr–Arg–OH, an extra spot, presumably corresponding to H–Ile–Tyr(MBS)–Arg–OH, was detected on TLC and its amount was estimated in more than 34%. We were reminded that during the purification of synthetic duck glucagon[14] on Sephadex G-25, we separated a small peak in front of the main peak. This fraction was less soluble in water and though we did not examine it further, it must contain such a side product, besides a tryptophan modified substance.

We therefore next examined whether this type of side reaction may take place in an instance of Arg(Mts), the sulfonyl moiety of which seems to be under somewhat sterically

Table III. Effect of Various Scavengers

Scavenger	Ile-Tyr-Arg *Rf* 0.48	By-product *Rf* 0.60
Anisole	70.7%	29.3%
Thioanisole	73.0	27.0
Dimethylsulfide	41.8	58.2
Phenol	75.2	24.8
Guaiacol	61.7	38.3
Cresol (*o*)	83.3	16.7
Anisole–Thioanisole (1:1)	74.0	26.0
Anisole–phenol (1:1)	88.8	11.2
Anisole–cresol (1:1)	91.0	9.0
Thioanisole–cresol (1:1)	91.3	8.7
Anisole–thioanisole–cresol (1:1:1)	96.1	3.9

17) G. Pfleiderer and G.P. Celliers, *Biochem. Z.*, **339**, 186 (1963).
18) R.J. Simpson, M.R. Neuberger, and T.Y. Liu, *J. Biol. Chem.*, **251**, 1936 (1976).

hindered circumstance. When Z(OMe)–Ile–Tyr–Arg(Mts)–OH was similarly treated with MSA-anisole, an undesired extra spot was still detected on TLC. This side product was now isolated by partition chromatography on Sephadex G-25[19] and characterized as H–Ile–Tyr-(Mts)–Arg–OH, as predicted.

Facing to the fact that acid labile protecting groups afford generally stable cations which are apt to participate in undesired side reactions, more efficient scavenger systems than anisole were next examined. Among scavengers tested (Table III), phenolic compounds, such as *o*-cresol, improved this situation in a great extent and thio compounds, such as thioanisole, in a certain degree. Such in the system of anisole–thioanisole–*o*-cresol (1:1:1, v/v), this type of side reaction was found to be suppressed in less than 4%. Keeping this side reaction and possibly the O-sulfonation of serine and threonine also in mind, we wish to examine the usefulness of Arg(Mts) to the practical peptide synthesis in future.

At this occasion, we wish to add one comment on the partition chromatography in the solvent system of *n*-butanol–acetic acid–water (4:1:5). During the course of the above isolation of the by-product, we were able to separate a small amount of the 3-(*p*-methoxybenzyl)-tyrosine derivative, which exhibited the identical *Rf* value with that of H–Ile–Tyr-(Mts)–Arg–OH. Formation of this side product is predictable, since the similar 3-(benzyl)-tyrosine from Z-derivative is known in literatures.[20] The main product, H–Ile–Tyr–Arg–OH could not be eluted with the upper phase of the above solvent system. After two fractions mentioned above were emerged from the column by the upper phase, the column was then developed with the lower phase in order to elute H–Ile–Tyr–Arg–OH. The result indicated that when a substance containing basic components is applied to the partition chromatography on Sephadex, the upper phase and subsequently the lower phase also have to be applied to achieve the complete elution.

Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 *n*-BuOH–AcOH–pyridine–H_2O (30:6:20:24), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). ^{1}H-NMR spectra were determined by the Varian HA-100 spectrometer.

Z(OMe)-Arg(Mts)-OH·CHA Salt——Under cooling with ice-NaCl, mesitylene-2-sulfonyl chloride (67.0 g, 0.3 mol) in acetone (300 ml) was added dropwise to a stirred solution of Z(OMe)–Arg–OH[21] (50.0 g, 0.15 mol) in a mixture of 4 N NaOH (150 ml, 0.6 mol) and acetone (750 ml) during a period of 40 min. After stirring for 30 min in an ice-bath and an additional 2 hr at room temperature, the solvent was evaporated *in vacuo* at the bath temperature below 30° and the residue was dissolved in H_2O. An aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt and the extract was washed with ice-chilled 0.2 N HCl and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. An oily residue was dissolved in CH_3CN and CHA (17.3 ml, 0.15 mol) was added. The resulting solid was recrystallized twice from MeOH and CH_3CN; yield 56.2 g (61%), mp 125—128°, $[\alpha]_D^{22}$ +5.5° (c=0.7, MeOH), Rf_1 0.45. *Anal.* Calcd. for $C_{24}H_{32}N_4O_7S \cdot C_6H_{13}N$: C, 58.14; H, 7.32; N, 11.30. Found: C, 58.15; H, 7.42; N, 11.31.

Boc-Arg(Mts)-OH·CHA Salt——The title compound was prepared in essentially the same manner as described above; yield 68%, mp 122—125°, $[\alpha]_D^{22}$ +15.1° (c=0.5, MeOH), Rf_1 0.21. *Anal.* Calcd. for $C_{20}H_{32}N_4O_6S \cdot C_6H_{13}N$: C, 56.19; H, 8.16; N, 12.60. Found: C, 56.05; H, 8.15; N, 12.64.

Z-Arg(Mts)-OH·CHA Salt——The title compound was similarly prepared; yield 62%, mp 113—114°, $[\alpha]_D^{22}$ +5.5° (c=1.1, MeOH), Rf_1 0.24. *Anal.* Calcd. for $C_{23}H_{30}N_4O_6S \cdot C_6H_{13}N$: C, 59.06; H, 7.35; N, 11.88. Found: C, 59.22; H, 7.37; N, 11.88.

H-Arg(Mts)-OH——(a) Z(OMe)–Arg(Mts)–OH·CHA (10.5 g) was dissolved in MeOH (100 ml) and 1 N HCl (17 ml) was added. The solvent was evaporated and the residue was extracted with AcOEt. The organic phase was washed with 10% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was treated with TFA (25 ml) in the presence of anisole (5 ml) in an ice-bath for 60 min and dry

19) D. Yamashiro, *Nature* (London), **201**, 76 (1964).
20) B. Iselin, *Helv. Chim. Acta*, **45**, 1510 (1962); B.W. Erickson and R.B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3750 (1973).
21) F. Weygand and E. Nintz, *Z. Naturforsch. B.*, **20**, 429 (1965).

ether was added. The resulting powder was dissolved in a small amount of H_2O and the pH of the solution was adjusted at 6 with 5% NH_4OH. The resulting solid was recrystallized from hot H_2O; yield 5.40 g (88%). The identical compound was obtained from Boc–Arg(Mts)–OH·CHA salt, after similar α-deprotection with TFA; yield 89%, mp 158—161°, $[\alpha]_D^{22}$ −5.2° (c=0.4, MeOH), Rf_3 0.60. *Anal.* Calcd. for $C_{15}H_{24}N_4O_4S \cdot H_2O$: C, 48.11; H, 7.00; N, 14.96. Found: C, 48.06; H, 7.32; N, 14.74.

(b) Z–Arg(Mts)–OH (derived from 6.90 g of the CHA salt as stated above) in MeOH (50 ml) containing a few drops of AcOH was hydrogenated over a Pd catalyst for 6 hr. The solution was filtered, the filtrate was condensed and the residue was recrystallized from hot H_2O to give the compound identical with that obtained in (a); yield 4.15 g (99%).

Stability of Arg(Mts)——H–Arg(Mts)–OH (19 mg, 50 μmol) was exposed to TFA (0.16 ml), 1 N NaOH (0.15 ml), 80% hydrazine hydrate (0.03 ml) in MeOH (0.5 ml), N-hydroxybenzotriazole (15 mg) in MeOH (0.5 ml) and 25% HBr–AcOH (0.13 ml) under various conditions and a part of the solutions was examined by TLC using a Shimadzu dual wavelength scanner. The results were listed in Table I. The Na-liq. NH_3 treatment of H–Arg(Mts)–OH (19 mg) was performed according to du Vigneaud *et al.*[22] The blue color was persisted for 1 min and discharged by NH_4Cl. After evaporation of NH_3, the residue was dissolved in H_2O (1 ml) and a part of the solution was similarly examined. The Tos group was removed completely under this condition. The HF treatment of H–Arg(Mts)–OH (17 mg) was performed according to Sakakibara *et al.*[11] and the product was examined as mentioned above.

Removal of the Mts Group by MSA and TFMSA——Treatment of H–Arg(Mts)–OH (10 μmol each) with MSA (50 equiv) or TFMSA–TFA (50 equiv. each) was performed in the presence of anisole (5 equiv.) at 20°. After 30 or 60 min, each solution was neutralized with 4 N NaOH and a part of the solution was subjected to an amino acid analyzer (Hitachi KLA-5). As references, H–Arg(NO_2)–OH, H–Arg(Tos)–OH, and H–Arg(MBS)–OH (10 μmol each) were similarly treated with MSA. The results were listed in Table II.

[8-Tyr(MBS)]-Granuliberin-R——As reported previously, the protected dodecapeptide amide, Z(OMe)–Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg(MBS)–Arg(MBS)–Pro–Ala–Ser(Bzl)–NH_2 (0.50 g) was treated with MSA-anisole and the deprotected peptide was purified by CM-cellulose (2.5×19 cm) using the gradient elution with 0.2 M $AcONH_4$ buffer at pH 6.9. A shoulder part of the main peak[12] (tube No. 61—80) was collected and the solvent was removed by lyophilization. The residue was desalted by column chromatography on Sephadex G-15 and lyophilization gave a fluffy white powder; yield 65 mg (16%), $[\alpha]_D^{25}$ −93.9° (c=0.1, 0.5 N AcOH), Rf_2 0.78 (indistinguishable from grnuliberin-R). Amino acid ratios in 6 N HCl, 4 N MSA (in parenthesis) and AP–M (in bracket) hydrolysate: Phe 1.99 (2.04) [1.93], Gly 1.00 (1.00) [1.00], Leu 0.95 (1.06) [1.01], Pro 2.15 (2.03) [1.60], Ile 0.95 (1.00) [1.04], Arg 1.72 (2.22) [1.97], Ala 0.97 (1.01) [1.00], Ser 0.69 (0.83) [0.90], Tyr 0.98 (0) [0], average recovery 86%, (85%), [86%]. *Anal.* Calcd. for $C_{76}H_{109}N_{19}O_{17}S \cdot 2CH_3COOH \cdot 4H_2O$: C, 52.76; H, 7.14; N, 14.61; S, 1.76. Found: C, 52.42; H, 6.92; N, 14.39; S, 1.74.

Z(OMe)-Ile-Tyr-Arg(MBS)-OH——To an ice-chilled solution of Z(OMe)–Ile–Tyr–$NHNH_2$[13] (1.56 g) in DMF (15 ml) was added 2.63 N HCl–DMF (2.9 ml) followed by isoamylnitrite (0.6 ml). After stirring for 10 min, the solution was neutralized with Et_3N (1.06 ml) and combined with a solution of H–Arg(MBS)–OH (1.50 g) in DMF (15 ml) containing Et_3N (1.06 ml). After stirring at 4° overnight, the solution was condensed and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and recrystallized from EtOH and ether; yield 2.0 g (84%), mp 163—164°, $[\alpha]_D^{22}$ +6.5° (c=0.3, DMF), Rf_1 0.32, Rf_2 0.08. Amino acid ratios in 6 N HCl (with phenol): Ile 1.01, Tyr 0.92, Arg 1.00 (average recovery 92%). *Anal.* Calcd. for $C_{37}H_{48}N_6O_{11}S$: C, 56.62; H, 6.16; N, 10.71. Found: C, 56.43; H, 5.97; N, 10.56.

Z(OMe)–Ile–Tyr–Arg(MBS)–OH (7 mg) was treated with MSA-anisole (0.1—0.03 ml) at 25° for 60 min and dry ether was added. The residue was dissolved in a small amount of H_2O and a few pieces of Amberlite CG-4B (acetate form) was added. The supernatant was examined by TLC. Two ninhydrin positive spots were detected and their relative color intensities were measured by a Shimadzu dualwavelength scanner; Rf_3 0.48: Rf_3 0.74=65.4: 34.6. The former is positive to Pauly, Sakaguchi and ninhydrin tests. The latter is positive to Sakaguchi and ninhydrin, but negative to Pauly test.

Z(OMe)-Ile-Tyr-Arg(Mts)-OH——The azide (prepared from 2.36 g of Z(OMe)–Ile–Tyr–$NHNH_2$ with 2.1 ml of 5.86 N HC–DMF, 0.7 ml of isoamylnitrite and 2.4 ml of Et_3N) in DMF (20 ml) was added to an ice-chilled solution of H–Arg(Mts)–OH (2.85 g) in DMF (15 ml) containing Et_3N (1.1 ml) and the mixture was stirred at 4° overnight. The solvent was evaporated and the residue was dissolved in 5% Na_2CO_3. The aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt. The extract was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then condensed. Treatment of the residue with ether afforded a solid, which was recrystallized from AcOEt and ether; yield 3.50 g (88%), mp 122—125°, $[\alpha]_D^{22}$ −12.1° (c=0.4, MeOH). Rf_1 0.27. Amino acid ratios in 6 N HCl (with phenol): Ile 1.03, Tyr 0.99, Arg, 1.00 (average recovery 92%). *Anal.* Calcd. for $C_{39}H_{52}N_6O_{10}S$: C, 58.78; H, 6.58; N, 10.55. Found: C, 58.73; H, 6.51; N, 10.33.

22) R.H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

Isolation of the By-products derived from Z(OMe)-Ile-Tyr-Arg(Mts)-OH——Z(OMe)-Ile-Tyr-Arg(Mts)-OH (383 mg) was treated with MSA (2 ml) in the presence of anisole (0.3 ml, about 1/5 of the usual amount) at 25° for 60 min and dry ether was added. The resulting precipitate was dissolved in a small amount of H_2O and the solution, after treatment with Amberlite CG-4B (acetate form, apporoximately 1 g) for 30 min, was filtered. The filtrate was lyophilized to give a fluffy white powder (*ca.* 300 mg, two spots; Rf_3 0.48 and Rf_3 0.60). This powder was dissolved in the upper phase (3 ml) of the solvent consisting of *n*-BuOH–AcOH–H_2O (4: 1: 5) and the solution was applied to a column of Sephadex G-25 (3×95 cm) equilibrated with the lower phase of the above solvent system. Individual fractions (7.5 ml each) were collected and absorbancy at 275 mμ was determined. The substance of Rf_3 0.60 was soon emerged from the column (F_1 tube No. 35—47) and a small peak (F_2 tube No. 69—81) was detected. The substance obtained in the latter peak exhibited the identical *Rf* value (Rf_3 0.60) with that of F_1. The substance of Rf_3 0.48 was found to be still retained in the column. Thus the lower phase was employed for its elution (F_3 tube No. 121—131) as shown in Fig. 1. Fractions corresponding to these three peaks were lyophilized to give fluffy powders respectively.

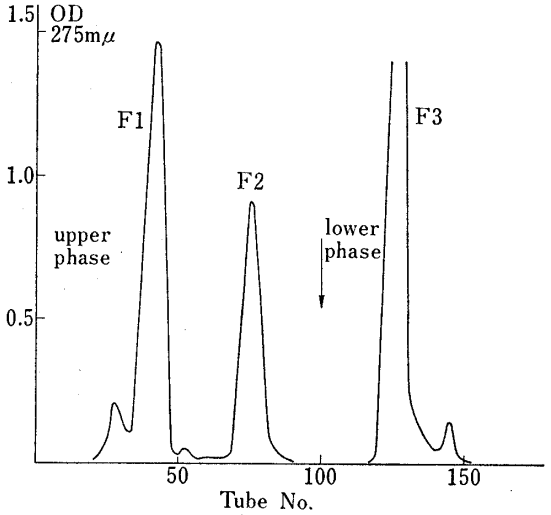


Fig. 1. Isolation of By-products derived from Z(OMe)-Ile-Tyr-Arg(Mts)-OH on Sephadex G-25

Sample 383 mg
Column: 3×95 cm
Solvent system: *n*-BuOH–AcOH–H_2O (4: 1: 5)

H–Ile–Tyr(Mts)–Arg–OH [F_1]: 95 mg (29%), Rf_3 0.60 (positive to ninhydrin and Sakaguchi, but negative to Pauly test), $[\alpha]_D^{25}$ +21.4° (*c*=0.1, 1 N AcOH). Amino acid ratios in 6 N HCl hydrolysate: Ile 1.00, Tyr 1.00, Arg, 0.94 (average recovery 82%). Amino acid ratios in AP–M digest: Ile 1.00, Tyr 0, Arg 1.00 (recovery of Ile 72%). NMR (CD_3OD): δ 7.05 (2H, s, mesitylene aromatic H), 6.80, 6.89, 7.18, 7.27 (total 4H, m, aromatic H). *Anal.* Calcd. for $C_{30}H_{44}N_6O_7S \cdot CH_3COOH \cdot 1/2H_2O$: C, 54.76; H, 7.04; N, 11.97; S, 4.57. Found: C, 54.76; H, 7.06; N, 12.59; S, 4.83.

H–Ile–Tyr(3-Bzl)–Arg–OH [F_2]: 10 mg (3.2%), Rf_3 0.60 (positive to ninhydrin, Sakaguchi and Pauly tests). Amino acid ratios in 6 N HCl hydrolysate: Ile 1.00, Tyr=0, Arg 1.10 (recovery of Ile 93%). Amino acid ratios in AP–M digest: Ile 1.00; Tyr 0, Arg 1.11 (recovery of Ile 98%). NMR (D_2O): δ 6.79—7.27 (total 7H, q, aromatic H). *Anal.* Calcd. for $C_{29}H_{42}N_6O_6 \cdot CH_3COOH \cdot 1/2H_2O$: C, 58.19; H, 7.46; N, 13.14. Found: C, 58.05; H, 7.17; N, 13.63; S, 0.

H–Ile–Tyr–Arg–OH [F_3]: 140 mg (49%), Rf_3 0.48 (positive to ninhydrin, Sakaguchi and Pauly tests), $[\alpha]_D^{21}$ +16.6° (*c*=0.3, 1 N AcOH). Amino acid ratios in 6 N HCl hydrolysate: Ile 0.94, Tyr 1.00, Arg 1.16 (average recovery 89%). Amino acid ratios in AP–M digest: Ile 1.00, Tyr 0.99, Arg 1.00 (average recovery 88%). *Anal.* Calcd. for $C_{21}H_{34}N_6O_5 \cdot CH_3COOH \cdot 1/2H_2O$: C, 53.16; H, 7.57; N, 16.18. Found: C, 53.02; H, 7.38; N, 16.87.

Effect of Various Scavengers——Z(OMe)–Ile–Tyr–Arg(Mts)–OH (40 mg, each) was treated with MSA (50 equiv.) in the presence of various scavengers (about 10 equiv.) in an ice-bath for 30 min and at room temperature for 30 min and dry ether was added. The resulting precipitate was dissolved in a small amount of H_2O and treated with Amberlite CG-4B (acetate form, approximately 300 mg). The filtrate was examined by TLC in the solvent system of *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2) and ninhydrin color intensities of detectable 2 spots were determined by a Shimadzu dual wavelength chromatography scanner. From the sum of the color intensities, percentage of each component was expressed in Table III.