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Studies on Peptides. L.[1,2] Acidolysis of Protecting Groups in Peptide Chemistry by Fluorosulphonic Acid and Methanesulphonic Acid

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Acidolytic cleavage of various protecting groups currently employed in peptide chemistry by fluorosulphonic and methanesulphonic acids was examined.

Recently we reported that various protecting groups currently employed in peptide chemistry could be removed by trifluoromethanesulphonic acid without significant side-reactions.[4] Similarly acidolysis of these groups by fluorosulphonic acid or methanesulphonic acid was next investigated.

In the presence of anisole, as a scavenger, each amino acid derivative was treated with the reagent at room temperature for 30 minutes and a part of the solution was subjected to quantitative amino acid analysis.

Fluorosulphonic acid removed most of the protecting groups listed in Table I, except for the Bzl and the formyl groups attached at the side functions of His and Lys respectively. Cleavage of the S-Bzl group of Cys was not quantitative even after 60 minutes treatment (83.1%). Recovery of Tyr from H-Tyr(Bzl)-OH was low, because of the formation of 3-benzyltyrosine.[5] Somewhat low recovery of Ser and Thr from Boc-Ser-OH, H-Ser(Bzl)-OH and H-Thr(Bzl)-OH seems to be the result from partial sulphonation of the hydroxyl group by this reagent.[6] Regeneration of Arg from H-Arg(NO_2)-OH or H-Arg(Tos)-OH was achieved quantitatively after treatment with this reagent at room temperature for 15 minutes (93.2 and 98.4% respectively), while under identical conditions, trifluoromethanesulphonic acid cleaved partially these protecting groups.[4]

Methanesulphonic acid cleaved also most of the protecting groups listed in Table I, but less tendency can be seen in cases of H-Arg(NO_2)-OH, H-Arg(Tos)-OH, H-His(Tos)-OH and H-Cys(Bzl)-OH as compared to the results of fluorosulphonic acid and trifluoromethanesulphonic acid. Low recovery of Tyr was also due to the formation of 3-benzyltyrosine. Recovery of Met was also low, when this treatment was performed without addition of thiols. Next application of methanesulphonic acid in solid phase synthesis was examined. H-Gly-Ala-OH was liberated from Z(OMe)-Gly-Ala-resin (Gly content 0.10 mmole/g)[7] in 84.2% yield, after treatment with this reagent in trifluoroacetic acid (1:1 v/v) at room temperature for 30 minutes. Trifluoromethanesulphonic acid gave comparable result.

1) Part XLIX: H. Watanabe, H. Ogawa and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 375 (1975).
2) Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemistry Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*t*-butoxycarbonyl, NPS=*o*-nitrophenylsulphenyl, Tos=tosyl, Bzl=benzyl, OBu^t=*t*-butyl ester, MBzl=*p*-methoxybenzyl.
3) Location: *Sakyo-ku, Kyoto.*
4) H. Yajima, N. Fujii, H. Ogawa and H. Kawatani, *J. Chem. Soc., Chem. Comm.*, **1974**, 107.
5) D. Yamashiro and C.H.Li, *J. Org. Chem.*, **38**, 591 (1973); B.W. Erickson and R.B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3750 (1973); P.A. Spanninger and J.L. von Rosenberg, *ibid.*, **94**, 1973 (1972).
6) U. Svanholm and V.D. Parker, *J. Chem. Soc., Perkin II*, **1972**, 962.
7) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1087 (1974).

Table I. Removal of Various Protecting Groups by FSO_3H or CH_3SO_3H (20°, 30 minutes)

Treated amino acid derivative	Parent amino acid regenerated (%)	
	FSO_3H	CH_3SO_3H
NPS–Val–OH	101.6	100.5
Boc–Ser–OH	72.7	96.5
Z(OMe)–Gly–OH	99.9	94.0
Z–Trp–OH	100.3	100.0
Z–Tyr–OH	99.8	100.1
Z–Met–OH	92.8[a)]	31.5
Z–Glu–OH	98.0	101.2
Z–Glu(OBu^t)–OH	98.0	99.5
H–Glu(OBzl)–OH	94.8	99.6
H–Asp(OBzl)–OH	100.1	100.8
H–Ser(Bzl)–OH	69.8	97.7
H–Thr(Bzl)–OH	78.4	101.6
H–Tyr(Bzl)–OH	31.0	30.2
H–Arg(NO_2)–OH	100.5	58.5
H–Arg(Tos)–OH	98.5	49.2
Z–Arg(Z_2)–OH	101.8	100.0
Z(OMe)–His(Tos)–OH	81.7	27.8
H–His(Bzl)–OH	0	0
H–Cys(Bzl)–OH	83.3	32.9
H–Cys(MBzl)–OH	100.0	94.6
H–Lys(For)–OH	0	0

a) reaction in the presence of dithiothreitol

At this convenience, we have examined the behaviour of the Z group toward the action of 3-bromocamphor-8- or 10-sulphonic acid. Regeneration of Glu from Z-Glu-OH was 85.9 or 82% respectively, after similar treatment with one of these reagents in trifluoroacetic acid at room temperature for 60 minutes. Acid labile protecting groups, such as NPS, Boc and Z(OMe), were cleaved completely under these conditions in methylene chloride.

Depending on the acidity of sulphonic acids so far examined, it may be possible to select the reagent to remove various protecting groups employed at the final stage of peptide synthesis.

Experimental

Treatment of Amino Acid Derivatives with Sulphonic Acids——Treatment of amino acid derivatives (1 mmole) with fluorosulphonic acid (10 equiv.) or bromocamphor sulphonic acids (10 equiv.) was performed in the presence of anisole (1.5 to 5 equiv.) at 20° for 30 min. As a solvent, CH_2Cl_2 (9.2 ml) was used for NPS, Boc and Z(OMe)-derivatives listed in Table I and trifluoroacetic acid (9.2 ml each) was employed for the rest of derivatives. A part of the solution was subjected to quantitative amino acid analysis and the results were listed in Table I. Treatment of amino acid derivatives with methanesulphonic acid (100 equiv.) was performed in essentially the same manner as described above, except that no solvent was employed. In an amino acid analyser, minor side reaction products could be detected when Ser and Thr derivatives were treated with fluorosulphonic acid and Z–Met–OH with methanesulphonic acid. Characterization of these products are under investigations.

Cleavage of the Peptide from the Polymer Support——Treatment of Z(OMe)-Gly-Ala-resin (peptide content 0.10 mM/g) (2.02 g) in trifluoroacetic acid (5 ml) was treated with methanesulphonic acid (5 ml) in the presence of anisole (0.5 ml) at room temperature for 30 min. The resin was removed by filtration and washed with trifluoroacetic acid (3 ml). The filtrate and washing were combined and the solution was condensed *in vacuo*. Ether was added and the resulting precipitate was dissolved in H_2O (5 ml), which after washing with AcOEt, was treated with Amberlite IR-4B (acetate form, 3 g) for 30 min. The resin was removed by filtration and the filtrate was condensed *in vacuo*. The residue was dried over KOH pellets overnight and then recry-

stallized from H_2O and EtOH; yield 35 mg (84.2%), mp 240—244°, $[\alpha]_D^{25}$ −45.4° (c=1.1, H_2O). (Lit.[8] $[\alpha]_D^{25}$ −50.4° in H_2O, Lit.[9] $[\alpha]_D^{22}$ −46.8° in H_2O). Parallel experiment was performed using trifluoromethanesulphonic acid (1 ml) in trifluoroacetic acid (5 ml). Yield of H-Gly-Ala-OH was 82.0%.

8) B.F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, 73, 3508 (1951).
9) F. Weygand and W. Steglich, *Chem. Ber.*, 93, 2983 (1960).

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Ennancement of Fluorescence Intensity of Dansyl Amino Acids on Silica Gel Plate using Cycloheptaamylose[1]

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Cycloheptaamylose (C7A) was found to greatly enhance and stabilize the fluorescence intensity of dansyl (DNS) amino acids on silicagel layer and this finding was applied to the detection and determination of these compounds on thin-layer chromatogram. Procedure: After the development of DNS-amino acids, the thin-layer plate is sprayed with 10 ml of 1.8% C7A, dried over phosphorus pentoxide and the spots are detected under a mercury lamp or determined using a scanning fluorometer.

Above 10 fold increase in the fluorescence was observed. The fluorescence was stable for 90 min under exposure to air in the diffused light and for 24 hr over phosphorus pentoxide under reduced pressure. Limits of detection for 24 DNS-amino acids were in the range of 0.1 to 0.5 pmole/spot. Standard curves for DNS-amino acids were linear in the range of 5 to 30 pmole. Samples from 1 to 5 pmole could also be determined semi-quantitatively.

Dansylation[3] coupled with thin-layer chromatography (TLC) has extensively been used for the detection or determination of low quantities of amines,[4] amino acids,[5] peptides,[6] and proteins.[7] However, the fluorescence of dansyl (1-dimethylaminonaphthalene-5-sulfonyl, DNS) derivatives on silica gel layers fades rapidly during storage.[3,8] Consequently, great care has been required in drying the thin-layer plates for the removal of developing solvents under strictly defined conditions to assure reproducibility, and the sensitivity was not always satisfactory.[3] Although the enhancement and stabilization of the fluorescence intensity was attained by spraying triethanolamine,[9] this reagent was susepeptible to oxidation on exposure to air and light, and not applicable to alkali-sensitive compounds. Recently,

1) This paper forms Part V of "Microanalysis of Proteins and Peptides." Preceding paper, Part IV: T. Kinoshita, F. Iinuma, A. Tsuji, and I. Moriguchi, *Chem. Pharm. Bull.* (Tokyo), 22, 2929 (1974).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) N. Seiler, "Methods of Biochemical Analysis," Vol. 18, ed. by D. Glick, Interscience Publishers, Inc., New York, 1970, pp. 259—337.
4) H. Tsuzuki, K. Kitani, K. Imai, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), 20, 1931 (1972).
5) L. Casola and G.D. Matteo, *Anal. Biochem.*, 49, 416 (1972).
6) Z. Tamura, *Japan Analyst*, 17, 908 (1968).
7) T. Nakajima, H. Endou, F. Sakai, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), 18, 1935 (1970).
8) J.F. Lawrence and R.W. Frei, *J. Chromatogr.*, 66, 93 (1972).
9) N. Seiler and M. Wiechmann, *Z. Anal. Chem.*, 220, 109 (1966).