Sulfanilyl Dipeptides for the Antiviral Agent

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From the view point of sulfanilamides for the anti-bacterial activity, amino acids were used instead of amine in this amido formation. In the initial experiments, acetylsulfanilyl amino acids were synthesized by the reaction of amino acids and acetylsulfanilyl chloride.

After the hydrolysis of acetylsulfanilyl amino acids, sulfanilyl amino acids were diazotized, and then, reacted with various active methylene compounds, such as 3-methyl-1-phenyl-5-pyrazolone, 2, 2-dimethyl-4, 6-dioxo-1, 3-dioxane, 2-acetonylbenzo-3, 1-oxazine-4-one and 4-hydroxycoumarin, to give the corresponding hydrazino derivatives.

The hydrazino derivatives obtained in suitable yields from the reaction with 4-hydroxycoumarin were used for the synthesis of dipeptides.

All sulfanilyl compounds were tested for the antiviral activity against HIV, FLUV (A), RSV, and HSV.

In 1932, Dogmagk began a study of a bright red dye later to be named Prontosil and found that it caused remarkable cures of streptococcal infections of mice.[1)]

However, Prontosil was inactive on bacterial cultures. Dogmagk's studies on Prontosil continued, and in 1933 the first of many human cures of severe staphyloccal septicemias was reported.[2)] Dogmagk even saved the life of his own daughter from a severe streptococcal infection. For his pioneering efforts in chemotherapy, Gerhard Dogmagk was awarded the Nobel prize for medicine and physiology in 1947.

Prontosil's inactivity in vitro but excellent activity in vivo attracted much attention. In 1935, Trefouel, Trefouel, Nitti, and Bovet[3)] reported their conclusion from a structure-activity study of sulfonamide azo dyes that the azo linkage was metabolically broken to release the active ingredient, sulfanilamide. Their reported finding was confirmed in 1937 when Fuller[4)] isolated sulfanilamide from the blood and urine of patients being treated with Prontosil. Modern chemotherapy and the concept of the prodrug were firmly established.

Following Prontosil's dramatic successes, a cascade of sulfanilamide derivatives began to be synthesized and tested—over 4500 by 1948 alone.[5)] From these only about two dozen have actually been used in clinical practice. In the late 1940's, penicillins began to replace the sulfonamides in chemotherapy. This was largely because of the sulfanilamides' toxicity for some patients and because sulfanilamide-resistant bacterial strains were becoming an increasing problem—the result of indiscriminant use worldwide.

Today, a few sulfonamide and especially sulfonamide - trimethoprim combinations are extensively used for urinary tract infections or for burn therapy.[6-10)] They are also the drugs of choice or alternates for a few other types of infections, but their overall use is otherwise quite limited in modern antimicrobial chemotherapy, having been largely replaced by antibiotics.

Recently, some dansyl amino acids were reported as antiviral agents. In this viewpoint, we considered the application of sulfonamides having amino acid moiety instead of aryl amines for the antiviral agents.

In general, virus life cycle consists of seven main steps. In the case of HIV (human immunodeficiency virus), during infection (1), the

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membrane of the virus fuses with that of the target cell, and the nucleic acid core of the nucleocapsid enters the cytoplasm of the host cell (2). Once there, the viral RNA is first transcribed into an RNA/DNA hybrid (3) and then into dsDNA (4). Reverse transcriptase, the enzyme catalyzing these reaction, is of viral (not host) origin. The dsDNA subsequently becomes integrated into the genome of the host cell (5), where it can remain in an inactive state for long period of time. When it becomes active, the DNA fragment corresponding to the viral genome is transcribed by the enzymes of the host cell (6). Not only does the host cell replicate the viral ssRNA, but it also transcribes the mRNA molecules that encode the precursors of the viral proteins. These precursors are integrated into the plasma membrane of the cell before undergoing proteolytic modification (7). The cycle comes to completion with the release of new virus particles.

One of the glycoproteins (fusion protein), which presents on the surface of virus, is involved in virus penetration, through fusion of viral and cell fusion and homolysis, activities which are activated by proteolytic cleavage of the protein by a host protease to yield two dislike-linked polypeptides, F_1 and F_2. F_1 peptide plays a most important role at the first stage of virus infection. We focused the inhibition of the viral infection at the first stage (1) by novel sulfanilyl dipeptides similar to the amino sequence of F_1 peptide. F_1 peptides of several viruses have hydrophobic amino acid sequences. We synthesized peptides using hydrophobic amino acids, such as Gly, Ala, Leu, Val. Pro, and Phe. This project may be connected with the development of vaccine.

Materials and Methods

Chemicals

Acetylsulfanily amino acids (**I**) were easily prepared by the reaction of acetylsulfanily chloride with sodium salt of amino acids in the presence of equimolar amount of triethyl amine. Amino acids used were hydrophobic species, such as Gly, Ala, Leu, Val, Pro, and Phe. Yields, melting points, and $[\alpha]_D$ of acetylsulfanilyl amino acids were shown at Table I.

The hydrazino adducts of active methylene compounds with diazonium salts derived from sulfanilyl amino acids, which were obtained by the hydrolysis of acetylsulfanilyl amino acids in alkaline aqueous solution, were obtained in suitable yields. Using active methylene compounds were 3-methyl-1-phenyl-5-pyrazolone (Pyz), 2, 2 -dimethyl-4, 6-dioxo-1, 3-dioxane (Dox), 2-acetonylbenzo-3, 1-oxazine-4-one (Box) and 4-hydroxycoumarin (Com), all of which were bioactive precompounds. Yields, melting points, and $[\alpha]_D$ of hydrazino adducts were shown at Table II.

The reaction of hydroxycoumarin with diazonium salts obtained from acetylsulfanilyl amino acids gave the corresponding hydrazino adducts (**V**) in good yields. These adducts were used for the synthesis of dipeptides in usual manner (Active Ester Method using dicyclohexylcarbodiimide and hydroxysuccinimide). Yields, melting points, and $[\alpha]_D$ of dipeptides obtained were shown at Table III-1 and III-2.

All products were identified with spectral measurements and elemental analyses.

Cells and Viruses

HIV type 1 and HIV type 2 were obtained from the culture supernatant of HUT-78 cell lines peristently infected with HTLV-IIIB and CEM cells peristently infected with LAV-2, respectively. HSV (herpes simplex virus) (KOS strain) was propagated in human embryonic fibroblasts (HEF). RSV (resiratory syncitial virus) (Long strain) was provided from Sendai National Hospital. FLUV-A (influenza-A type virus)/Ishikawa/7/98 (H3N2) was laboratory strain which was passed more than 10 times in hen's embryonated eggs and 3 times in MDCK cells.

Cytotoxicity of the medicine was determined by measuring of viability of infected each host cells by the corresponding viruses.[11,12] The concentration of the medicine that reduced the via-

bility of infected host cells to 50% of the control was estimated as the 50% cytotoxic concentration (CC_{50}).

Results and Discussion

In acid solution, an amino acid is protonated and exists primarily as a cation; in basic solution, an amino acid is deprotonated and exists primarily as an anion. Thus, at some intermediate point, the amino acid must be exactly balanced between anionic and cationic forms and exist primarily as the neutral, dipolar zwitterion. The amino group of amino acid has essentially basic property. However, in the N-protected product with acetylsulfanilyl chloride, the amino group was changed to acidic. This transformation of amino group property was possible to develop to the application for the antiviral reagents.

We obtained 24 hydrazinosulfonyl amino acids (**II–V**) by the reaction of active methylene compounds with diazonium salts derived from acetylsulfanilyl amino acids (**I**). These products showed inactivity against viruses of 4 species, such as HIV, FLUV-A, RSV, and HSV.

Thirty six Com-hydrazinosulfonyl dipeptides (**VI**) were obtained from the synthesis of peptide using coumarin-hydrazino adducts by means of active ester method. All dipeptides had no antiviral activity, however, Com-S-Pro-Phe had cytotoxicity against host cells infected with FLUV-A in a CC_{50} value of 78.786 mg/ml, and Com-S-Phe-Ala and Com-S-Phe-Pro had cytotoxicity against host cells infected with HSV in CC_{50} values of 55.388 mg/ml and 74.227 mg/ml, respectively. The results of pharmacological test were clarified that dipeptides had a low possibility for the antiviral activity.

The continuous study, the elongation of Com-S-dipeptides to tripeptides for the antiviral activity, is under investigation.

Acknowledgments

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Synthesis of Acetylsulfanilyl Amino Acids(AcS-AA)

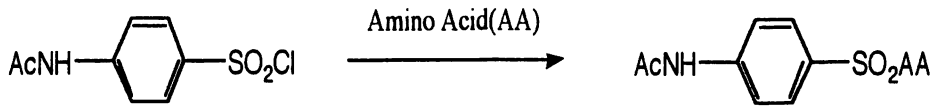
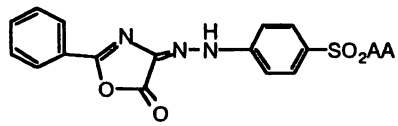


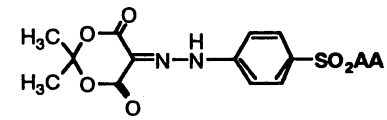
Table I. Data of Acetylsulfanilyl Amino Acids

	mp(℃)	Yield(%)	$[\alpha]_D$
AcS-Gly	237 - 238	51	-
AcS-Ala	179 - 181	43.5	-26.4°
AcS-Leu	220 - 223	41	4.4°
AcS-Val	239 - 242	43	19.6°
AcS-Phe	200 - 203	48	-3.2°
AcS-Pro	227 - 229	66	-152.4°

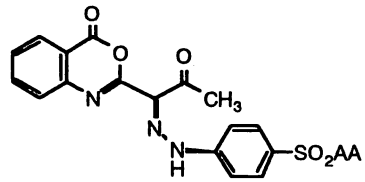
Structures of Hydrazinosulfonyl Amino Acids



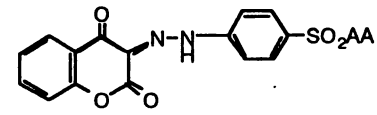
Pyz-S-AA(II)



Dox-S-AA(III)



Box-S-AA(IV)

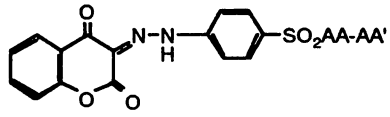


Com-S-AA(V)

Table II. Data of Hydrazinosulfonyl Amino Acids

	mp(℃)	Yield(%)	$[\alpha]_D$
Pyz-S-Gly	231 - 233	60	-
Pyz-S-Ala	205 - 208	53	7.2°
Pyz-S-Leu	115 - 118	48	59.6°
Pyz-S-Val	182 - 185	41	86.4°
Pyz-S-Phe	148 - 151	34	30.8°
Pyz-S-Pro	119 - 122	41	-63.6°
Dox-S-Gly	169 - 172	43	-
Dox-S-Ala	155 - 157	11	-6.4°
Dox-S-Leu	175 - 177	43	43.2°
Dox-S-Val	203 - 206	40	16.4°
Dox-S-Phe	175 - 179	47.5	38.8°
Box-S-Gly	128 - 131	70	-
Box-S-Ala	139 - 142	29	-2.4°
Box-S-Leu	141 - 144	64	14.4°
Box-S-Val	138 - 141	67	16.0°
Box-S-Phe	136 - 139	48	46.0°
Box-S-Pro	167 - 170	53	-56.8°
Com-S-Gly	263 - 265	52	-
Com-S-Ala	265 - 268	60	-100.0°
Com-S-Leu	220 - 223	67	-224.4°
Com-S-Val	241 - 244	63	-208.8°
Com-S-Phe	264 - 265	64	-13.2°
Com-S-Pro	254 - 256	64	-310.4°

Structures of Coumarinyl-hydrazinosulfonyl Dipeptides



Com-S-Dipetides(VI)

Table III-1. Data of Com-S-Dipeptides

Dipeptide	mp(℃)	Yield(%)	$[\alpha]_D$
Gly-Gly	208 - 210	57.5	-
Gly-Ala	220 - 221	91	192.0°
Gly-Leu	183 - 185	54	17.6°
Gly-Val	178 - 181	84	116.4°
Gly-Phe	165 - 167	80	151.2°
Gly-Pro	175 - 178	55.5	-141.2°
Ala-Gly	173 - 175	70.5	-
Ala-Ala	235 - 238	62	47.6°
Ala-Leu	116 - 118	64	15.2°
Ala-Val	146 - 148	54	24.4°
Ala-Phe	132 - 135	73	9.2°
Ala-Pro	145 - 146	62	-27.6°
Leu-Gly	132 - 135	68	-
Leu-Ala	164 - 168	64	28.0°
Leu-Leu	141 - 143	85.5	-12.4°
Leu-Val	121 - 124	67	-83.6°
Leu-Phe	115 - 118	78	-15.2°
Leu-Pro	135 - 138	54	-90.4°
Val-Gly	209 - 211	80	-
Val-Ala	175 - 178	75	31.6°
Val-Leu	187 - 190	39	-28.4°
Val-Val	143 - 146	63.5	79.6°
Val-Phe	122 - 124	57	-24.8°
Val-Pro	136 - 139	92	59.6°

Table III-2. Data of Com-S-Dipeptides

Dipeptides	mp(℃)	Yield(%)	$[\alpha]_D$
Phe-Gly	153 - 158	54.5	-
Phe-Ala	150 - 153	38	39.6°
Phe-Leu	141 - 143	73	105.6°
Phe-Val	148 - 151	64.5	-63.2°
Phe-Phe	104 - 107	86	53.6°
Phe-Pro	113 - 116	51	-96.0°
Pro-Gly	173 - 176	63.5	-
Pro-Ala	187 - 190	76.5	-109.6°
Pro-Leu	128 - 131	72	-65.6°
Pro-Val	149 - 152	80	-159.6°
Pro-Phe	115 - 118	61.5	-58.0°
Pro-Pro	253 - 255	53	-58.8°