[Chem. Pharm. Bull. 13(3) 261~267 (1965)]

UDC 615.778.25-092.21

31. Toyozo Uno*1, Teishiro Kushima, and Masahiko Fujimoto*2 : Studies on the Metabolism of Sulfadimethoxine. I.*3 On the Excreted Substance in the Human Urine after Oral Administration of Sulfadimethoxine.

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Regarding to the reports as the metabolism of sulfadimethoxine, N^1-(2,6-dimethoxy-4-pyrimidinyl)sulfanylamide, one of the long acting sulfa-drugs, the existence of glucuronide including glucuronolactone[1] in human urine has been reported, and also there have been some reports[2~5] regarding its determination in blood and in urine as free form and acetylated form by Bratton-Marshall method. It was mentioned that there existed only 15~20% acetylated form in urine and above 70% of the rest was glucuronide.[6~8]

Previously we reported*3 that sulfadimethoxine, N^4-acetylsulfadimethoxine, and sulfadimethoxine-N-glucuronide were found in the human urine, and the ultraviolet and infrared spectra of that isolated glucuronide suggested to be sulfadimethoxine-N^1-glucuronide.

Shortly after our report, Bridges, *et al.*[9] claimed that ammonium salt of sulfadimethoxine-N^1-glucuronide was isolated, and its S-benzyl thiuronium salt was identical with the synthetic material. They also found sulfadimethoxine-N^4-glucuronide in addition to the above three substances.

In the present paper, further examination above the sulfadimethoxine-N^1-glucuronide are reported with the hitherto investigation.

The sample solution (in Experimental) was examined by paper chromatography using several kinds of solvents. Three spots were detected and named Nos. 1, 2, and 3 respectively.

The standard substances dissolved in normal urine and in the sample urine were developed at the same time. The Rf values of the excrements and the standard substances are shown on Table I.

Spot No. 1. developed yellow color promptly by Ehrlich's reagent and red color by Tsuda's reagent. Its Rf values corresponded with those of standard sulfadimethoxine on the each solvent. Then the spot No. 1 was confirmed as unchanged sulfadimethoxine.

Spot No. 2 turned to yellow by spraying the Ehrlich's reagent after hydrolysis with 10% hydrochloric acid, and its Rf values corresponded with those of N^4-acetylsulfadimethoxine on the each solvent. The sample solution was developed on a filter paper,

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*3 A part of this work was reported at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, November 2, 1963.

1) B. A. Koechlin, W. Kern, R. Engelberg : Antibiotic Med. and Clin. Therapy, **6**, (supple. 1.), 22~31 (1959).
2) T. Sakuma : Am. J. Med. Sci., **1960**, 142.
3) W. P. Boger : Antibiotic Annual., **1958~1959**, 48.
4) S. T. Madsen : Antibiotic Med. and Clin. Therapy, 8, 87~97 (1961).
5) H. Takahashi, *et al.* : Chemotherapy, **7**, 259 (1959).
6) H. Nishimura, *et al.* : *Ibid.*, 8, 14 (1960).
7) K. Sakai, *et al.* : *Ibid.*, 8, 406 (1960).
8) H. Naoshita, *et al.* : *Ibid.*, 8, 521 (1960).
9) J. W. Bridges, M. R. Kibby, R. T. Williams : Biochem. J., **9**, 12 P. (1964).

Table I. Rf Values of Standards and Excrements in Human Urine after Administration of Sulfadimethoxine

	BuOH satd. with H_2O	BuOH–Pr. OH–H_2O (2:1:1)	BuOH satd. with 3% NH_4OH	BuOH–Pr. OH–0.1*N* NH_4OH (2:1:1)	BuOH–AcOH–H_2O (5:1:4)	BuOH satd. with *N*HCl
S. D.	0. 85	0. 86	0. 37	0. 49	0. 89	0. 83
N^4-acetyl-S. D.	0. 86	0. 87	0. 50	0. 63	0. 89	decomp.
N^1,N^4-diacetyl-S. D.	0. 85	0. 85	tailing	0. 71	0. 89	〃
S. D-N^4-sulfonate	0. 35	0. 51	0. 02～0. 21	0. 00	0. 53	〃
Sulfanilamide	0. 55	0. 66	0. 53	0. 63	0. 58	—
No. 1	0. 84	0. 86	0. 37	0. 49	0. 87	0. 83
No. 2	0. 84	0. 86	0. 52	0. 63	0. 89	decomp.
No. 3	0. 15	0. 35	0. 16	0. 35	0. 52	0. 42

S. D. =sulfadimethoxine

40×40 cm., using butanol saturated with 3% ammonium hydroxide solvent and the section of No. 2 was cut off, and eluted by 3% ammonium hydroxide solution. After hydrolysis with 10% sodium hydroxide solution on a steam bath and neutralization with hydrochloric acid, the Rf value corresponded with that of sulfadimethoxine.

In the paper electrophoresis using 1% ammonium hydroxide solution, the substance extracted from the spot No. 2 travelled to positive as well as the standard N^4-acetylsulfadimethoxine, however N^1,N^4-diacetylsulfadimethoxine stayed on the original point. Therefore it was confirmed from these facts that the spot No. 2 was N^4-acetylsulfadimethoxine.

Spot No. 3 was the largest of the three spots, and promptly developed yellow color by spraying Ehrlich's reagent and red by Tsuda's reagent.

As it colored by naphthoresorcinol reagent, it was pressumed a sort of glucuronide. By extracting spot No. 3 a slightly yellowish non-crystalline substance was obtained. This was very soluble in water, soluble in methanol, and not soluble in the organic solvents as ethanol, acetone, ethyl ether and chloroform.

The substance travelled to positive in the paper electrophoresis using 1% ammonium hydroxide solution. In spite of various attempts, purification was unsuccessful and on ignition about 2% of ash was remained. But it was shown to be chromatographically pure. Its ultraviolet and infrared spectra are shown in Fig. 1 and Fig. 2 respectively.

From these facts, the spot No. 3 was pressumed N-glucuronide.

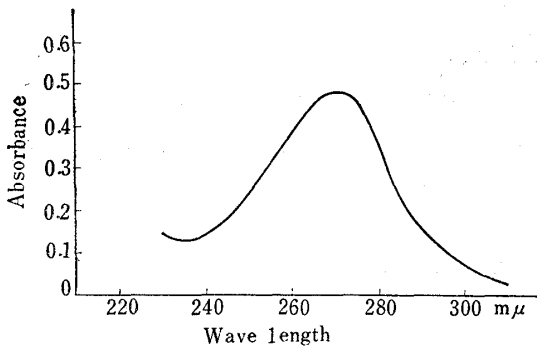

Fig. 1. Ultraviolet Absorption Spectrum of Extract of Spot No. 3

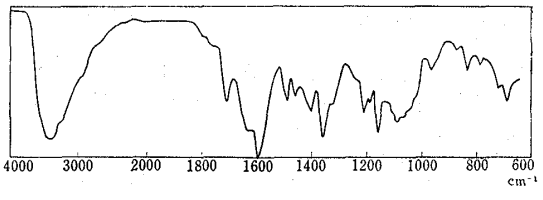

Fig. 2. Infrared Absorption Spectrum of Extract of Spot No. 3

Generally, sulfa-drugs including nitrogen hetero ring are considered to be in two forms, amido form and imido form. Uno and Ueda[10] reported regarding to infrared spectra of N-pyridine, thiazole and pyrimidine derivatives of sulfanilamide, that SO_2 symmetric stretching bands were located between 1170 cm^{-1} to 1145 cm^{-1} in amido form and 1145 cm^{-1} to 1130 cm^{-1} in imido form.

On the other hand, it was found at 1150 cm^{-1} in sulfadimethoxine, and at 1157 cm^{-1} in the spot No. 3 from our spectra. From these facts, it is considered that the substance separated from the spot No. 3 has amido form and N^1-glucuronide.

For the isolation of the glucuronide, a new method in experimental was employed to increase its yield. Attempts to crystallize the isolated glucuronide were unsuccessful, but it was shown to be chromatographically pure. The isolated glucuronide by this method and the extract of No. 3 spot above mentioned had identical infrared spectra, and the Rf values of the isolated glucuronide were in good agreement with those of No. 3 on Table I.

Attempts to crystallize the glucuronide were not successful. Then the glucuronide was methylated using absolute methanol saturated with anhydrous hydrogen chloride. The methyl derivative was isolated as faint yellowish amorphous substance, but failed to crystallize, and it was chromatographically pure. The ultraviolet absorption spectrum of the methyl derivative exhibited only one peak at 268 mμ, and was essentially identical with the spectrum of the isolated glucuronide before methylation.

Table II. Rf Values of the Methyl Derivatives of the Glucuronide

	BuOH satd. with H_2O	BuOH–AcOH–H_2O (5:1:4)	BuOH satd. with 3% NH_4OH
Rf	0.60	0.72	0.11～0.45 (tailing)

Toyo Roshi No. 51, 2×40 cm. 16 hr.

Table III. Rf Values of the Methyl Acetyl Derivative of the Glucuronide and IV

	BuOH satd. with H_2O	BuOH–AcOH–H_2O (5:1:4)	BuOH satd. with 3% NH_4OH
Methyl-acetyl derivative	0.90	0.89	0.60～0.85
IV	0.85	0.90	0.58～0.88

Toyo Roshi No. 51, 2×40 cm. 16 hr.

The methyl derivative was acetylated with acetic anhydride and pyridine, and the methyl acetyl derivative of the glucuronide was purified by liquid chromatography using silica gel and chloroform, and the white amorphous substance, m.p. 104～106°(decomp.) was obtained. The ultraviolet absorption spectrum of the methyl acetyl derivative exhibited only one peak at 266 mμ, and essentially identical with the spectrum of the methyl derivative. Table III and Fig. 3 show the Rf values and the infrared spectrum of the methyl acetyl derivative respectively.

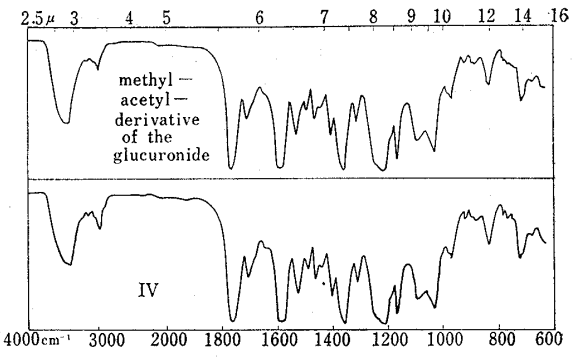

Fig. 3. Infrared Spectra of the Methyl Acetyl Derivative of the Glucuronide and IV

10) T. Uno, K. Machida, K. Hanai, S. Sasaki, M. Ueda : This Bulletin, 11, 704 (1963).

On the other hand, sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)-uronate (Ⅲ) was synthesized from potassium salt of sulfadimethoxine (I), and methyl-(1-bromo-1-deoxy-tri-O-acetyl-α-D-glucopyranosid)uronate (Ⅱ) according to Okumura's method.[11] By the N^4-acetylation of Ⅲ with acetic anhydride and pyridine, sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate-N^4-acetate (Ⅳ) was synthesized. The ultraviolet absorption spectrum and the paper chromatograms of Ⅳ were in good agreement with those of the methyl acetyl derivative of the glucuronide in human urine.

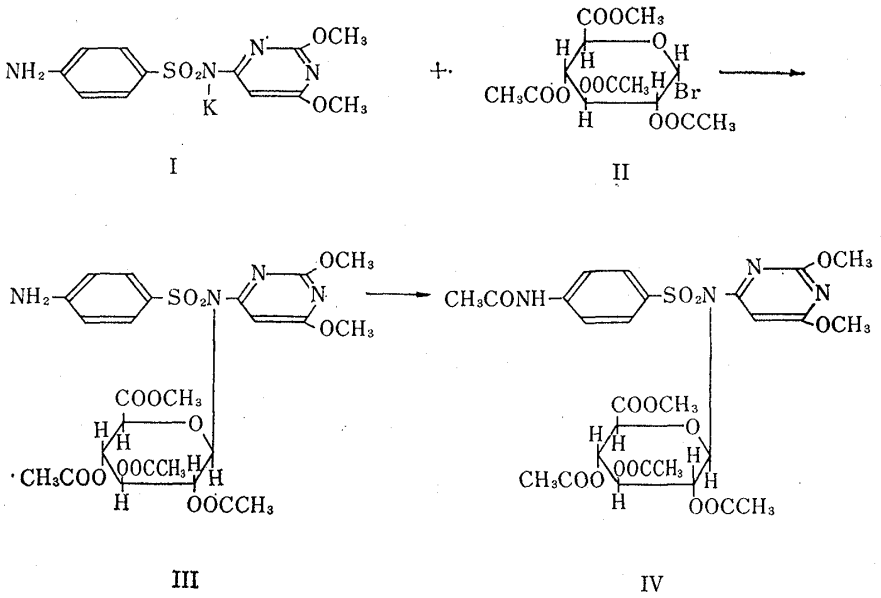

Chart 1.

Moreover, Ⅳ and the methyl acetyl derivative of the glucuronide extracted from human urine had identical infrared spectra.

As described above, the methyl acetyl derivative of the glucuronide in human urine, was confirmed sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate-N^4-acetate.

From this facts and the infrared spectrum of the glucuronide (Fig. 2), the glucuronide found in human urine after oral administration of sulfadimethoxine, was confirmed to be sufadimethoxine-N^1-β-D-glucopyranoside or its lactone form.

Furthermore, in order to examine phenolic substance, paper chromatography was carried out using butanol saturated with water, butanol saturated with 3% ammonium hydroxide, and butanol-acetic acid-water (5:1:4). Also, paper electrophoresis was carried out using 1% borax as electrolytic solution. Ferric chloride reagent and phosphomolibdic acid reagent were used as detecting reagents, but no phenolic substances were found.

Experimental

Preparation of Sample Solution——Human urine was collected for 0~60 hr. after oral administration of 1 g. of sulfadimethoxine, and was concentrated to about 1/30 of its volume under reduced pressure at temperature below 30°. Salts deposited were removed with centrifugation after addition of about the same volume of EtOH. The supernatant solution was used for the sample solution. The sample solution for testing phenolic substance was prepared according to the following processes.

11) K. Okumura : Ann. Rep. Tanabe Seiyaku Co. Ltd., 6, 61 (1961); Yakugakukenkyu, 34, 36 (1962).

10 ml. of 5% HCl was added to 5 ml. of the above supernatant solution and the mixture was heated for 30 min. on the boiling bath, neutralized with 5% NaOH, condensed to about 1/5 volume under reduced pressure, and the salt deposited was removed with centrifugation after addition of about the same volume of EtOH. The supernatant solution was used for the sample solution for testing phenolic substance.

Identification of Each Excrement——Paperchromatography and paper electrophoresis were employed for the identification. Metabolites were compared with synthetic substance using various developing solvents.

a) Paper chromatography.

Paper : Toyo Roshi No. 51, 2×40 cm.
Solvent : BuOH saturated with H_2O, BuOH–AcOH–H_2O (5:1:4), BuOH saturated with 3% NH_4OH, BuOH–PrOH–H_2O (2:1:1), BuOH–PrOH–0.1*N* NH_4OH (2:1:1), BuOH saturated with *N* HCl.
Method : One dimensional ascending method.
Developing hour : 16 hr.
Temperature : 22~26°.

Detecting reagent : Ehrlich's reagent (for detection of amino group) : 2% *p*-dimethylaminobenzaldehyde EtOH solution containing a volume of 1/50 of conc. HCl, Tsuda's reagent (for detection of amino group) : 0.1% N-(2-dimethylaminoethyl)-1-naphthylamine oxalate EtOH solution, naphthoresorcinol reagent (for detection of glucuronide) : mixed solution of the same volume of 0.2% 1,3-dihydroxynaphthalene EtOH solution and 2% trichloroacetic acid aqueous solution, Ferric chloride reagent (for detection of phenolic hydroxyl group) : 2% $FeCl_3$ solution, phosphomolibdic acid reagent (for detection of phenolic hydroxyl group) : conc. NH_4OH was sprayed after spraying 2% phosphomolibdic acid aqueous solution.

There existed considerable salts and normal urine ingredients in the sample solution treated with EtOH. Because they may change Rf values, it was treated as follows.

1. The sample solution.
2. The sample solution containing the standard substance.
3. The solution of normal urine treated with the same method as the sample solution.
4. The solution of normal urine containing the standard substance.

These solution were put on the filter paper, and developed simultaneously.

b) Paper electrophoresis.

Paper : Toyo Roshi No. 51, 2×40 cm.
Solution : 1% NH_4OH, 1% borax.
Method : 20~25 v./cm. 2 hr.

Preparation of Standard Substance——Sulfadimethoxine : Commercially available sulfadimethoxine was purified by recrystallization from EtOH. m.p. 202°.

Sulfanilamide : Commercially available sulfanilamide was purified by recrystallization from EtOH. m.p. 164.5°.

N^4-acetylsulfadimethoxine[12] : Prepared by acetylation of sulfadimethoxine. m.p. 208~210°. *Anal.* Calcd. for $C_{14}H_{16}O_5N_4S$: C, 47.71; H, 4.57; N, 15.90. Found : C, 47.69; H, 4.59; N, 15.78.

N^1,N^4-diacetylsulfadimethoxine[12] : Prepared by diacetylation of sulfadimethoxine. m.p. 200~202°. *Anal.* Calcd. for $C_{16}H_{18}O_6N_4S$: C, 48.72; H, 4.59; N, 14.20. Found : C, 48.98; H, 4.43; N, 14.11.

Dipotassium sulfadimethoxine-N^4-sulfonate : The method of Uno and Kono[13] was applied to the preparation of this compound. 4 g. of $ClSO_2H$ was slowly added to 40 ml. of anhydrous pyridine under stirring and cooling with ice. To the mixture, 10 g. sulfadimethoxine was added, and was allowed to stand overnight. The mixture was poured into 80 ml. of *N* KOH, and pyridine was removed by extraction with ether. Aqueous layer was concentrated under reduced pressure to about 20 ml., and was allowed to stand in a refrigerator. A white precipitate was collected. *Anal.* Calcd. for $C_{12}H_{12}O_7N_4S_2K_2$ (Dipotassium sulfadimethoxine-N^4-sulfonate) : C, 30.82; H, 2.80; N, 11.98. Found : C, 30.41; H, 3.22; N, 11.68.

Separation of Spot No. 3——4.8 L. of human urine was collected for 0~60 hr. after administration of 2 g. of sulfadimethoxine for every 2 days, and filtered. To the filtrate, 80 g. of charcoal was added, filtered and washed after allowing to stand for several hours. 2 L. of MeOH–H_2O–NH_4OH (1:1:0.08) solution was added on the separated charcoal, and filtered after stirring for 30 min. at 35°. The filtrate was condensed to syrup under reduced pressure, and fixed on the 16 leaves of Toyo Roshi No. 50 (filter paper) 40×40 cm. which had been previously well washed. Paper chromatography was carried out using BuOH satd. with H_2O as developing solvent by Uno and Ueda's two dimensional developing box.[14]

The part of spot No. 3 was extracted with H_2O, condensed under reduced pressure, and the chromatography was repeated on 8 leaves of the same filter paper using BuOH–PrOH–H_2O (2:1:1) as develop-

12) Y. Kawashima : Yukigoseikagaku Kyokaishi, **20**, 395 (1962).
13) T. Uno, T. Kono : Yakugaku Zasshi, **80**, 201 (1960).
14) T. Uno, M. Ueda : *Ibid.*, **80**, 1785 (1960).

ing solvent. The H_2O extract was condensed to dryness, and 130 mg. of brownish amorphous substance was obtained, which was dissolved in 2 ml. of H_2O, and absorbed in the column of ion exchange resin Dia ion SK # 1A (H form) (radius : 1 cm., length : 10 cm.), well washed with 300 ml. of H_2O and eluted with 2% NH_4OH. The eluate was condensed to dryness under reduced pressure, and 59 mg. of faint brownish amorphous substance was obtained. 59 mg. of which was dissolved in 1 ml. of MeOH, and filtered. To the filtrate, 3 ml. of EtOH was added and the precipitate was filtered off. 27 mg. of faint yellowish amorphous substance was obtained. Attempts to recrystallize the substance from several solvents were unsuccessful. *Anal.* Calcd. for $C_{18}H_{25}O_{10}N_5S \cdot H_2O$ (ammonium sulfadimethoxine-N^1-glucosiduronate$\cdot H_2O$) : C, 41.45; H, 5.22. Found : C, 41.15; H, 5.53.

To increase the yield, we also employed next method. Urine of three men was collected for 0~94 hr. after administration of 1.5 g. of sulfadimethoxine once a day for two days, and filtered. 86 g. of activated charcoal was added to the filtrate. After standing 4 hr., charcoal was filtered and washed with H_2O. This charcoal was mixed with 27 g. of cellulose powder, packed to chromato-tube (radius : 5.5 cm.), and extracted with the mixed solvent of MeOH-H_2O-conc. NH_4OH (1:1:0.08). The positive portion for aromatic amine was collected. (about 4.7 L.). The following evaporation procedure was carried out below 37°. The extracted solution was concentrated to syrup under reduced pressure. To the syrup, 200 ml. of abs. MeOH was added, cooled with ice, and the precipitate was filtered off. The filtrate was again concentrated to syrup, 300 ml. of abs. MeOH was adeed, cooled with ice and the precipitate was filtered off. The filtrate was concentrated and the crude gum (34 g.) was obtained. The crude gum was dissolved in about 40 ml. of H_2O, then the solution was adjusted to pH 4 with AcOH, and treated with saturated $(AcO)_2Pb$ solution. The filtrate separated from the precipitate was adjusted to pH 7.5 with NH_4OH and saturated basic lead solution was added in excess. The basic lead salt was collected, washed with H_2O, decomposed with H_2S in MeOH and the filtrate was evaporated to dryness under reduced pressure. The faint red brownish substance (0.8 g.) was obtained.

The Methyl Derivative of the Glucuronide——126.5 mg. of the glucuronide described above was dissolved in 4 ml. of abs. MeOH saturated with anhydrous HCl gas at 0°, the mixture was allowed to stand for 30 min., and the solvent was removed by evaporation under reduced pressure at below 0°. The residue was dissolved in a small amount of MeOH, the solution was added to the silica gel column (radius: 1 cm., length : 10 cm.) and developed with the mixed solvent of MeOH-$CHCl_3$ (1:7). The positive portion for aromatic amine reaction was collected and concentrated under reduced pressure to dryness (15 mg. obtained). 15 mg. of the residue was dissolved in a small amount of MeOH. The solution was fixed to the filter paper that had been washed with H_2O (Toyo Roshi No. 50, 40×40 cm.) and were developed with the mixed solvent of BuOH-AcOH-H_2O (5:1:4) to the top of paper. After drying in air, the methyl derivative portion was extracted with MeOH. The extracted solution was concentrated to dryness (6.2 mg.), and it was dissolved in a small amount of abs. MeOH and filtered. Ether was added in excess and the precipitate was collected. The faint yellowish amorphous substance was obtained (1.4 mg.).

The Methyl Acetyl Derivative of the Glucuronide——125.6 mg. of the glucuronide was methylated as described above and the reaction mixture was evaporated to dryness under reduced pressure. Without further purification, the residue was dissolved in 366 mg. of pyridine and 702 mg. of Ac_2O. The mixture was allowed to stand overnight at room temperature, and the solvent was removed under reduced pressure (6 mm. Hg) at 40°. The residue was dissolved in 30 ml. of $CHCl_3$, extracted sufficiently with H_2O, then it was dried over anhydrous Na_2SO_4, and the solution was evaporated to dryness under reduced pressure (140.3 mg.). 140.3 mg. of the residue was purified by liquid chromatography using silica gel column (radius : 1 cm., length : 15 cm.) and $CHCl_3$. This liquid chromatography was repeated three times and a white amorphous substance, m.p. 104~106° (decomp.), was obtained (25 mg.). *Anal.* Calcd. for $C_{27}H_{32}O_{14}N_4S \cdot H_2O$: C, 47.23; H, 4.99; N, 8.16. Found : C, 47.69; H, 4.81; N, 7.82.

Sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate (III)——6.2 g. of potassium salt of sulfadimethoxine (I) was dissolved in 4 ml. of H_2O, 7.5 g. of methyl (1-bromo-1-deoxy-tri-O-acetyl-α-D-glucopyranosid)uronate (II) was dissolved in 22.5 ml. of acetone, and the two solutions were mixed. The mixture was allowed to stand overnight at room temperature.

Addition of H_2O (46 ml.) to the mixture gave brownish gum substance which was purified by liquid chromatography using silica gel column and $CHCl_3$. A white amorphous substance m.p. 103° (decomp.), was obtained. Yield : 3.43 g. $[\alpha]_D^{15}$ +77.8 (c=1.47, MeOH). *Anal.* Calcd. for $C_{25}H_{30}O_{13}N_4S$: C, 47.92; H, 4.83; N, 8.94. Found : C, 48.05; H, 5.00; N, 8.78.

Sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate-N^4-acetate (IV)——319.6 mg. of III was dissolved in the mixture of 694 mg. of pyridine and 624.9 mg. of Ac_2O. The mixture was allowed to stand overnight at room temperature and the solvent was removed under reduced pressure (6 mm. Hg) at 40°. The residue was dissolved in 30 ml. of $CHCl_3$, extracted sufficiently with H_2O, then it was dried over anhydrous Na_2SO_4 and the solution was evaporated to dryness under reduced pressure. A part of the obtained substance (140.3 mg. in 339.2 mg.) was purified by liquid chromatography using silica gel column and $CHCl_3$. This chromatography was repeated twice and a white amorphous substance, m.p. 104~106° (decomp.), was obtained. *Anal.* Calcd. for $C_{27}H_{32}O_{14}N_4S \cdot H_2O$: C, 47.23; H, 4.99; N, 8.16. Found : C, 47.71; H, 4.74; N, 8.26.

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Summary

Metabolic products of sulfadimethoxine excreted in human urine were examined and three metabolites were separated. Unchanged sulfadimethoxine and N^4-acetylsulfadimethoxine were confirmed by paper chromatography and electrophoresis.

And, the other one was sulfadimethoxine-N-glucuronide, and the methyl acetyl derivative of the glucuronide was confirmed sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate-N^4-acetate.

Sulfadimethoxine-N-glucuronide found in human urine was decided to be sulfadimethoxine-N^1-β-D-glucopyranoside or its lactone form.

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32. Hiroshi Mitsuhashi,*1 Tadashi Sato,*2 Taro Nomura, and Ikuko Takemori*1 : Studies on the Constituents of Asclepiadaceae Plants. XIV.*3 Structure of Tomentogenin.*4

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The isolation of sarcostin and tomentogenin from the stems of *Marsdenia tomentosa* DECNE (Asclepiadaceae) has been reported previously[1] from these laboratories and a tentative structure of tomentogenin was proposed.*4

In the present paper, some later findings on the structure of tomentogenin are reported. The plant material was collected at Amatsu, Chiba, in June 1962, and dried at 60°. The powdered material was treated as shown in Chart 1 and described in the experimental part. "Tomentogenin" was thus obtained and showed properties very similar to those reported before.*3 By careful examination with paper chromatography ($CHCl_3$/formamide),[2] tomentogenin was found to be separated into two very closely situated spots, and the results of elemental analysis indicated $C_{21}H_{34}O_5$ or $C_{21}H_{36}O_5$. Crude tomentogenin, upon catalytic hydrogenation took up about 0.3 mole hydrogen and gave tomentogenin (I), which was identical with the major spot of crude tomentogenin on paper chromatography. Elemental analysis of tomentogenin (I), $[\alpha]_D^{16}$ +36° (c=0.95, MeOH) suggested a molecular formula of $C_{21}H_{36}O_5$. Infrared bands at 3400 and 3150 cm^{-1} showed the presence of hydroxyl groups, but there were no bands assignable to

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*3 Part XIII : Steroids, **4**, 483 (1964).

*4 Part of this work was reported at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, and published in this Bulletin, **12**, 981 (1964).

1) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, E. Yamada : This Bulletin, **10**, 804 (1962).

2) H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, T. Nomura : *Ibid.*, **10**, 808 (1962).