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121. Toyozo Uno*1 and Michihiro Ueda*2 : Studies on the Metabolism of Sulfathiazole. IV.[1] A New Sulfathiazole-glucuronic Acid Conjugate in the Human Urine.

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In the preceding paper,[2] it was reported that a new glucuronic acid conjugate of sulfathiazole which is different from sulfathiazole-N^4-glucuronide and is decomposed to sulfathiazole and glucuronic acid by hydrolysis, was excreted in human urine after administration of sulfathiazole. Previously Kishiwada[3] reported the excretion of glucuronic acid complex in urine after administration of sulfisomezole. The isolation of the compound, however, was not carried out, and the structure of the compound was not obvious.

In the present work, for the isolation and purification of ammonium salt of the conjugate, a new method described in experimental was employed to increase its yield. By the recrystalisation from water, colorless small needles with the melting point at 176~178° were obtained. This compound corresponded to the monohydrate of ammonium sulfathiazole glucosiduronate $C_{15}H_{20}O_8N_4S_2 \cdot H_2O$, and was soluble in water, methanol and insoluble in ethanol, ether, acetone and benzene.

Molar Ratio of Glucuronic Acid and Sulfathiazole in the Conjugate

Determination of glucuronic acid was carried out by the carbazole method,[4,5] and sulfatiazole was determined by Bratton-Marshall's method[6,7] described in the previous paper.[8] From the results of the experiment, it was confirmed that glucuronic acid combined with sulfathiazole with the molar ratio of one to one in the conjugate.

Substituted Position of Glucuronic Acid in the Conjugate

Judging from the studies on the glucuronides of sulfanilamides,[9] in the case of sulfathiazole, either O-glucuronide or N-glucuronide is possibly considered. In sulfathiazole, the formation of 3-hydroxysulfathiazole was anticipated by Bray.[10] To make this point clear, the glucuronide was hydrolysed with *N*HCl and the product was investigated by paper chromatography and infrared spectroscopy. Rf value and infrared spectrum of the hydrolysis product agreed with those of sulfathiazole. Phenolic hydroxyl group was not detected. No depression of melting point was observed with sulfathiazole. Therefore the hydrolysis product is not 3-hydroxysulfathiazole which Bray anticipated, but sulfathiazole itself. Accordingly, the glucuronide isolated from urine should be N-glucuronide. As for the N-glucuronide of sulfathiazole three types of conjugates, namely N^4-substituent, N^1-substituent and N-substituent of heterocycle would be expected. Among them, N^4-substituent was already reported to be isolated

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in the previous paper[2] and is not this conjugate. Therefore, this conjugate should be either N^1-substituent or N-substituent of heterocycle. In order to determine the structure of this conjugate, ultraviolet and infrared spectra were investigated in detail.

Discussion on the Structure by Ultraviolet Spectroscopy

Ultraviolet absorption spectra of sulfathiazole or sulfapyridine which have a substituent at N^1 or N of heterocycle were investigated by Shephered *et al.*[11~17] and it was concluded that N^1-substituents of sulfathiazole and sulfapyridine (amido form) have one absorption maximum at the region of 260~290 mμ, while their heterocycle N-substituents (imido form) have two absorption maxima. Same sort of investigations were made on several derivatives of sulfathiazole, and the results were shown in Figs. 1 and 2.

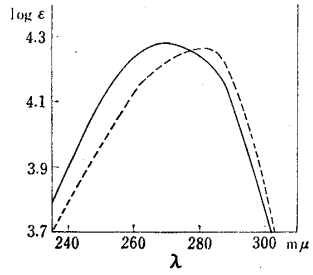

Fig. 1.

——— glucuronic acid conjugate

------ H_2N-C$_6$H$_4$-SO_2-N(CH$_3$)-C$_3$H$_2$S-N

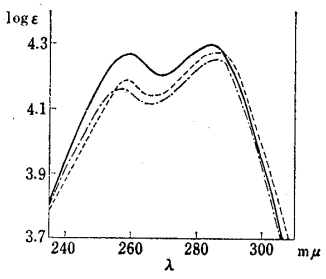

Fig. 2.

——— sulfathiazole

------ H_2N-C$_6$H$_4$-SO_2-N=C$_3$H$_2$S-N-CH_3

-·-·- H_2N-C$_6$H$_4$-SO_2-N=C$_3$H$_2$S-N-CH_2-CH_2-OH

N^1-methyl-N^1-(2-thiazolyl)sulfanilamide has only one absorption maximum at 282 mμ, while N^1-(3-methyl-4-thiazolin-2-ylidene)sulfanilamide, N^1-3-(2-hydroxyethyl)-4-thiazolin-2-ylidene)sulfanilamide which are N-substituents of heterocycle and sulfathiazole have two absorption maxima at about 260 mμ, and 290 mμ. On the other hand, the conjugate obtained by the authors has only one absorption maximum at 272 mμ. This fact supports that the conjugate is N^1-substituent of sulfathiazole.

Discussion of the Structure by Infrared Spectroscopy

The tautomery of the derivatives of sulfathiazole (amido-form, imido-form) was investigated with infrared spectra by Sheĭker,[18] and it was concluded that absorption band at about 940 cm^{-1} was observed in the derivative of imido-form and not in the amido-form. On the other hand, as previously reported by authors,[19] among many kinds of derivatives of sulfanilamide, SO_2 symmetrical stretching bands were

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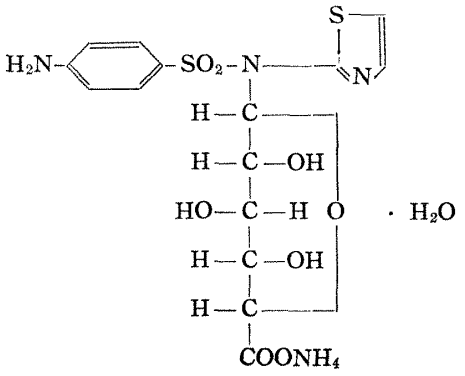
observed at higher frequency region than 1145 cm^{-1} in amido form, and at lower than 1145 cm^{-1} in imido form. On the glucuronide isolated from the urine, the absorption band of ν_{SO_2} was observed at 1158 cm^{-1} and no absorption was observed at about 940 cm^{-1}. Judging from these facts the glucuronide must be present in the amido form.

Based on the above mentioned discussions, the glucuronide obtained was concluded to be sulfathiazole-N^1-glucuronide having the following formula.



Experimental

Isolation of N-Glucuronide from Human Urine——Urine of three men after administration of 1 g. of sulfathiazole three times daily for three days was collected and filtered. 20~30 ml. of glacial AcOH and 300 g. of activated charcoal were added to each 10 L. of the filtrate and stirred. After standing over night, charcoal was filtered and washed with H_2O. This charcoal was extracted with 1.5 L. of BuOH-MeOH-conc.NH_4OH-H_2O (1:1:0.4:8) at 40° for 30 min. and the procedure was repeated three times. The following evaporated procedure was carried out below 40°. Extracted solutions were collected and concentrated to syrup under reduced pressure, removing the sulfathiazole and acetylsulfathiazole separated. To the residual syrup, 75 ml., 150 ml. and 300 ml. of abs. MeOH added in turn, cooling with ice and precipitate was filtered off on each time. The filtrate was concentrated under a reduced pressure and the residue was dissolved in a small amount of water. The solution was passed through the column (radius: 1.5 cm., length: 25 cm.) filled with 100~200 mesh of Dowex 50-X8 (H-from). Rinsed with water until the eluate becomes to pH 5, and then with 0.1*N* HCl for 3 hr. Water eluate and the fraction which is positive to aromatic amine test was collected. The solution was neutralized with *N* NH_4OH and concentrated to 5~10 ml. under a reduced pressure. The concentrated solution was fixed to the filter papers that had been washed with water and dried (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 papers. After dried in air, the glucuronide portion was cut off and extracted with water three times. The extracted solution was again passed through the column (radius: 0.5 cm., lengh: 20 cm.) filled with the same ion exchange resin described above and adsorbed. After continuous rinse of water, eluted with 0.5*N* NH_4OH, and the positive portion for aromatic amine reaction was collected and concentrated under a reduced pressure to dryness (about 230 mg.). The residue was dissolved in a small amount of abs. MeOH and filtered. 1.5 times of EtOH was added and the precipitate was separated with filtration, washed with abs. EtOH. After dryness dissolved in small amount of water and dried *in vacuo* on P_2O_5, colorless small needles were obtained, yield, 50 mg. *Anal.* Calcd. for $C_{15}H_{20}N_4O_8S_2 \cdot H_2O$: C, 38.62; H, 4.62; N, 12.01. Found: C, 38.01; H, 5.04; N, 11.81.

Hydrolysis with Hydrochloric Acid——To 20 mg. of crystal described above, 0.5 ml. of *N* HCl was added. The mixture was heated on the steambath for 90 min. and filtrated, The filtrate was neutralized with NaOH and was passed through the column packed with 100~200 mesh of H-form Dowex 50W-X8 (radius: 2.5 mm., length: 6 cm.) and adsorbed. The column was rinsed with 0.1*N* HCl for 2 hr. and again rinsed with H_2O until the eluate became to neutral. Then eluted with *N* NH_4OH and the fraction positive for aromatic amine test was collected and evaporated to dryness under reduced pressure. Recrystallized from EtOH. Yield, 7 mg. colorless prismatic rod crystal, m.p. 202°. This crystal corresponded with sulfathiazole.

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Summary

A new glucuronide of sulfathiazole was isolated from human urine after administration of sulfathiazole. This glucuronide was isolated as its ammonium salt with melting point 176~178° (decomp.). It was confirmed that the conjugate is sulfatiazole-N^1-glucuronide by the data of ultraviolet and infrared spectroscopy.

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122. Mikio Honjo, Yoshiyasu Furukawa, Hiroki Moriyama, and Kuniyoshi Tanaka : Synthesis of Nicotinamide Adenine Dinucleotide Analogs and their Coenzymatic Activities.*1

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Many NAD*3 analogs have so far been prepared enzymatically,[1~8] but the analogs synthesized chemically are only NMN-IMP,[9] the N-hydroxyethyl derivative of NAD[10] and nicotinic acid adenine dinucleotide.[11]

This paper describes the chemical synthesis of NAD analogs in which the adenosine portion is replaced by other naturally occurring nucleosides derived from RNA and DNA, and their reaction with dehydrogenases.

Among the compounds of this kind, only NMN-dAMP has so far been prepared enzymatically.[6]

In general, the phosphoramidate method is superior to the DCC method in the synthesis of nucleotide coenzymes such as FAD, UDPG, etc.[12] Therefore, the synthesis

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*1 After our publication of the short communication in this Bulletin, **10**, 73 (1962), a similar paper on the synthesis of nicotinamide adenine dinucleotide analogs and their properties was published in J. Biol. Chem., **237**, 1709 (1962) by C.P. Fawcett and N.O. Kaplan.

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*3 Abbreviations used : NAD, Nicotinamide adenine dinucleotide; NMN-IMP, Nicotinamide hypoxanthine dinucleotide; RNA, ribonucleic acid; DNA, Deoxyribonucleic acid; NMN-dAMP, 2′-Deoxyadenosine analog of NAD; FAD, Flavin adenine dinucleotide; UDPG, Uridine diphosphate glucose; NMN, Nicotinamide mononucleotide; AMP-NH_2, Adenosine 5′-phosphoramidate; DCC, Dicyclohexylcarbodiimide; dAMP, 2′-Deoxyadenosine 5′-phosphate; GMP, Guanosine 5′-phosphate; CMP, Cytidine 5′-phosphate; UMP, Uridine 5′-phosphate; TMP, Thymidine 5′-phosphate; NMN-GMP, Nicotinamide guanine dinucleotide; NMN-CMP, Nicotinamide cytosine dinucleotide; NMN-UMP, Nicotinamide uracil dinucleotide; NMN-TMP, Nicotinamide thymine dinucleotide; NADH, Reduced NAD.

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