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Studies on Metabolism of Drugs. IX.[1] On the Excreted Substances in the Human Urine after Oral Administration of Sulfamethomidine[2]

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Metabolic products of sulfamethomidine excreted in human urine were examined by paper chromatography and three metabolites were detected. Unchanged sulfamethomidine and N^4-acetylsulfamethomidine were confirmed by paper chromatography.

And, the other one was isolated and identified to be sodium sulfamethomidine-N^1-D-glucopyranosiduronate containing 1 mole of water of crystallization by the comparison of infrared and ultraviolet spectrographic data, and *Rf* values with the synthetic specimen.

There have been some reports[4–6] with regard to the metabolites of N^1-(6-methoxy-2-methyl-4-pyrimidinyl)sulfanilamide namely sulfamethomidine, one of the long acting sulfa-drugs, which only described that there existed both acetylated form and free from as the metabolites.

On the other hand, Frederick J. DiCarlo, *et al.*[7,8] suggested that in addition to the unchanged form and the acetylated form, the glucuronide of sulfamethomidine was present in the human urine, which was an ether glucuronide having the glucuronic acid moiety linked the pyrimidine nucleus. The isolation of this metabolite, however, was not carried out.

In the present paper, it is described that the identification of the metabolites of sulfamethomidine in human was done by comparison with the synthetic specimens in paper chromatography, infrared and ultraviolet spectra.

Experimental

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

Paper Chromatography: Paper chromatography was carried out with Tōyō filter paper No. 51 (2×40 cm) by the one dimensional ascending method with the solvent systems of BuOH–MeOH–H_2O (3:1:1), BuOH–MeOH–N HCl (3:1:1), BuOH–MeOH–3% NH_4OH (3:1:1), BuOH–AcOH–H_2O (5:1:4) and BuOH saturated with 3% NH_4OH.

Detecting Reagent: Ehrlich's reagent (for detection of amino group): 2% *p*-dimethylaminobenzaldehyde EtOH solution containing a volume of conc. HCl, Tsuda's reagent[9] (for detection of amino group):

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9) K. Tsuda and I. Matsunaga, *Yakugaku Zasshi*, **62**, 362 (1942).

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, Aniline hydrogen phthalate reagent[10] (for detection of glucuronic acid): To 100 ml of butanol saturated with H_2O, 0.93 g of aniline and 1.66 g of phthalic acid are added. The *Rf* values of the glucuronic acid are 0.26, 0.17 and 0.14 on the solvent systems of BuOH–AcOH–H_2O (4:1:2), BuOH–Pyridine–H_2O (6:4:3) and PhOH–H_2O–conc. NH_4OH (79:20:1), respectively.

Method of Determination: Sulfamethomidine was determined by authors' procedure[11] according to the Bratton–Marshall's method.[12] Glucuronic acid was determined by the carbazole method.[13]

Preparation of Standard Substance——Sulfamethomidine: Commercially available sulfamethomidine was purified by recrystallization from H_2O, mp 146°. N^4-Acetylsulfamethomidine: To the mixture of 4.3 g of acetic anhydride and 14 ml of acetic acid, 2.1 g of sulfamethomidine was added and allowed to stand for 1 hr. To the mixture, 5% NaOH was added to adjust approximately to pH 5 on cooling. A crystalline substance was separated by filtration, washed with water and dried in air. The crude material was dissolved in 60 ml of abs. MeOH and allowed to stand in a refrigerator to afford 1.8 g of colorless needles, mp 216—218°. *Anal.* Calcd. for $C_{14}H_{16}O_4N_4S$: C, 50.22; H, 4.77; N, 16.58. Found: C, 50.09; H, 4.91; N, 16.80.

Isolation of N-Glucuronide from Human Urine——The human urine (10 liter) of three normal men after administration of 1 g of sulfamethomidine twice daily for three days was collected. To the urine adjusted to pH 4.5 with glacial AcOH, was added 300 g of active charcoal (Norit SX-II), and the mixture was stirred and filtered. The charcoal was extracted three times at 40° for 25 min with 2, 1.5 and 1.5 liter of ammoniacal alkaline solvents of MeOH–NH_4OH–H_2O (5:1:20), respectively. All the following evaporation or concentration procedure was carried out below 40° in a N_2 atmosphere under reduced pressure. Extracted solutions were combined and concentrated to syrup, removing insoluble material. To the residual syrup, 130 ml of MeOH was added, and insoluble material were removed by filtration. The filtrate was concentrated and added to about 200 ml of water. The solution was applied to Dowex 50 W–X 8 (H–form), 100—200 mesh(radius: 1.5 cm, length: 34 cm). The column was washed with water thoroughly and eluted with N NH_4OH. The eluate was concentrated to about 80 ml, and the concentrated solution was applied to Amberlite IRA–68 (OH–form), 20—50 mesh (radius: 2.5 cm, length: 109 cm). The column was washed with water and eluted with 0.01 N NH_4OH. The eluate was concentrated to about 10 ml, adjusted to pH 4.5 with 10% AcOH, and 50% $(AcO)_2Pb$ solution was added. The filtrate separated from the precipitate was adjusted to pH 7 with N NH_4OH, and 10% basic lead acetate solution was added. The precipitates were collected, washed with water and decomposed with H_2S in MeOH. After removal of PbS, the filtrate was applied to Dowex 50 W–X 8 (H–form), 100—200 mesh (radius: 0.9 cm, length: 18 cm). The eluate was concentrated to about 10 ml, applied onto the 20 sheets of the water–washed filter papers (Tōyō filter paper No. 50, 40×40 cm) and developed with the mixed solvent of BuOH–MeOH–H_2O (3:1:1) to the top of the paper. After drying in air, the glucuronide portion was cut off and extracted three times with water. The extracted solution was concentrated to about 50 ml and applied to Amberlite IRA–68 (OH–form), 20—50 mesh (radius: 0.5 cm, length: 43 cm). The column was washed with water and eluted with 0.01N NH_4OH. The eluate was concentrated to about 20 ml, applied to SE–Sephadex C–25 medium (H–form), (radius: 1 cm, length: 14.5 cm)and eluted with water. The main fraction which showed positive to Ehrlich's reagent was collected and neutralized with 0.1N $NaHCO_3$. The neutralized solution was concentrated to about 50 ml and passed through Dowex 4 (OH–form), 100—200 mesh (radius: 0.2 cm, length: 7 cm). The effluent was concentrated to dryness. The residue was dissolved in a small amount of abs. MeOH and filtered. To the filtrate, abs. EtOH (2 times) was added, and the precipitate was separated by filtration and washed with abs. EtOH. After dryness, the colorless powder was dissolved in a small amount of water and dried *in vacuo* over P_2O_5, a colorless crystalline residue was obtained. Yield: 1.2 g. *Anal.* Calcd. for $C_{15}H_{21}O_9N_4SNa\cdot H_2O$: C, 42.35; H, 4.54; N, 10.98. Found: C, 42.31; H, 4.42; N, 10.73. UV $\lambda_{max}^{H_2O}$ mμ (log ε): 268 (4.25). IR cm^{-1}: ν_{SO_2} 1162 (KBr).

Sulfamethomidine-N^1-[methyl(tri-O-acetyl-β-D-glucopyranosid)uronate]——To 24 ml of water containing 5.8 g of KOH, 31.2 g of sulfamethomidine was added. After sulfamethomidine was dissolved, 50 ml of acetone was added. To the mixture, 40 g of methyl (2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate[14] was added and allowed to stand for three days at room temperature. Acetone was evaporated and then yellowish gum was afforded. The yellowish gum was separated by filtration and dissolved in 80 ml of abs. MeOH. The MeOH solution was poured gradually into 1.2 liter of water with stirring, and a colorless precipitate was afforded. The precipitate was collected and dissolved in 800 ml of MeOH. The solution was passed through Amberlite IRA–68 (OH–form), 50—100 mesh (radius: 1.5 cm, length: 40 cm). The effluent was concentrated to leave an amorphous residue, and this residue was recry-

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11) T. Uno and M. Ueda, *Yakugaku Zasshi*, **82**, 759 (1962).
12) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
13) Z. Dishe, *J. Biol. Chem.*, **167**, 189 (1947); *Idem, ibid.*, **183**, 489 (1950).
14) G.N. Bollenback, John W. Long, D.G. Benjamin, and J.A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).

stallized from iso-PrOH to colorless fine crystals, mp 108—109°. Yield: 17.5 g. *Anal.* Calcd. for $C_{25}H_{30}O_{12}N_4S$: C, 49.12; H, 5.27; N, 8.90. Found: C, 49.18; H, 5.05; N, 9.18. UV λ_{max}^{EtOH} mμ (log ε): 273 (4.37). IR cm^{-1}: ν_{SO_2} 1160 (KBr).

Sodium Sulfamethomidine-N^1-D-glucopyranosiduronate——The methyl-acetyl derivative of the glucuronide described above (16 g) was hydrolyzed with 800 ml of 2N NH_4OH for four days at room temperature and later concentrated to about 50 ml. The solution was applied to Amberlite IRA-68 (OH-form), 20—50 mesh (radius: 1.5 cm, length: 60 cm). The column was washed with water and eluted with 0.01N NH_4OH. The fraction which showed positive to Ehrlich's reagent was collected and applied to Dowex 50 W-X 8 (H-form), 100—200 mesh (radius: 1.5 cm, length: 17 cm). The column was washed with water and eluted with N NH_4OH. The eluate was applied to SE-Sephadex C-25 medium (H-form) (radius: 1.5 cm, length: 25 cm) and eluted with water. The eluate was neutralized with 0.1N $NaHCO_3$, concentrated to about 50 ml and passed through Dowex 4 (OH-form), 100—200 mesh (radius: 0.5 cm, length: 10 cm). The effluent was concentrated to dryness. The residue was reprecipitated from abs. MeOH-abs. EtOH (1:2) to colorless powder. This was dissolved in a small amount of water and dried *in vacuo* over P_2O_5, a colorless crystalline residue was obtained. Yield: 5 g. *Anal.* Calcd. for $C_{18}H_{21}O_9N_4SNa\cdot H_2O$: C, 42.35; H, 4.54; N, 10.98. Found: C, 42.13; H, 4.48; N, 10.69. UV $\lambda_{max}^{H_2O}$ mμ (log ε): 268 (4.25). IR cm^{-1}: ν_{SO_2} 1162 (KBr).

Results and Discussion

Paper Chromatography of Metabolites

The human urine after oral administration of sulfamethomidine was examined by paper chromatography using several kinds of solvents. Three spots were detected and named No. 1, 2 and 3 respectively as shown in Table I.

Spot No. 1 showed yellow promptly with Ehrlich's reagent and reddish purple immediately with Tsuda's reagent after the diazo reaction. Its *Rf* values corresponded with those of standard sulfamethomidine on the each solvent. Then the spot No. 1 was confirmed as unchanged sulfamethomidine.

Spot No. 2 showed negative to Ehrlich's reagent, but it turned to yellow by spraying the Ehrlich's reagent after hydrolysis with 4N hydrochloric acid in an oven at 60° for one hour. The sample solution was developed on a filter paper (Tōyō filter paper No. 50, 40×40 cm), using butanol saturated with 3% ammonium hydroxide, and the section of No. 2 was cut off and extracted with methanol. The extracted solution was concentrated and examined by paper chromatography. The *Rf* values corresponded with those of synthetic N^4-acetylsulfamethomidine on the each solvent. Therefore, the spot No. 2 was confirmed as N^4-acetylsulfamethomidine.

Table I. *Rf* Values of Excrements in Human Urine after Administration of Sulfamethomidine

Solvent	BuOH MeOH H_2O (3:1:1)	BuOH MeOH N HCl (3:1:1)	BuOH MeOH 3% NH_4OH (3:1:1)	BuOH AcOH H_2O (5:1:4)	BuOH satd. with 3% NH_4OH	
No. 1	0.84	0.83	0.57	0.85	0.32	Sulfamethomidine
No. 2	0.87	0.88	0.66	0.91	0.41	N^4-Acetylsulfamethomidine
No. 3	0.46	0.56	0.50	0.40	0.14	Sulfamethomidine-N^1-glucopyranosiduronate

Spot No. 3 was the largest of the three spots, and developed yellow promptly with the Ehrlich's reagent and reddish purple with Tsuda's reagent after the diazo reaction. As it colored by naphthoresorcinol reagent and its *Rf* values were the lowest of the three on the each solvent, it was presumed a sort of glucuronide.

Isolation of Substance of Spot No. 3

In order to determine the structure of the spot No. 3, it was isolated according to the following extraction method as shown in Chart 1, which was characterized by the suitable

application of several kinds of ion exchange resins, and a colorless crystalline substance was obtained. This compound is unstable under acid conditions and its ammonium salt tend toward the acid with the vaporization of its ammonia. Therefore, this compound was isolated as the sodium salt. This was soluble in water, methanol, and insoluble in the organic solvents, such as ethanol, ether and benzene, *etc.* By paper chromatography, it was confirmed that

Urine (10 liter)
| added charcoal activated at pH 4.5
charcoal — filtrate
| treated with MeOH–NH_4OH–H_2O (5:1:20) at 40°
filtrate — charcoal
| evapd. to syrup, dissolved in abs. MeOH
MeOH soln. — ppt.
| evapd., dissolved in H_2O
Dowex 50 W-X8 100—200 mesh
| washed with H_2O, eluted with 1 N NH_4OH
aq. soln.
| evapd. to about 80 ml
Amberlite IRA-68 20—50 mesh
| washed with H_2O, eluted with 0.01 N NH_4OH
aq. soln.
| evapd. to about 10 ml, added with 50% $(AcO)_2Pb$ at pH 4.5
filtrate — ppt.
| added with 10% basic lead acetate at pH 7
ppt. — filtrate
| treated with H_2S in MeOH
filtrate — PbS
| neutralized with NH_4OH
Dowex 50 W-X8 100—200 mesh
| washed with H_2O, eluted with 0.5 N NH_4OH and evapd.
PPC (BuOH–MeOH–H_2O (3:1:1))
| extracted with H_2O, evapd. to about 50 ml
Amberlite IRA-68 20—50 mesh
| washed with H_2O, eluted with 0.01 N NH_4OH, and evapd.
SE-Sephadex C-25 medium
| eluted with H_2O, neutralized with 0.1 N $NaHCO_3$
Dowex 4 100—200 mesh
| washed with H_2O
aq. soln.
| evapd. to dryness, reprecipitated from abs. MeOH–abs. EtOH
crystal (1.2 g)

Chart 1. Isolation of Sulfamethomidine-N^1-glucuronide

hydrolysis of this substance with N hydrochloric acid at 60° for 2 hours gave sulfamethomidine and glucuronic acid. Besides, the determination of hydrolyzate indicated that glucuronic acid combined with sulfamethomidine with the molar ratio of one to one in the conjugate. The elementary analytical values of this compound corresponded to those of the monohydrate of sodium sulfamethomidine glucosiduronate.

Generally, sulfa-drugs including nitrogen hetero ring are considered to be in two forms, amido form and imido form. The authors[15] reported regarding to infrared spectra of N-pyridine, thiazole and pyrimidine derivatives of sulfanilamide, that the SO_2 symmetric stretching frequencies are divided into the regions, and sulfanilamides which take the amido form absorb in the region 1170—1145 cm^{-1} while sulfanilamides which take the imido form absorb in the region 1145—1130 cm^{-1}. On the other hand, it was found at 1162 cm^{-1} in the spot No. 3. This fact suggested that the substance of the spot No. 3 was N^1-glucuronide.

Synthesis of Sodium Sulfamethomidine-N^1-D-Glucopyranosiduronate

In order to confirm the structure of the substance of the spot No. 3, sulfamethomidine-N^1-[methyl(tri-O-acetyl-β-D-glucopyranosid)uronate]was synthesized according to Okumura's method,[16] which was hydrolyzed with ammonium hydroxide, and later passed through SE-Sephadex C-25 medium. The eluate was neutralized with 0.1N sodium bicarbonate and crystallized to sodium sulfamethomidine-N^1-D-glucopyranosiduronate containing one mole of water of crystallization.

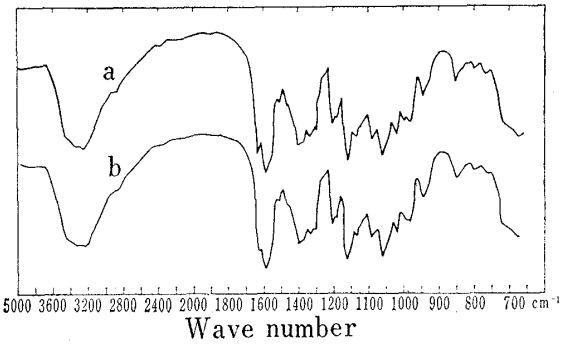

Fig. 1. Infrared Absorption Spectra (KBr)
a: Synthetic sample b: Extracted sample

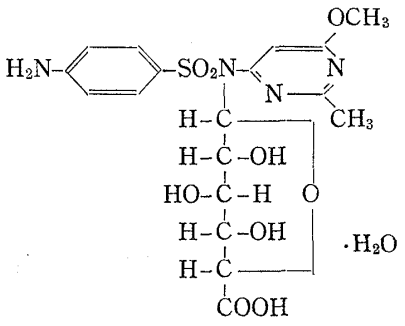

Chart 2

Identification of Substance of Spot No. 3

The *Rf* values of the extracted sample were in good agreement with those of the synthetic sample on each solvent as shown in Table I. Both compounds extracted and synthesized had identical infrared spectra as shown in Fig. 1. Moreover, the ultraviolet absorption spectrum of the extracted sample exhibited only one peak at 268 mμ, and was essentially identical with the spectrum of the synthetic sample.

As described above, the substance of the spot No. 3 was confirmed to be sulfamethomidine-N^1-D-glucopyranosiduronic acid having the following formula.

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