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Studies on Metabolism of Drugs. VIII.[1] On the Glucuronide of Sulfaphenazole in Human[2]

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The metabolism of sulfaphenazole (SP), *i.e.* N^1-(1-phenylpyrazole-5-yl)sulfanilamide in the human body was investigated. The main metabolite was isolated and confirmed to be the N-glucuronide of SP. In order to determine the structure of this glucuronide, SP-N^1-[methyl (tri-O-acetyl-β-D-glucopyranosid)uronate] (III) was synthesized from potassium salt of SP and methyl (2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate. Hydrolysis of III with NH_4OH gave both N^1-glucuronide and ring N-glucuronide of SP in the ratio of about 2 to 1. Furthermore, N^1-methyl and ring N-methyl derivatives of SP were prepared with CH_2N_2, and their infrared and ultraviolet spectra were compared with those of the conjugate to confirm the substituted position of the glucuronic acid.

These evidences indicated that the extracted compound is an imido-type conjugate, carrying the glucuronic acid attached to the nitrogen in the pyrazole ring while SP itself takes the amido form. Therefore, the main metabolite of SP is a sodium 1-deoxy-[1-phenyl-5-(sulfanilimino)-3-pyrazolin-2-yl]-D-glucopyranosiduronate containing 1 mole of water of crystallization.

Sulfaphenazole, one of the long acting sulfa-drugs, the synthesis of which was carried out by P. Schmidt, *et al.*[4] in 1958, is a unique sulfa-drug, including the phenylpyrazole ring combined with the N^1-position of sulfanilamide, and have been investigated[5–8] from a clinical point of view.

With regard to the metabolism of sulfaphenazole in human, Riess, *et al.*[9] described that they attempted to isolate the main metabolite, and its methyl-acetyl derivative was prepared, and it was considered to be an imido-type conjugate, carrying the glucuronic aicd attached to the N-2 of the pyrazole ring on the basis of ultraviolet spectrographic data chiefly. The structure of this metabolite, however, was not proved determinately by synthesis.

On the other hand, the studies on N^1-methyl derivative of sulfaphenazole have been reported,[10–13] in connection to the structure of sulfaphenazole.

When the metabolites of sulfaphenazole in human was investigated by paper chromatography, apart from above mentioned reports, authors took an interest in the fact that a sort of large quantities of metabolite was formed. Then, this main metabolite was isolated accord-

1) Part VII: M. Ueda, N. Murakami, H. Atsumura, and K. Furuki, *Yakugaku Zasshi*, **87**, 455 (1967).
2) A part of this work was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan at Toyama, April, 1966.
3) Location: *Gofuku, Toyama.*
4) P. Schmidt and J. Druey, *Helv. Chim. Acta*, **41**, 306 (1958).
5) E. Pasargiklian, G. Ibba, and G. Pinna, *Minerva med.*, 4699 (1958) (*C.A.*, **53**, 8410 (1959)).
6) N. Gargano and S. Grazi, *Minerva med.*, 4707 (1958) (*C.A.*, **53**, 8410 (1959)).
7) O. Zangaglia, A. Ferrata, and F. Cambieri, *Minerva med.*, 4713 (1958) (*C.A.*, **53**, 8410 (1959)).
8) G. Fontana, *Minerva med.*, 4726 (1958) (*C.A.*, **53**, 8411 (1959)).
9) W. Riess, K. Schmid, and H. Keberle, *Klin. Wschr.*, **43**, 740 (1965).
10) J. Seydel, *Naturwissenschaften*, **50**, 663 (1963).
11) J. Seydel and E. Krüger-Tiemer, *Arzneimittel-Forsch.*, **14**, 1294 (1964).
12) J. Seydel, H. Wolter, E. Krüger-Thiemer, and Ellen Wempe, *Klin. Wschr.*, **41**, 1067 (1963).
13) K. Eichenberger, R.F. Zurcher, A. Rossi, M. Wilhelm, and P. Schmidt, *Helv. Chim. Acta*, **48**, 524 (1965).

ing to authors' method, and its elementary analytical values were in good agreement with the analytical calculated values of the N-glucuronide fo sulfaphenazole.

Further, the synthesis of this substance was carried out to establish its structure. And, in order to determine the substituted position of the glucuronic acid definitely, the tautomerism of sulfaphenazole was investigated, and the isomeric N-methyl derivatives were prepared and compared with this metabolite in infrared and ultraviolet data.

Experimental

Analytical Methods——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–AcOH–H_2O (5:1:4) and BuOH saturated with 5N NH_4OH.

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[14] (for detection of amino group): 0.1% N-(2-dimethylaminoethyl)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% trichloro acetic acid aqueous solution.

Method of Determination: Sulfaphenazole was determined according to the modified Bratton–Marshall's method[15] described by Padowetz, *et al.*[16] Glucuronic acid was determined by the carbazole method.[17]

Isolation of N-Glucuronide from Human Urine——The human urine (13.5 liter) after administration of sulfaphenazole, 2 g of which was given orally to four normal men as the initial dose and successively 1 g as the maintenance dose five times at intervals of 12 hr, was collected. To the urine adjusted to pH 4.5 with glacial AcOH was added 350 g of active charcoal (Norit SX-II) with stirring, allowed to stand for 1 hr, filtered and washed with H_2O. The charcoal was extracted three times at 40° for 25 min with 2, 1.6 and 1.4 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, concentrated to about 400 ml and filtered. The filtrate was applied to Dowex 50 W-X 8 (H-form), 100—200 mesh (radius: 1.5 cm, length: 40 cm). The column was washed with H_2O and later eluted with N NH_4OH. The eluate was concentrated to about 100 ml, adjusted to pH 4.5 with AcOH and then 30% $(AcO)_2Pb$ solution added until precipitation was complete. After removal of the precipitate by filtration, the filtrate was adjusted to pH 7 with N NH_4OH and 30% basic lead acetate solution was added. The basic lead precipitate was collected and washed with H_2O. After drying in air, the lead salt was pulverized, made into a fine suspension in 10% NH_4OH and decomposed by treatment with H_2S. After removal of PbS by filtration, the filtrate was concentrated and applied to Dowex 50 W-X 8 (H-form), 100—200 mesh (radius: 0.85 cm, length: 15.5 cm). The column was washed with H_2O and later eluted with N NH_4OH. The eluate was concentrated to about 10 ml, applied in a line onto the water-washed filter papers (Tōyō filter paper No. 50, 40×40 cm) and developed with the mixed solvent of BuOH–MeOH–N NH_4OH (2:1:1) to the top of the paper. After drying in air, the glucuronide portion was cut off, extracted with H_2O and then concentrated to about 50 ml. The solution was passed through Amberlite IRP-64 (H-form), 100—200 mesh (radius: 0.5 cm, length: 5.5 cm). The effluent was applied to Duolite C-10 (H-form), 50—100 mesh (radius: 1.6 cm, length: 6 cm) and eluted with H_2O. The main fraction which showed positive to Ehrlich's reagent was collected, neutralized with 0.1N $NaHCO_3$ and concentrated to about 10 ml. The solution was passed through Dowex 4 (OH-form), 100—200 mesh (radius: 0.5 cm, length: 5 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: 3 g. *Anal.* Calcd. for $C_{21}H_{21}O_8N_4SNa \cdot H_2O$: C, 47.54; H, 4.37; N, 10.56. Found: C, 47.25; H, 4.38; N, 10.37. UV $\lambda_{max}^{H_2O}$ mμ (log ε): 274 (4.38). λ_{max}^{MeOH} mμ (log ε): 268 (4.23), 284 (4.23). IR cm^{-1}: ν_{SO_2} 1133 (KBr).

Sulfaphenazole-N^1-[methyl(tri-O-acetyl-β-D-glucopyranosid)uronate](III)——To 24 ml of water containing 5.8 g of KOH, 31.4 g of sulfaphenazole was added. After sulfaphenazole was dissolved, 100 ml of acetone was added. To the mixture, 30.2 g of methyl (2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)-uronate[18] was added, and allowed to stand for 4 days at room temperature. The solvent was evaporated

14) K. Tsuda and I. Matsunage, *Yakugaku Zasshi*, **62**, 362 (1942).
15) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
16) W. Padowetz, K. Schmid, and J. Druey, *Helv. Chim. Acta*, **44**, 89 (1960).
17) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947); *Idem, ibid.*, **183**, 489 (1950).
18) G.N. Bollenback, J.W. Long, D.G. Benjamin, and J.A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).

to about 40 ml 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 then a colorless precipitate was afforded. To the precipitate, $CHCl_3$ was added, and an insoluble material was removed by filtration. The evaporation of $CHCl_3$ gave 13 g of the residue, and this material was dissolved in a small amount of abs. MeOH. The MeOH solution was passed through Amberlite IRA-68 (OH-form), 100—200 mesh (radius: 1.1 cm, length: 23.5 cm) to remove sulfaphenazole and later concentrated to about 70 ml. The solution was applied to Duolite C-10 (H-form), 50—100 mesh (radius: 1.1 cm, length: 6.6 cm). The column was washed with abs. MeOH and later eluted with 0.3N ammoniacal MeOH. The MeOH solution was concentrated to dryness. The obtained amorphous substance was recrystallized from EtOH to yield 2.7 g of colorless needles, mp 251—252° (decomp.). This intermediate was submitted to the thin-layer chromatography (Wakogel B-5, contaning 5% gypsum, Wako Pure Chemical Co., Tokyo) with chloroform,[a] ethyl acetate[b] and acetone,[c] and the paper chromatography with the solvent systems of BuOH–MeOH–H_2O (3:1:1)[d] and BuOH–AcOH–H_2O (5:1:4),[e] and the TLC and the PPC gave a single spot in all cases. (Its *Rf* values were 0.03,[a] 0.4,[b] 0.6,[c] 0.97[d] and 0.98[e] on the each solvent.) *Anal.* Calcd. for $C_{28}H_{30}O_{11}N_4S$: C, 53.33; H, 4.80; N, 8.89. Found: C, 53.48; H, 4.93; N, 8.64. UV λ_{max}^{MeOH} mμ (log ε): 278 (4.31). IR cm^{-1}: ν_{SO_2} 1165 (KBr).

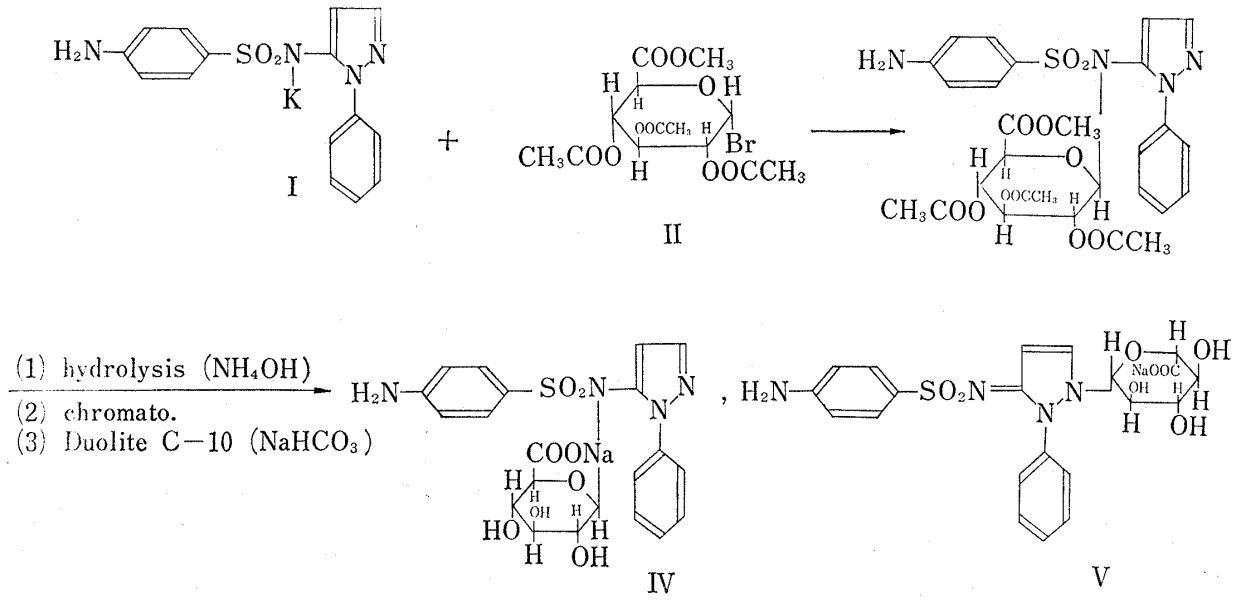


Chart 1

Sodium N^1-[N^1-(1-Phenyl-pyrazole-5-yl)sulfanilamido]-D-glucopyranosiduronate (IV) and Sodium 1-Deoxy-[1-phenyl-5-(sulfanilimino)-3-pyrazolin-2-yl]-D-glucopyranosiduronate (V)——A mixture of 1.4 g of III and 54.6 ml of 2N NH_4OH was allowed to stand for three days at room temperature in order to hydrolyze III. The solution was evaporated until it was neutral, applied to Duolite C-10 (H-form), 50—100 mesh (radius: 1.1 cm, length: 6 cm). The column was washed with H_2O and later eluted with 0.01N NH_4OH. The main fraction which showed positive for aromatic amine reaction was collected and then concentrated to about 10 ml. The solution was applied in a line onto 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 N^1-glucuronide portion (*Rf* value: 0.43) and the ring N-glucuronide portion (*Rf* value: 0.25) were cut off, respectively.

The ring N-glucuronide portion containing ammonium 1-deoxy-[1-phenyl-5-(sulfanilimino)-3-pyrazolin-2-yl]-D-glucopyranosiduronate was extracted with H_2O. The extract was concentrated to about 50 ml and passed through Amberlite IRP-64 (H-form), 100—200 mesh (radius: 0.3 cm, length: 4 cm). The effluent was applied to Duolite C-10 (H-form), 50—100 mesh (radius: 0.5 cm, length: 5 cm) and eluted with H_2O. The main fraction which showed positive to Ehrlich's reagent was collected, neutralized with 0.1N $NaHCO_3$ and later concentrated to about 20 ml. The solution was passed through Dowex 4 (OH-form), 100—200 mesh (radius: 0.3 cm, length: 4 cm). The effluent was concentrated to dryness and the residue was reprecipitated from abs. MeOH–abs. EtOH (1:2) as described in "Isolation of N-Glucuronide." The precipitate was separated by filtration and washed with abs. EtOH. After drying, 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: 120 mg. *Anal.* Calcd. for $C_{21}H_{21}O_8N_4SNa \cdot H_2O$: C, 47.54; H, 4.37; N, 10.56. Found: C, 47.35;

H, 4.25; N, 10.47. UV $\lambda_{max}^{H_2O}$ mμ (log ε): 274 (4.38). λ_{max}^{MeOH} mμ (log ε): 268 (4.23), 284 (4.23). IR cm^{-1}: ν_{SO_2} 1133 (KBr).

The N^1–glucuronide portion containing ammonium N^1–[N^1–(1–phenylpyrazole–5–yl)sulfanilamido]–D–glucopyranosiduronate was cut off from the above mentioned developed filter papers and extracted with H_2O, and the extract was concentrated to about 50 ml. The following purification procedure was carried out according to that described for (V). Yield: 200 mg. *Anal.* Calcd. for $C_{21}H_{21}O_8N_4SNa \cdot 2H_2O$: C, 45.98; H, 4.59; N, 10.22. Found: C, 46.23; H, 4.42; N, 10.33 UV $\lambda_{max}^{H_2O}$ mμ (log ε): 271 (4.27). λ_{max}^{MeOH} mμ (log ε): 273 (4.32). IR cm^{-1}: ν_{SO_2} 1156 (KBr).

Table I. *Rf* Values of the Isomeric N–Glucuronides of Sulfaphenazole

	BuOH–AcOH–H_2O (5:1:1)	BuOH–MeOH–H_2O (3:1:1)	BuOH satd. with 5 N NH_4OH
IV	0.56	0.43	0.20
V	0.28	0.26	0.08
extracted sample	0.28	0.26	0.08

Tōyō filter paper No. 51, 2×40 cm

Detection of Rearrangement of Glucuronic Acid——1.5% solutions of IV and V in 2N NH_4OH were prepared and allowed to stand at 20° and 40° for three days. In order to detect the rearrangement of the glucuronic acid between IV and V, paper chromatography was carried out at intervals of 24 hr after the preparation of the sample solution. However, no appreciable rearrangement took place under the above mentioned conditions.

Preparation of N-Deuterated Compound——N–Deuterated species were prepared by the exchange reaction with deuterium oxide in Me_2CO. Its infrared spectrum was compared with that of ordinary compound which was treated with hydrogen oxide under the same conditions as the case of deuteration. The measurements of IR spectra were carried out for hexachlorobutadiene (3600—1800 and 1500—1300 cm^{-1}) and Nujol (1800—1500 and 1300—1650 cm^{-1}) pastes.

Preparation of 2-Methyl-1-phenyl-5-sulfanilimino-3-pyrazolin——Sulfaphenazole (15 g) in a small amount of abs. MeOH was added dropwise to 1 liter of the ether solution containing CH_2N_2 prepared from 20 ml of nitrosomethylurethane. The mixture was subsequently stirred until the evolution of nitrogen ceased. The reaction mixture was concentrated to about 4 ml and filtered. The insoluble N^1–methyl derivative (12 g) was obtained and this material was recrystallized from MeOH, mp 215—217°. The filtrate was applied in a line onto the silica gel plates (Wakogel B–5, 20×20 cm) and developed with the mixed solvent of $CHCl_3$–6% ammoniacal MeOH (80:15). After drying in air, the ring–nitrogen derivative portion (*Rf* value: 0.3) which was positive to Ehrlich's reagent was raked together, and extracted with MeOH. The MeOH soln. was concentrated, applied in a line onto the silica gel plates again and developed with the mixed solvent of Ether–abs. MeOH (90:10). After drying in air, the corresponding area containing the ring N–methyl derivative was raked together and extracted with MeOH. The MeOH solution was concentrated to about 10 ml and decolorized with charcoal. The solution was applied to Dowex 50 W–X 8 (H–form), 100—200 mesh (radius: 0.85 cm, length: 4.5 cm). The column was washed with MeOH and later eluted with 0.5N ammoniacal MeOH. The eluate was passed through Dowex 1–X 8 (OH–form), 100—200 mesh (radius: 0.5 cm, length: 10 cm). The effluent was concentrated to dryness and a colorless crystalline residue, mp 107—108°, was obtained. Yield: 120 mg. *Anal.* Calcd. for $C_{16}H_{16}O_2N_4S$:C, 58.52; H, 4.91; N, 17.06. Found: C, 58.47; H, 4.75; N, 16.98. UV λ_{max}^{MeOH} mμ (log ε): 267 (4.36), 283 shoulder (4.32). IR cm^{-1}: ν_{SO_2} 1131 (KBr).

Table II. *Rf* Values of the Isomeric N–Methyl Derivatives of Sulfaphenazole

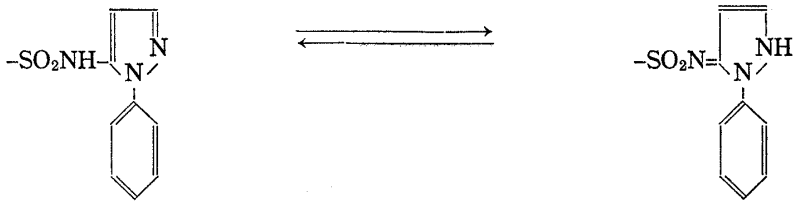
	BuOH–AcOH–H_2O (5:1:4)	BuOH–MeOH–H_2O (3:1:1)	BuOH satd. with 5 N NH_4OH
Ring N–methyl derivative	0.62	0.68	0.54
N^1–Methyl derivative	0.97	0.96	0.94

Tōyō filter paper No. 51, 2×40 cm

Results and Discussion

Tautomerism of Sulfaphenazole

Generally, in the sulfa-drugs a heterocyclic ring containing nitrogen, the tautomerism between the amido form and the imido form is considered. In the case of sulfaphenazole, the tautomerism is considered as follows.



amido form imido form

In relation to the sturcture of sulfaphenazole derivatives, Seydel, *et al.*[10–12] reported that sulfaphenazole are present in the imido form. In this investigation, they claimed that not only by methylation of sulfaphenazole in alkaline medium, but also by reaction with diazomethane,only ring N–methyl derivative of sulfaphenazole was isolated, and the hypothesis that sulfaphenazole are in the imido form based their arguments on this fact.

Shortly after their report, Eichenberger, *et al.*[13] succeeded in the synthesis of N^1–methyl derivative of sulfaphenazole by using 1–phenyl–5–acetylaminopyrazole as a starting material, and reported that by methylation of sulfaphenazole in alkaline medium, not ring N–methyl derivative but N^1–methyl derivative was isolated, the structure of which was proved by an unambiguous synthesis, in opposition to Seydel's conclusion.

To make this point clear, sulfaphenazole was methylated with diazomethane according to the usual manner. By methylation, authors were successful in isolation of the ring N–methyl derivative of sulfaphenazole, mp 107—108°, in a small amount, and the N^1–methyl derivative, the structure of which was conclusively proved by Eichenberger, *et al.*[13]

Further, authors investigated the structure of sulfaphenazole by comparison with the infrared spectra of sulfaphenazole and its N–deuterated species according to Uno's method.[19] Uno and Ueda, *et al.*[19] reported regarding to infrared spectra of N–pyridine, thiazole and pyrimidine derivatives of sulfonamides and its deuterated species that monosubstituted sulfonamides which take the imido form show the prominent spectral change on N–deuteration in the region 1600 to 1200 cm^{-1}, and contrarily, the spectra of sulfonamides which take the amido form show the remarkable spectral change on N–deuteration in the region 1000 to 700 cm^{-1}. Moreover, it was described that the SO_2 symmetric stretching bands of sulfanilamide derivatives are located between 1170 to 1145 cm^{-1} in the amido form and 1145 to 1130 cm^{-1} in the imido form.

In sulfaphenazole, the spectral change on N–deuteration occurred remarkably in the region 1000 to 700 cm^{-1}, but the bands around 1600 cm^{-1} hardly affected by N–deuteration. In addition, the SO_2 symmetric stretching band was found at 1150 cm^{-1} from our spectra.

These results indicated the conclusion that sulfaphenazole is mostly in the amido form with regard to the tautomerism between the amido form and the imido form.

Isolation of N-Glucuronide from Human Urine

The isolation of N–glucuronide from the human urine after oral administration of sulfaphenazole was carried out mostly with the basic lead acetate method and the suitable combination of the ion–exchange column chromatography to afford a colorless crystalline substance.

19) T. Uno, K. Machida, K. Hanai, M. Ueda, and S. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **11**, 704 (1963).

The applied ion-exchange resins in the isolation procedure were Dowex 50 W-X 8 ($RSO_3^-H^+$) 100—200 mesh, Duolite C-10 ($RCH_2SO_3^-H^+$) 50—100 mesh, Amberlite IRP-64 ($RCOO^-H^+$) 100—200 mesh and Dowex 4(OH^- form) 100—200 mesh. Hydrolysis of this substance with N hydrochloric acid at 60° for three hours gave glucuronic acid and sulfaphenazole, and this solution was used for their determination,[16,17] and it was confirmed that the glucuronic acid combined with sulfaphenazole with the molar ratio of one to one in the conjugate.

The elementary analytical values of the conjugate corresponded to those of the monohydrate of sodium sulfaphenazole glucosiduronate.

Substituted Position of Glucuronic Acid in the Isolated Compound

As for the N-glucuronide of sulfaphenazole, three types of conjugates, namely N^4-substituent, N^1-substituent and N-substituent of heterocycle would be expected, judging from the studies on the glucuronides of sulfanilamide derivatives.

On the other hand, this conjugate showed positive to Ehrlich's reagent and promptly developed reddish purple by Tsuda's reagent after the diazo reaction. Therefore, this conjugate is not N^4-substituent, but should be either N^1-substituent or N-substituent of heterocycle.

Regarding to the infrared spectra of sulfanilamide derivatives,[18] the location of the SO_2 symmetric stretching bands have been already described. In the isolated glucuronide by this method, it was observed at about 1133 cm^{-1}, that is, at lower frequency region than 1145 cm^{-1}, and the finger print region of this conjugate resembles closely that of the ring N-methyl derivative of sulfaphenazole as shown in Fig. 2.

Moreover, regarding to the ultraviolet spectra of sulfanilamide derivatives, it has been reported[20,21] that there are one maximum for the amido form and two maxima for the imido form. This conjugate has only one absorption maximum at about 274 mμ in aqueous solution and two absorption maxima at about 268 mμ and 284 mμ in absolute methanol solution.

These facts suggests that this conjugate is N-substituent of the pyrazole ring of sulfaphenazole.

Syntheses of Isomeric N-Glucuronides of Sulfaphenazole

In order to determine the structure of the isolated N-glucuronide, two isomeric N-glucuronides of sulfaphenazole were prepared as shown in Chart 1.

Sulfaphenazole-N^1-[methyl(tri-O-acetyl-β-D-glucopyranosid)uronate](III) was synthesized from potassium salt of sulfaphenazole (I) and methyl(2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate (II). By hydrolysis of III with ammonium hydroxide, both sodium N^1-[N^1-(1-phenylpyrazole-5-yl)sulfanilamido]-D-glucopyranosiduronate (IV) and sodium 1-deoxy-[1-phenyl-5-(sulfanilimino)-3-pyrazolin-2-yl]-D-glucopyranosiduronate (V) were obtained in the ratio of about 2 to 1.

Then, IV and V were compared in respect of infrared spectra as shown in Fig. 1 and 2, ultraviolet spectra and paper chromatography as shown in Table I.

In the infrared spectra of the N^1-glucuronide (IV), the SO_2 symmetric stretching band is obsereved in 1156 cm^{-1} and on the other hand, in the ring N-glucuronide (V), it is observed in about 1133 cm^{-1}.

In the ultraviolet spectra, IV has one absorption maximum at about 271 mμ in aqueous solution and about 273 mμ in absolute methanol solution, respectively. On the contrary, V has one absorption maximum at about 274 mμ in aqueous solution and two absorption maxima at about 268 mμ and 284 mμ in absolute methanol solution.

20) R.G. Shepherd, A.C. Bratton, and K.C. Blanchard, *J. Am. Chem. Soc.*, **64**, 2532 (1942).
21) T. Uno and M. Ueda, *Chem. Pharm. Bull.* (Tokyo), **11**, 709 (1963).

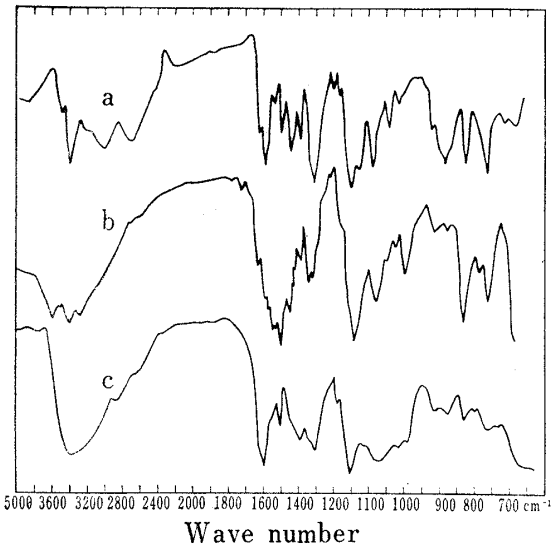


Fig. 1. Infrared Absorption Spectra (KBr)

	SO_2 sym. stretch. (cm^{-1})
a: Sulfaphenazole (SP)	1150
b: N^1-Methyl derivative of SP	1146
c: N^1-Glucuronide of SP (IV)	1156

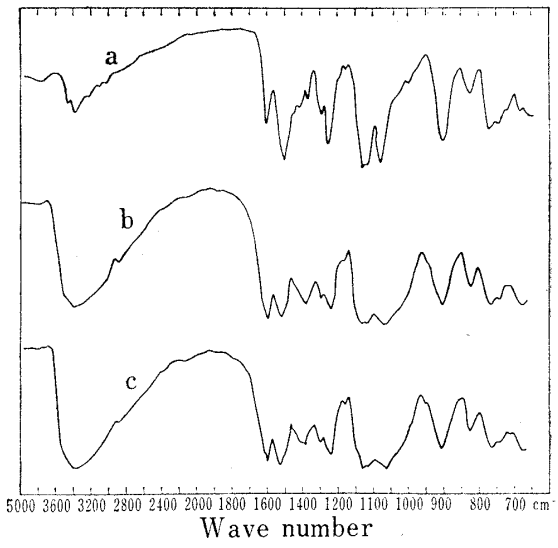


Fig. 2. Infrared Absorption Spectra (KBr)

	SO_2 sym. stretch. (cm^{-1})
a: Ring N-methyl derivative of SP	1131
b: Extracted sample	1133
c: Synthetic sample (V)	1133

Previously authors reported[22] that the partition coefficient of the N^1-glucuronide of sulfa-drugs may be larger than those of the ring N-glucuronide. In the isomeric N-glucuronides of sulfaphenazole, the *Rf* values of IV are higher than those of V on each solvent as given in Table I.

Identification of Isolated N-Glucuronide

The extracted sample and the synthetic sample were compared in respect of infrared spectra as shown in Fig. 1 and 2, ultraviolet spectra and paper chromatography as shown in Table I. The data of the extracted sample are in good agreement with those of V.

In view of the above mentioned facts, we concluded that the isolated glucuronide from the human urine after oral administration of sulfaphenazole are sodium 1-deoxy-[1-phenyl-5-(sulfanilimino)-3-pyrazolin-2-yl]-D-glucopyranosiduronate containing one molecule of water of crystallization.

It is very interesting that sulfaphenazole itself is in the amido form while in the glucuronide of the metabolite of sulfaphenazole in human, the glucuronic acid is introduced to the N-2 position of the pyrazole ring of sulfaphenazole. Such a case has been reported for both sulfisoxazole[23] and sulfisomezole.[22]

It attracts attention that these sulfa-drugs are common in respect of carrying the heteropentacyclic ring moiety.

The syntheses of the ring N-glucuronides of monosubstituted sulfanilamides have never been reported. This time, for sulfaphenazole, the synthesis of the ring N-glucuronide was carried out.

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