Studies on Metabolism of Drugs. X.¹⁾ New Metabolite of Sulfisomezole in Man. (3)

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In order to elucidate the remaining one minor metabolic product of sulfisomezole in human urine after its ingestion, the human urine was treated with activated carbon, and the adsorbed product was purified through Dowex 50W-X8 and Amberlite IRA-68 columns, paper partition chromatography, and Sephadex G-25 and SE-Sephadex, and the product was obtained as colorless prisms, mp 123—125°. This product was examined for solubility, presence of alcoholic hydroxyl, hydrolysis, and the usual chemical and physical analyses. Nuclear magnetic resonance spectrum indicated the loss of a methyl group from sulfisomezole and appearance of a methylene and hydroxyl. Acetylation of this product with acetic anhydride gave a triacetylated compound. These experimental results indicated the *in vivo* oxidation of CH₃ in sulfisomezole to CH₂OH and the product was confirmed to be N¹-(5-hydroxymethyl-3-isoxazolyl)sulfanilamide.

Sulfisomezole (I), N¹-(5-methyl-3-isoxazolyl) sulfanilamide, is a widely used long-duration sulfa drug first developed by Kanô and others.³⁾ Previous papers of this series reported the isolation of sulfisomezole N¹-glucuronide⁴⁾ and 2'-glucuronide⁵⁾ as the new metabolites from human urine after ingestion of I. Besides the above two, unchanged I and N⁴-acetyl-sulfisomezole⁶⁾ were also detected. Paper partition chromatography (PPC) of the human urine, developed with a solvent system of butanol-methanol-water (3:1:1) revealed the presence of another new substance at *Rf* 0.77, differing from *Rf* 0.87 of I. In order to establish the identity of this substance, metabolic products of I were extracted and examination was made on the structure of this new product. Experimental results indicated that this metabolite was formed by oxidation of the methyl group in I, to N¹-(5-hydroxymethyl-3-isoxazolyl) sulfanilamide (I-CH₂OH).

Experimental

One-dimensional Paper Chromatography—Toyo Roshi No. 51 filter paper (2×4 cm) was used. Developing Solvent: BuOH-MeOH-H₂O (3:1:1), BuOH-MeOH-1N NH₄OH (3:1:1), BuOH-AcOH-H₂O (4:1:5), BuOH saturated with 3% NH₄OH.

Detection Reagents—Ehrlich reagent⁷⁾ (for detection of amino group). Tsuda reagent⁸⁾ (for detection of aromatic amino group). Nessler reagent⁹⁾ (for detection of alcohol). Xanthogenic acid reaction¹⁰⁾ (for detection of alcohol). Bitto method¹¹⁾ (for detection of alcohol).

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Apparatus—Spectrophotometers used were Hitachi-Perkin Elmer Model 139 and Hitachi Model EPS-2 recording spectrophotometer. Infrared (IR) spectra were measured with Japan Optics Model IR-E, nuclear magnetic resonance (NMR) spectra with Varian Model 60 and Japan Electron Model JUM-C-60H, and mass spectra with Hitachi Model RMU-7. DC generator and paper electrophoresis apparatus were Toyo Kagaku Model II-C and C-I, respectively.

Extraction of I-CH₂OH from Human Urine—Urine (30 liters) was collected from 5 persons who had ingested 1.5 g of I every 12 hr for a total of 10 times. The urine was adjusted to pH 4.5 with AcOH, 600 g of activated carbon (Norit SX-II) was added, the whole was stirred, and the carbon was collected by filtration. The carbon was extracted consecutively with 3.5, 2.5, and 2.0 liters of a mixed solvent of MeOH-16N NH₄OH-H₂O (5:1:20) at 45°, and the extract solution was concentrated under a reduced pressure to about 400 ml. Subsequent concentration procedures were all carried out in N₂ stream at below 45°. I that precipitated out was removed from the concentrate and the residual solution was passed through a column (diameter × length: 4.1 × 40 cm) packed with Dowex 50W-X8 (100–200 mesh, H form). The column was eluted with H₂O until the effluent became neutral, then washed with 2 liters of 0.1N HCl for 5 hr. The column next washed with 17 liters of H₂O by which urea, sulfisomezole N¹-glucuronide, and sulfisomezole 2'-glucuronide eluted out in that order. The column was washed with 1.2 liters of 0.17N HCl and then with 10 liters of H₂O by which I-CH₂OH eluted out with small contamination of I. This last effluent was neutralized with 1N NH₄OH and concentrated under a reduced pressure to ca. 20 ml. This solution was passed through a column (2.3 × 30 cm) of Amberlite IRA-68, the column was washed with 1 liter of 0.005 N NH₄OH for 8 hr, and eluted slowly with 2 liters of 0.01 N NH₄OH for 15 hr. The last effluent was streaked on five filter papers (40 × 40 cm) of Toyo Roshi No. 50, preliminarily washed with H₂O, and developed with BuOH-MeOH-H₂O (3:1:1). The portion containing I-CH₂OH was cut out, extracted with H₂O, and the aqueous solution was concentrated under a reduced pressure.

This concentrated solution was passed through a column (11 × 15 cm) of Sephadex G-25 Fine, the column was eluted with H₂O, and the aqueous effluent was concentrated under a reduced pressure. This concentrate was then adsorbed on 24 ml of SE-Sephadex C-25 Medium, the gel was eluted with H₂O, and the effluent was concentrated under a reduced pressure. This concentrate was adsorbed on 5 ml of Dowex 50W-X8 (100–200 mesh), which was washed with water, and eluted with 0.5N NH₄OH. The effluent was concentrated under a reduced pressure and the residue was dried over P₂O₅, affording 300 mg of I-CH₂OH. Its recrystallization from H₂O gave colorless prisms, mp 123–125°. *Anal.* Calcd. for C₁₀H₁₁O₄N₃S: C, 44.60; H, 4.12; O, 23.77; N, 15.61; S, 11.90. Found: C, 44.52; H, 4.19; O, 23.53; N, 15.48; S, 11.70. UV $\lambda_{\max}^{\text{EtOH}}$ m μ (log ϵ): 266 (4.24). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1465 (CH₂), 1154 (SO₂), 1310 (SO₂). NMR δ ppm (DMSO-*d*₆): 4.40 (2H, singlet, CH₂), 5.50 (1H, broad, OH), 5.95 (2H, singlet, NH₂), 6.15 (1H, singlet, isoxazole ring CH), 6.55 (2H, doublet, *J*=8.5 cps, benzene proton at 3 and 5), 7.44 (2H, doublet, *J*=8.5 cps, benzene proton at 2 and 4), 10.90 (1H, broad, NH).

Synthesis of N¹,N⁴,5-Triacetyl-N¹-(5-hydroxymethyl-3-isoxazolyl)-sulfanilamide—A mixture of 40 mg of I-CH₂OH and 0.2 ml (about 5 times the calculated amount) of Ac₂O was gently heated at ca. 140° for 2 hr, allowed to cool, and evaporated to dryness over NaOH under a reduced pressure. The residue was dissolved in MeOH, a small amount of H₂O and activated carbon were added, and the mixture was filtered. The filtrate was evaporated to dryness and the residue was recrystallized from MeOH to 15 mg of fine needles, mp 145–146°. *Anal.* Calcd. for C₁₆H₁₇O₇N₃S: C, 48.60; H, 4.34; N, 10.63. Found: C, 48.85; H, 4.50; N, 10.51.

Result and Discussion

Extraction of I-CH₂OH from human urine is illustrated in Chart 1 and the product was purified by the consecutive use of activated carbon, Dowex 50W-X8 (RSO₃⁻H⁺), Amberlite IRA-68 (OH⁻ form), paper chromatography, Sephadex G-25 (gel filtration), and SE-Sephadex (RC₂H₄SO₃⁻H⁺), and 300 mg of I-CH₂OH was obtained as colorless prisms, mp 123–125°. This substance is soluble in MeOH and EtOH, slightly soluble in PrOH, EtOAc, and H₂O, and sparingly soluble in benzene and ether. H₂O was used for its recrystallization. The substance colors immediately with the Ehrlich reagent, but not with Ninhydrin, aniline-phthalic acid, and FeCl₃ reagent. It is also negative to the iron hydroxamate reaction for identification of carboxylic acid.

I-CH₂OH was heated with 5N HCl or 5N NaOH for 8 hr on a boiling water-bath and the neutralized solution was submitted to paper chromatography. Majority of the product was the unchanged compound but sulfanilamide and sulfanilic acid were detected as the hydrolyzed product.

Chart 1. Isolation of Hydroxymethyl-sulfisomezole

When the solvent system used in paper chromatography was BuOH-MeOH-1N NH₄OH (3:1:1), similar to BuOH-MeOH-H₂O (3:1:1), the *R_f* value lowered from 0.77 to 0.32, suggesting the presence of an acidic hydrogen in N¹-position which formed an ammonium salt in NH₄OH-alkaline solvent and the compound became soluble in water, resulting in the lowering of the *R_f* value. Electrophoresis of the metabolite with 0.2N AcOH as an electrolyte solution, at 1000 V and 3–5 mA, showed the same electrophoretic movement as that of I, suggesting that there is no sulfonate or phosphate group.

Elemental analysis of the well-dried extract of the metabolite gave values agreeing with the empirical formula of C₁₀H₁₁O₄N₂S. Comparison of ultraviolet (UV) spectra of I and the extracted product showed the presence of a maximum absorption at 267 mμ in I and at 266 mμ in this substance, the curve being similar in both.

Comparison of the IR spectra of I and the extracted product showed that I exhibited a strong absorption for methyl at 1370 cm⁻¹ while this absorption was absent in that of the extracted product, as shown in Fig. 1, and in its stead an absorption for a methylene had appeared at 1465 cm⁻¹. Absorption for hydroxyl was not clarified due to a strong absorption at 3300 cm⁻¹ in the spectrum of I. Presence of an absorption for SO₂ at 1154 cm⁻¹ in the spectrum of the extracted product indicated that this product was in an amide type.¹²⁾

In the NMR spectra of the extracted product shown in Fig. 2, examinations were made by dissolving the product in CD₃SOCD₃ (A) and with an addition of D₂O (B). The singlet

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Fig. 1. Infrared Absorption Spectra (KBr)

a: hydroxymethyl-sulfisomezole
b: sulfisomezole

Fig. 2. NMR Spectra of the new Metabolite, Hydroxymethyl-sulfisomezole (A) and Addition of D₂O to A (B) in d₆-DMSO at 60 Mc

(2H) at 4.40 ppm is assigned to the methylene, and the slightly broad peak (1H) at 5.50 and the singlet (2H) at 5.59 ppm are assigned to OH and NH₂ group, respectively, since they almost disappear by the addition of D₂O. The presence of CH₂OH now became certain. The sharp singlet (1H) at 6.15 ppm is the proton at 4-position in the isoxazole ring, and the doublets ($J=8.5$ cps) at 6.55 (2H) and 7.44 are respectively *ortho* and *meta* to the NH₂ group in the *p*-disubstituted benzene. The broad peak (1H) at 10.90 ppm disappears on the addition of D₂O and is therefore assigned to the NH proton.

In the mass spectrum, the product underwent decomposition because of the high temperature volatilization (200°) and the molecular ion with highest mass was not detected but the largest amount (signal) in the decomposition product was at m/e 31 corresponding to CH₂OH followed by that at m/e 18 corresponding to H₂O. This result agrees well with the

Chart 2. The Metabolic Pathways of Sulfisomezole

observations of Ohno and Hatanaka,¹³⁾ and of Ahlquist, *et al.*¹⁴⁾ that CH_2OH is easily liberated from β -branched primary alcohols and the dehydration peak appears markedly. The product was positive to the Nessler reagent, xanthogenic acid reaction, and the Bitto method, all for the detection of primary alcohol. It formed N^1 , N^4 , 5-triacetylated compound when warmed with acetic anhydride. These experimental results suggested that I- CH_2OH has the structure of N^1 -(5-hydroxymethyl-3-isoxazolyl) sulfanilamide, as shown in Chart 2.

An attempt was made to synthesize this substance from I and its oxidation in glacial acetic acid with 30% hydrogen peroxide or selenium dioxide, and other reagents was carried out but the oxidation reaction in this case was a liberation of the isoxazole to form sulfanilamide, or the starting material was recovered. Five metabolic products obtained from human urine after ingestion of I are shown in Chart 2.

Oxidation of the methyl group in sulfisomezole to CH_2OH *in vivo* is the first instance in sulfa drugs and it is interesting that minor I- CH_2OH is thus changed *in vivo* although the chemical oxidation of I is rather difficult.

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