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# Amino Acids and Peptides. V. Synthesis of Amino Acid Derivatives Containing a Sulfonamide Bond[1)]

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Amino acid derivatives containing a sulfonamide bond were synthesized and their biological activities were examined. Some sulfonyllysine derivatives exhibited fibrinolytic activity. $N^4$-Lysylsulfanilamide was also prepared and treated with trypsin. The results suggest that a drug acylated with an amino acid might have improved solubility in water and might act as a kind of pro-drug.

**Keywords**——sulfonylamino acid; sulfonyllysine; sulfanilylamino acid; fibrinolytic activity; pro-drug

In general, amino acids have good solubility in water, and furthermore their salts (HCl salt, Na salt, *etc.*) are more hydrophilic. Therefore acylation of a water-insoluble drug with an amino acid and salt-formation of the acylated drug may increase the solubility in water. Administration of a water-insoluble drug by aqueous injection might then be possible, and the acylated drug might be hydrolyzed by an enzyme in the body to exhibit its medicinal effect. Therefore a drug acylated with an amino acid may work as a kind of pro-drug and may exhibit prolongation of the medicinal effect. We tested this possibility by using sulfanilamide and Lys. Sulfanilamide is sparingly soluble in water and Lys is known to be a site of trypsin cleavage. $N^{\alpha}$,$N^{\varepsilon}$-diZ-Lys[2)] was coupled with sulfanilamide by the DCC method[3)] followed by hydrogenation to afford $N^4$-lysylsulfanilamide (I). Compound I was sparingly soluble in

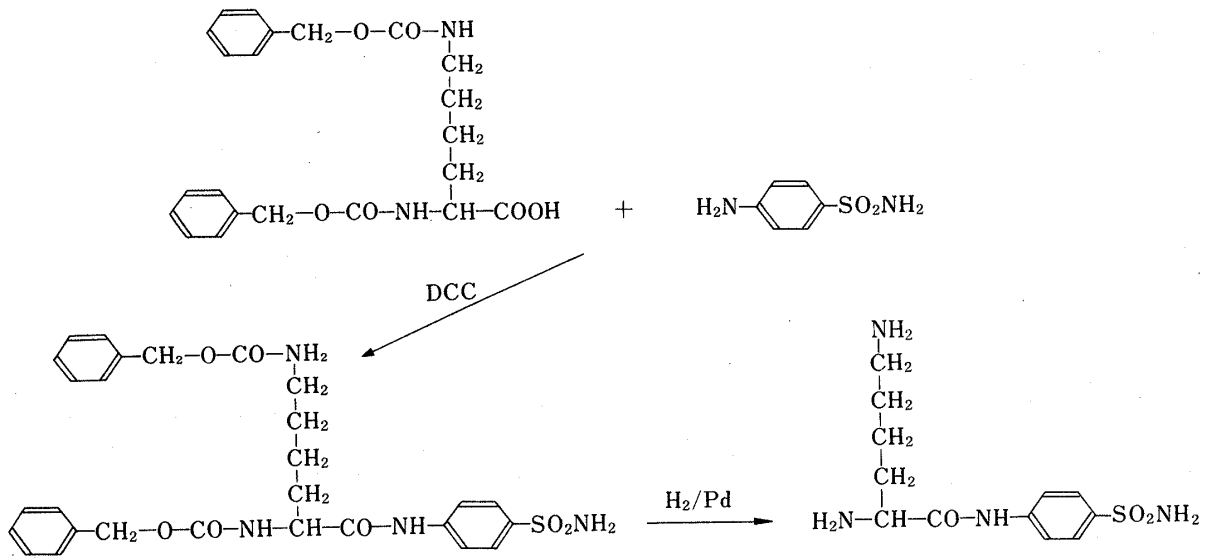



Fig. 1. Synthetic Scheme for Lysylsulfamine

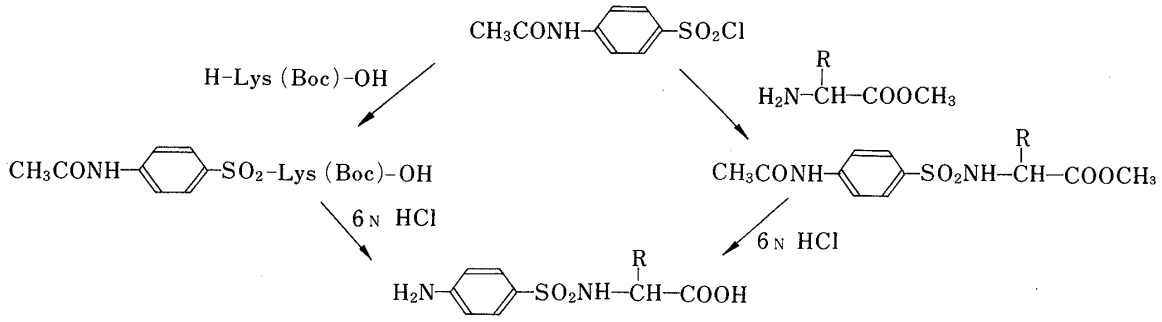



Fig. 2. Synthetic Scheme for Sulfanilylamino Acids

TABLE I. Fibrinolytic Activities of Sulfonylamino Acids

| | Dose (mg/kg, *i.v.*) | Fibrinolytic activity (%) 2 min | 10 min |
|---|---|---|---|
| Lys–Sulamine | 10 | 103 | 116 |
| Sulfanilyl–Lys | 10 | 107 | 109 |
| Asul–Lys(Boc)–OH | 10 | 142 | 131 |
| | 5 | 162 | 131 |
| | 2.5 | 124 | 116 |
| Asul–Lys(Z)–OH | 10 | 150 | 121 |
| | 5 | 181 | 133 |
| | 2.5 | 166 | 118 |
| | 1.25 | 124 | 110 |
| Asul–Lys(Asul)–OH | 10 | 163 | 146 |
| | 5 | 206 | 153 |
| | 2.5 | 151 | 151 |
| | 1.25 | 155 | 124 |
| | 0.625 | 158 | 133 |
| Dns–Lys(Boc)–OH | 10 | 193 | 143 |
| | 5 | 149 | 120 |
| | 2.5 | 165 | 121 |
| | 1.25 | 149 | 142 |
| Tos–Lys(Boc)–OH | 10 | 194 | 144 |
| | 5 | 202 | 138 |
| | 2.5 | 190 | 136 |
| | 1.25 | 144 | 124 |
| | 0.625 | 133 | 108 |
| Ms–Lys(Boc)–OH | 10 | 150 | 102 |
| | 5 | 103 | 92 |
| Tos–Lys(Tos)–OH | 10 | 176 | 164 |
| | 5 | 210 | 126 |
| | 2.5 | 159 | 140 |
| | 1.25 | 169 | 123 |

$$\text{Fibrinolytic activity (\%)} = \frac{\text{euglobulin lysis time preadministration}}{\text{euglobulin lysis time postadministration}} \times 100$$

water, contrary to our expectation, but its HCl salt was readily soluble. Release of Lys was observed when I was treated with trypsin. About 60% of I was hydrolyzed by the enzyme within 24 h. The hydrolysis was slow, but this supports the view that a drug acylated with an amino acid may act as a kind of pro-drug.

Next, various amino acids were sulfanilated as shown in Fig. 2. *N*-Acetylsulfanilylchloride[4] was coupled with an amino acid methyl ester followed by acid hy-

drolysis with 6 N HCl to afford a sulfanilylamino acid. $N^{\alpha}$-Sulfanilyl-Lys was prepared in two different ways with a Z group[5] or Boc group[6] as the protecting group for the ε-amino group of Lys. $N^{\alpha}$-Sulfanilyl-Arg was prepared by hydrogenolysis of $N^{\alpha}$-Asul-$N^{G}$-$NO_2$-Arg-OMe followed by acid hydrolysis. Optical isomers of sulfanilyl-Ala were prepared; their rotations in water were $-36.9°$ and $+36.2°$. Antibacterial effects of the synthetic sulfanilyl-amino acids were examined but no effect was observed against various gram-positive and gram-negative organisms.

It is known that some sulfonylamino acids can affect blood coagulation. For example, some arenesulfonylarginines were reported to be thrombin inhibitors.[7] We examined the fibrinolytic activities of I, $N^{\alpha}$-sulfanilyl-Lys and $N^{\alpha}$-Asul-$N^{\varepsilon}$-Boc-Lys. Among these samples, $N^{\alpha}$-Asul-$N^{\varepsilon}$-Boc-Lys exhibited fibrinolytic activity, so we prepared various sulfonyllysine derivatives to examine them for fibrinolytic activity. The sulfonyllysine derivatives were synthesized from Lys (or its derivative) and the corresponding sulfonylchloride. The synthetic sulfonyllysine derivatives and their fibrinolytic activities are summarized in Table I.

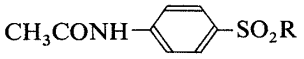
Comparing the fibrinolytic activities of these compounds, $N^{\alpha}$-Ms-$N^{\varepsilon}$-Boc-Lys was less active than other $N^{\alpha}$-arenesulfonyl-$N^{\varepsilon}$-Boc-lysines. An $N^{\alpha}$-arenesulfonyl group may be more effective than an $N^{\alpha}$-alkanesulfonyl group. Among $N^{\alpha}$-arenesulfonyl-$N^{\varepsilon}$-Boc-lysines, $N^{\alpha}$-dansyl and $N^{\alpha}$-tosyl compounds exhibited high activity. Among $N^{\alpha}$-Asul-lysines, $N^{\alpha}$,$N^{\varepsilon}$-diAsul-Lys was the most effective, but the $N^{\varepsilon}$-sulfonyl group seemed not to be necessary for the activity considering the activities of $N^{\alpha}$-sulfonyl-$N^{\varepsilon}$-Boc-lysines and $N^{\alpha}$-sulfonyl-$N^{\varepsilon}$-Z-lysines. Some of the synthetic sulfonyllysine derivatives exhibited fibrinolytic activity but their effects were not sufficient for medical purposes. Further synthetic studies on the fibrinolytic activities of sulfonyllysine derivatives are planned.

## Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography on Silica gel G (type 60, E. Merck) are indicated as follows: $Rf^1$ = *n*-BuOH–AcOH–$H_2O$ (4 : 1 : 5, upper phase), $Rf^2$ = *n*-BuOH–pyridine–AcOH–$H_2O$ (4 : 1 : 1 : 2), $Rf^3$ = $CHCl_3$–MeOH–$H_2O$ (8 : 3 : 1, lower phase), $Rf^4$ = AcOEt–benzene (1 : 1).

**$N^4$-($N^{\alpha}$,$N^{\varepsilon}$-diZ-Lys)sulfanilamide**——$N^{\alpha}$,$N^{\varepsilon}$-diZ-Lys (1.1 g) and sulfanilamide (0.45 g) were coupled in THF by

TABLE II. Yields and Physical Constants of Asul-amino Acid Methylesters




| R (Yields %) | mp (°C) | $[\alpha]_D^t$, $t$ (°C) $c=1.0$ | Analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| L-Ala–OMe (69) | 129—132 | −33.6°, $t$=21 MeOH | 48.0 (47.7 | 5.4 5.4 | 9.3 9.2) |
| D-Ala–OMe (62) | 133—134 | +34.2°, $t$=24 MeOH | 48.0 (47.8 | 5.4 5.3 | 9.3 9.3) |
| Pro–OMe (71) | 117—123 | −118.2°, $t$=26 MeOH | 51.5 (51.5 | 5.6 5.7 | 8.6 8.4) |
| $N^{\varepsilon}$–Z–Lys–OMe (52) | 145—147 | +2.4°, $t$=25 MeOH | 55.8 (56.0 | 6.0 5.9 | 8.6 8.3) |
| Asp–$(OMe)_2$ (67) | 113—114 | −1.6°, $t$=26 MeOH | 46.9 (46.6 | 5.1 5.4 | 7.8 7.5) |
| $N^{G}$–$NO_2$–Arg–OMe (55) | 211—213 | −10.0°, $t$=26 DMF | 41.2 (41.3 | 5.1 5.2 | 16.2 16.3) |

the DCC method[3] in the usual manner. Recrystallized from AcOEt. Yield 1.11 g (74%), mp 177—179 °C, $[\alpha]_D^{26}$ −6.4° ($c$=1.0, MeOH), $Rf^3$ 0.64, $Rf^4$ 0.41. *Anal.* Calcd for $C_{28}H_{32}N_4O_7S$: C, 59.1; H, 5.7; N, 9.9. Found: C, 59.4; H, 5.8; N, 9.7.

**$N^4$-Lysylsulfanilamide (I)**——Prepared by hydrogenation of $N^4$-($N^\alpha$,$N^\varepsilon$-diZ-Lys)sulfanilamide (1 g) over a Pd catalyst in MeOH in the usual manner. Recrystallized from MeOH. Yield 375 mg (71%), mp 171—174 °C, $[\alpha]_D^{28}$ +51.9° ($c$=1.0, 1 N HCl), $Rf^1$ 0.26, $Rf^2$ 0.40. *Anal.* Calcd for $C_{12}H_{20}N_4O_3S$: C, 48.0; H, 6.7; N, 18.6. Found: C, 47.8; H, 6.9; N, 18.3.

**Trypsin Treatment of I**——To increase its solubility in water, I was converted into the dihydrochloride salt by lyophilization from 1 N HCl. The hydrochloride salt was treated with trypsin according to the procedure reported by Hirs *et al.*[8] Liberated Lys was analyzed periodically and the following results were obtained.

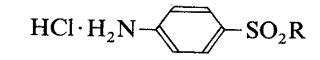
| Time (h) | 2 | 5 | 10 | 24 |
|---|---|---|---|---|
| Liberated Lys | 26% | 48% | 56% | 60% |

**General Procedure for Preparation of Asul-amino Acid Methyl Esters**——An amino acid methyl ester hydrochloride (0.03 mol) was dissolved in a mixture of DMF and $H_2O$. The solution was adjusted to pH 9 with $Et_3N$ and combined with *N*-acetylsulfanilylchloride (0.036 mol) at 0 °C. The reaction mixture was stirred overnight at room temperature and the solvent was evaporated off. The residue was dissolved in $CHCl_3$ and $H_2O$, and the $CHCl_3$ layer was washed successively with 5% $NaHCO_3$, $H_2O$, 10% citric acid and $H_2O$. The $CHCl_3$ layer was dried over $Na_2SO_4$ and evaporated down. The residue was recrystallized from AcOEt or MeOH. Yields and physical constants of the products are shown in Table II.

**$N^\alpha$-Asul-$N^\varepsilon$-Boc-Lys**——Prepared from $N^\varepsilon$-Boc-Lys (2.46 g), *N*-acetylsulfanilyl chloride (2.8 g) and $Et_3N$ (3 ml) in a mixture of DMF and $H_2O$. Recrystallized from a mixture of AcOEt and MeOH. Yield 1.8 g (41%), mp 172—175 °C, $[\alpha]_D^{25}$ +19.2° ($c$=1.0, MeOH), $Rf^3$ 0.71, $Rf^4$ 0.16. *Anal.* Calcd for $C_{19}H_{29}N_3O_7S$: C, 51.5; H, 6.6; N, 9.5. Found: C, 51.2; H, 6.6; N, 9.5.

**General Procedure for Preparation of Sulfanilylamino Acids**——Asul-amino acid methyl ester (1 g) was suspended in 6 N HCl (5 ml) and the mixture was kept at 100 °C for 40 min. The mixture became a clear solution, which was diluted with $H_2O$, washed with AcOEt and evaporated down. The residue was recrystallized from a small amount of $H_2O$. Asul-Asp was purified by Dowex 50 ($H^+$) column chromatography using the pH gradient method with pyridine–acetate buffer (0.1 mol, pH 3.8—5.8). The nitro group on the Arg derivative was removed by hydrogenation in dioxane before hydrolysis. $N^\alpha$-Sulfanilyl-Lys was prepared by hydrolysis of $N^\alpha$-Asul-$N^\varepsilon$-Boc-Lys and $N^\alpha$-Asul-$N^\varepsilon$-Z-Lys-OMe. The products were identical. Yields and physical constants of the products are shown

TABLE III. Yields and Physical Constants of Sulfanilylamino Acids



| R (Yields %) | mp (°C) | $[\alpha]_D^t$, $t$ (°C), $c$=1.0 | Analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| | | | +$H_2O$ | | |
| L-Ala (63) | 128—133 | −36.9°, $t$=27 | 36.2 | 5.1 | 9.4 |
| | | $H_2O$ | (36.1 | 5.2 | 9.3) |
| | | | +$H_2O$ | | |
| D-Ala (59) | 126—131 | +36.2°, $t$=27 | 36.2 | 5.1 | 9.4 |
| | | $H_2O$ | (36.1 | 5.1 | 9.2) |
| Pro (68) | 133—135 | −93.5°, $t$=25 | 43.2 | 4.6 | 9.2 |
| | | MeOH | (43.5 | 4.7 | 9.5) |
| | | | +$H_2O$ | | |
| Lys·HCl (66) | 195—212 | +6.9°, $t$=27 | 36.7 | 5.9 | 10.7 |
| | | $H_2O$ | (36.5 | 5.9 | 10.4) |
| | | | +1/2$H_2O$[a] | | |
| Asp (68) | 169—176 | −2.9°, $t$=33 | 40.4 | 4.4 | 9.4 |
| | | 1 N HCl | (40.6 | 4.2 | 9.5) |
| | | | +2$H_2O$ | | |
| Arg·HCl (60) | 140—153 | +5.7°, $t$=27 | 32.9 | 5.8 | 10.2 |
| | | $H_2O$ | (32.6 | 6.0 | 10.0) |

a) Not HCl salt. Purified by ion-exchange column chromatography.

Table IV. Yields and Physical Constants of Sulfonyllysines

| Materials (Yields %) | mp (°C) | $[\alpha]_D^t$, $t$ (°C) $c=1.0$ | Analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| Z<br>Asul–Lys<br>(54) | 167—170 | +17.3°, $t=26$<br>MeOH | 55.3<br>(55.4 | 5.7<br>5.7 | 8.8<br>8.6) |
| Asul<br>Asul–Lys<br>(51) | 168—171 | +16.6°, $t=26$<br>MeOH | 48.9<br>(48.8 | 5.2<br>5.3 | 10.4<br>10.1) |
| Boc<br>Dns–Lys<br>(76) | 214 | −3.0°, $t=23$<br>MeOH | 57.6<br>(57.8 | 7.0<br>6.9 | 8.8<br>8.8) |
| Boc<br>Tos–Lys<br>(63) | 128—130 | −10.6°, $t=25$<br>MeOH | 54.0<br>(54.4 | 7.1<br>7.0 | 7.0<br>7.1) |
| Boc<br>Ms–Lys·DCHA<br>(50) | 188—192 | −5.5°, $t=25$<br>MeOH | 57.0<br>(57.0 | 9.4<br>9.6 | 8.3<br>8.7) |
| Tos<br>Tos–Lys·DCHA<br>(64) | 200—205 | +34.9°, $t=25$<br>MeOH | 60.4<br>(60.4 | 7.8<br>7.9 | 6.6<br>6.7) |

in Table III.

**Antibacterial Effects of the Sulfanilylamino Acids**——No antibacterial effect against various gram-positive and gram-negative organisms was observed even at a concentration of 100 $\mu$g/ml.

**General Procedure for Preparation of Sulfonyllysine Derivatives**——Lys (or a Lys derivative) was sulfonylated with the appropriate sulfonylchloride (10% excess) in a mixture of $H_2O$, DMF and $Et_3N$ at pH 9. After the reaction, the solvent was evaporated off and the residue was extracted with $H_2O$. The aqueous layer was washed with AcOEt and acidified with citric acid. The resulting precipitate was extracted with AcOEt and the AcOEt layer was washed with $H_2O$, dried over $Na_2SO_4$ and evaporated down. The residue was precipitated from AcOEt–petroleum ether. If the precipitate was syrupy, the product was crystallized as the dicyclohexylamine salt. Yields and physical constants of the products are summarized in Table IV.

**Fibrinolytic Activities of Sulfonyllysine Derivatives**——The activities were measured by the euglobulin clot lysis time (ELT) method reported by Von Kaulla and Schultz.[9] Samples were administered to rats by intravenous injection.

**Acknowledgement** The authors are grateful to the staff of the Department of Biochemistry, Research Institute, Daiichi Seiyaku Co., Ltd. for carrying out the fibrinolytic and antibacterial assays.

## References and Notes

1) Standard abbreviations for amino acids and protecting groups are used [*J. Biol. Chem.*, **247**, 977 (1972)]. Other abbreviations include: Asul, *N*-acetylsulfanilyl; Dns, dansyl; Ms, mesyl; Tos, tosyl; DCHA, dicyclohexylamine; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide.
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