[Chem. Pharm. Bull. 19(10)2041—2045(1971)]

UDC 547.551.52.09 : 615.31.076.9

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

Michihiro Ueda, Ikuko Takegoshi, and Tamotsu Koizumi

Faculty of Pharmaceutical Sciences, University of Toyama[2]

(Received March 1, 1971)

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 CH_3 in sulfisomezole to CH_2OH and the product was confirmed to be N^1-(5-hydroxymethyl-3-isoxazolyl)sulfanilamide.

Sulfisomezole (I), N^1-(5-metyl-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 N^1-glucuronide[4] and 2′-glucuronide[5] as the new metabolites from human urine after ingestion of I. Besides the above two, unchanged I and N^4-acetyl-sulfisomezole[6] 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 N^1-(5-hydroxymethyl-3-isoxazolyl) sulfanilamide (I-CH_2OH).

Experimental

One-dimensional Paper Chromatography——Toyo Roshi No. 51 fiter paper (2×4 cm) was used. Developing Solvent: BuOH–MeOH–H_2O (3:1:1), BuOH–MeOH–1N NH_4OH (3:1:1), BuOH–AcOH–H_2O (4:1:5), BuOH saturated with 3% NH_4OH.

Detection Reagents——Ehrlich reagent[7] (for detection of amino group). Tsuda reagent[8] (for detection of aromatic amino group). Nessler reagent[9] (for detection of alcohol). Xanthogenic acid reaction[10] (for detection of alcohol). Bitto method[11] (for detection of alcohol).

1) Part of this work was presented at the 24th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, June 1967. Part IX: M. Ueda, N. Murakami, K. Furuki, and H. Atsumura, *Chem. Pharm. Bull.* (Tokyo), **16**, 352 (1968).
2) Location: *3190 Gofuku, Toyama.*
3) H. Kanô and K. Ogata, *Shionogi Kenkyusho Nenpo*, **7**, 1 (1957); H. Nishimura, K. Nakajima, and N. Shimaoka, *ibid.*, **7**, 7 (1957).
4) M. Ueda and K. Kuribayashi, *Yakugaku Zasshi*, **84**, 1104 (1964).
5) M. Ueda, Y. Nakagawa, and N. Murakami, *Yakugaku Zasshi*, **87**, 451 (1967).
6) H. Nishimura, K. Nakajima, S. Okamoto, N. Shimaoka, and K. Sasaki, *Shionogi Kenkyusho Nenpo*, **8**, 5 (1958); Y. Kishiwada, *Osaka Shiritsu Daigaku Igaku Zasshi*, **9**, 719 (1960).
7) A.E.A. Werner, *Lancet*, **236**, 18(1939); T. Uno and M. Ueda, *Yakugaku Zasshi*, **80**, 1785 (1960).
8) K. Tsuda and S. Matsunaga, *Yakugaku Zasshi*, **62**, 362 (1942).
9) L. Rosenthaler, *Arch. Pharm.*, **244**, 373 (1906).
10) E. Bamberger and W. Lodter, *Chem. Ber.*, **23**, 213 (1890).
11) B. Bitto, *Chemiker Ztg.*, **17**, 611 (1893).

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-CH_2OH 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 NH_4OH–H_2O (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 N_2 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 H_2O 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 H_2O by which urea, sulfisomezole N^1-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 H_2O by which I-CH_2OH eluted out with small contamination of I. This last effluent was neutralized with 1N NH_4OH and concentrated under a reduced pressure to *ca.* 20 ml. This solution was passed through a colunn (2.3 × 30 cm) of Amberlite IRA-68, the column was washed with 1 liter of 0.005 N NH_4OH for 8 hr, and eluted slowly with 2 liters of 0.01 N NH_4OH for 15 hr. The last effluent was streaked on five filter papers (40 × 40 cm) of Toyo Roshi No. 50, preliminarily washed with H_2O, and developed with BuOH–MeOH–H_2O (3: 1: 1). The portion containing I-CH_2OH was cut out, extracted with H_2O, 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 H_2O, 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 H_2O, 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 NH_4OH. The effluent was concentrated under a reduced pressure and the residue was dried over P_2O_5, affording 300 mg of I-CH_2OH. Its recrystallization from H_2O gave colorless prisms, mp 123—125°. *Anal.* Calcd. for $C_{10}H_{11}O_4N_3S$: 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 λ_{max}^{EtOH} mμ (log ε): 266 (4.24). IR ν_{max}^{KBr} cm^{-1}: 1465 (CH_2), 1154 (SO_2), 1310 (SO_2). NMR δ ppm (DMSO-d_6): 4.40 (2H, singlet, CH_2), 5.50 (1H, broad, OH), 5.95 (2H, singlet, NH_2), 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 N^1,N^4,5-Triacetyl-N^1-(5-hydroxymethyl-3-isoxazolyl)-sulfanilamide——A mixture of 40 mg of I-CH_2OH and 0.2 ml (about 5 times the calculated amount) of Ac_2O 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 H_2O 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 $C_{16}H_{17}O_7N_3S$: 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-CH_2OH from human urine is illustrated in Chart 1 and the product was purified by the consecutive use of activated carbon, Dowex 50W-X8 ($RSO_3^-H^+$), Amberlite IRA-68 (OH^- form), paper chromatography, Sephadex G-25 (gel filtration), and SE-Sephadex ($RC_2H_4SO_3^-H^+$), and 300 mg of I-CH_2OH was obtained as colorless prisms, mp 123—125°. This substance is soluble in MeOH and EtOH, slightly soluble in PrOH, EtOAc, and H_2O, and sparingly soluble in benzene and ether. H_2O was used for its recrystallization. The substance colors immediately with the Ehrlich reagent, but not with Ninhydrin, aniline–phthalic acid, and $FeCl_3$ reagent. It is also negative to the iron hydroxamate reaction for identification of carboxylic acid.

I-CH_2OH 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.

urine
| added activated charcoal at pH 4.5 (adjusted with AcOH)
charcoal
| treated with MeOH–16N NH_4OH–H_2O (5:1:20) at 45°
filtrate ← charcoal
| evapd. to about 100 ml
Dowex 50W-X8 (100—200 mesh, H type)
| 0.1N HCl 5 hr, washed with H_2O, 0.17N HCl 4 hr, eluted with H_2O
aq. soln.
| neutralized with 1N NH_4OH, evapd. to about 20 ml
Amberlite IRA-68
| eluted with 0.01N NH_4OH, evapd.
PPC BuOH–MeOH–H_2O (3:1:1)
| eluted with H_2O
Sephadex G 25
| eluted with H_2O
SE-Sephadex
| eluted with H_2O
Dowex 50W-X8 (100—200 mesh, H type)
| eluted with 0.5N NH_4OH
aq. soln.
| evapd. to dryness
crystals

Chart 1. Isolation of Hydroxymethyl-sulfisomezole

When the solvent system used in paper chromatography was BuOH–MeOH–1N NH_4OH (3:1:1), similar to BuOH–MeOH–H_2O (3:1:1), the *Rf* value lowered from 0.77 to 0.32, suggesting the presence of an acidic hydrogen in N^1-position which formed an ammonium salt in NH_4OH-alkaline solvent and the compound became soluble in water, resulting in the lowering of the *Rf* 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 $C_{10}H_{11}O_4N_2S$. 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 SO_2 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 CD_3SOCD_3 (A) and with an addition of D_2O (B). The singlet

12) T. Uno, K. Machida, K. Hanai, M. Ueda, and S. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **11**, 704 (1963); M. Ueda, M. Murakami, and Y. Nakagawa, *ibid.*, **16**, 345 (1968).

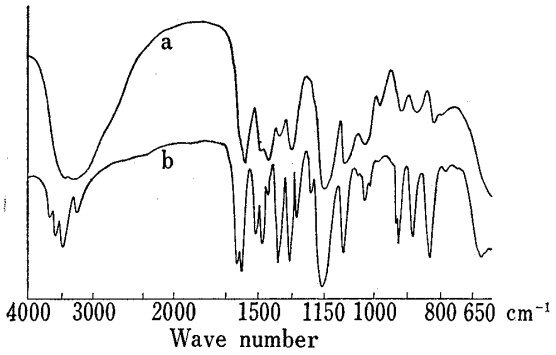

Fig. 1. Infrared Absorption Spectra (KBr)
a: hydroxymethyl-sulfisomezole
b: sulfisomezole

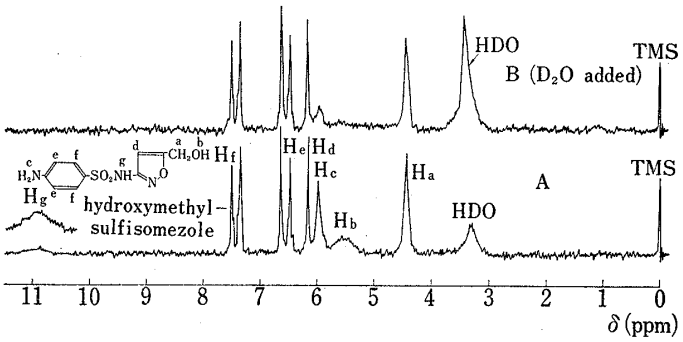

Fig. 2. NMR Spectra of the new Metabolite, Hydroxymethyl-sulfisomezole (A) and Addition of D_2O to A (B) in d_6-DMSO at 60 Mc

(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 NH_2 group, respectively, since they almost disappear by the addition of D_2O. The presence of CH_2OH 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 NH_2 group in the *p*-disubstituted benzene. The broad peak (1H) at 10.90 ppm disappears on the addition of D_2O 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 CH_2OH followed by that at *m/e* 18 corresponding to H_2O. This result agrees well with the

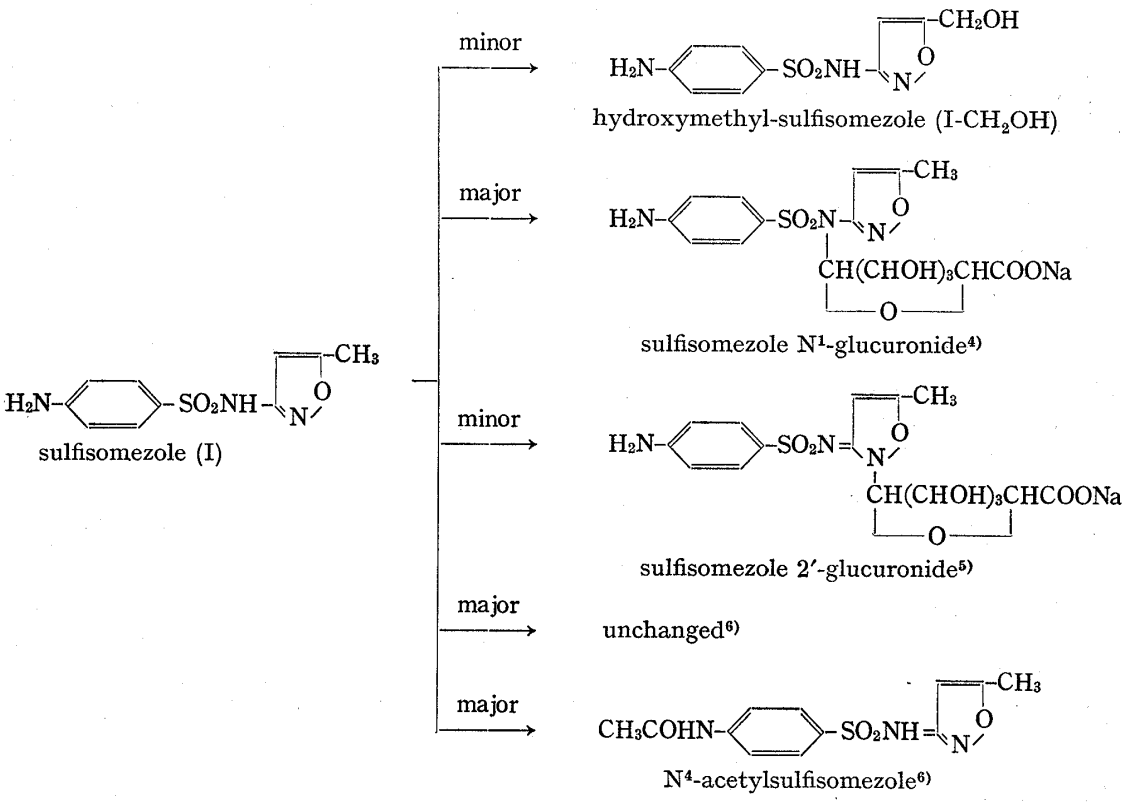

Chart 2. The Metabolic Pathways of Sulfisomezole

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.

Acknowledgement The authors are indebted to Dr. H. Kanô and Mr. I. Adachi of the Shionogi Research Laboratory for NMR spectral measurements, to the Elemental Analysis Center of the Kyoto University for the analyses of oxygen and sulfur, to the Naka Plant of Hitachi Ltd. for mass spectral measurements, and to Messrs. M. Morikoshi and H. Takami of this Faculty for elemental analyses and NMR spectal measurements.

13) M. Ohno and A. Hatanaka, *Acta Chem. Scand.*, **17**, 2051 (1963).
14) L. Ahlquist, R. Ryhage, E. Stenhagen, and E. Sydow, *Arkiv Kemi*, **14**, 211 (1959).