the bis(2-chloroethyl)amino moiety. In an attempt to gain further insight into this consideration, stereomodels and atomic models were constructed for the final products (IIIa–g). From both model types it appeared that the hexyl side chain was the appropriate length to allow the terminal methyl group to sterically affect the nitrogen atom. The ethyl and butyl side chains were too short for such an interaction and side chains longer than hexyl appeared subject to repulsion by the chloroethyl groups, thus, reducing their steric interaction with the nitrogen atom. Such an interaction between the nitrogen atom and the hexyl side chain could sterically hinder the participation of the nitrogen atom in the formation of the aziridinium ion intermediate. Such an interaction could reduce the reactivity of the hexyl derivative (IIIc) which would in turn reduce its antileukemic effectiveness and contribute to its relatively low toxicity.

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# Comparison of the Absorption, Excretion, and Metabolism of Suxibuzone and Phenylbutazone in Humans


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**Abstract** □ The absorption, excretion, and metabolism of a single oral dose of suxibuzone, a new nonsteroidal anti-inflammatory agent, in healthy male volunteers were compared with those of phenylbutazone. After oral administration of either suxibuzone or phenylbutazone, phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone were found in the plasma; phenylbutazone was the main metabolite of suxibuzone and phenylbutazone. In the urine, *p*-γ-dihydroxyphenylbutazone and several glucuronide conjugates also were found. Spectrometric and/or enzymatic analysis showed that these glucuronide conjugates were suxibuzone glucuronide, 4-hydroxymethylphenylbutazone glucuronide, 4-hydroxymethyloxyphenbutazone glucuronide, oxyphenbutazone glucuronide, and phenylbutazone glucuronides (two types: *O*-glucuronide and C-4-glucuroxide) after suxibuzone administration, and oxyphenbutazone glucuronide and phenylbutazone glucuronide after phenylbutazone administration. The conjugates specific to suxibuzone administration, suxibuzone glucuronide, 4-hydroxymethylphenylbutazone glucuronide, and 4-hydroxymethyloxyphenbutazone glucuronide, were excreted in the first 6 hr urine. These findings and the pharmacokinetics of these metabolites in the plasma and urine show that suxibuzone is a prodrug of phenylbutazone.



**Keyphrases** □ Suxibuzone—*in vivo* absorption, excretion, and metabolism compared to phenylbutazone, humans □ Phenylbutazone—*in vivo* absorption, excretion, and metabolism compared to suxibuzone, human □ Pharmacokinetics—suxibuzone and phenylbutazone, *in vivo* humans □ Anti-inflammatory agents—suxibuzone and phenylbutazone, *in vivo*, humans


Suxibuzone is a derivative of phenylbutazone, in which the proton at the C-4-position of the pyrazolidine ring is replaced by a β-carboxypropionyloxymethyl group. The outstanding feature of the drug is that it has extremely low ulcerogenicity (1, 2) although its anti-inflammatory, analgesic, and antipyretic properties are as strong as those of an equimolar dosage of phenylbutazone (3).

This feature of suxibuzone can be understood by comparing the biological fates of suxibuzone and phenylbutazone; however, there have been no studies on metabolism of suxibuzone in humans. Therefore, the present study compared the metabolic pathways and pharmacokinetics of suxibuzone and phenylbutazone in humans.

## EXPERIMENTAL

**Materials**—Suxibuzone[1], phenylbutazone[1], oxyphenbutazone[1], and γ-hydroxyphenylbutazone[1] were used as received. 4-Hydroxymethylphenylbutazone[2] was synthesized and purified.

**Chromatography**—HPLC was performed using a μ-Bondapak $C_{18}$ column (30 × 0.4-cm i.d.)[3], which was fitted with a 254-nm UV detector. For low-resolution liquid chromatography, a column (20 × 2.5 cm) packed with Amberlite XAD-2 resin (coarse grade 35–50 mesh)[4] or Dowex-1 resin (200–400 mesh)[5] was used.

GC was carried out under the following conditions: column 5% Silicon GE SE-30 on Chromosorb W AW-DMCS 60–80 mesh[6], 2 m × 3-mm i.d.; column temperature, 275°; nitrogen flow rate, 60 ml/min; and detector, flame ion detector.

TLC was carried out using commercial silica gel plates[7] with the following solvent systems (SS):

[1] S. A. Esteve Laboratory, Barcelona, Spain.
[2] Taiho Pharmaceutical Co., Research Laboratory, Tokushima, Japan.
[3] Waters Associates, Milford, Mass.
[4] Rohm and Haas Co.
[5] Dow Chemical Co.
[6] Shimadzu, Kyoto, Japan.
[7] Kieselgel 60 $F_{254}$, Precoated, 0.25-mm thick, Merck, West Germany.


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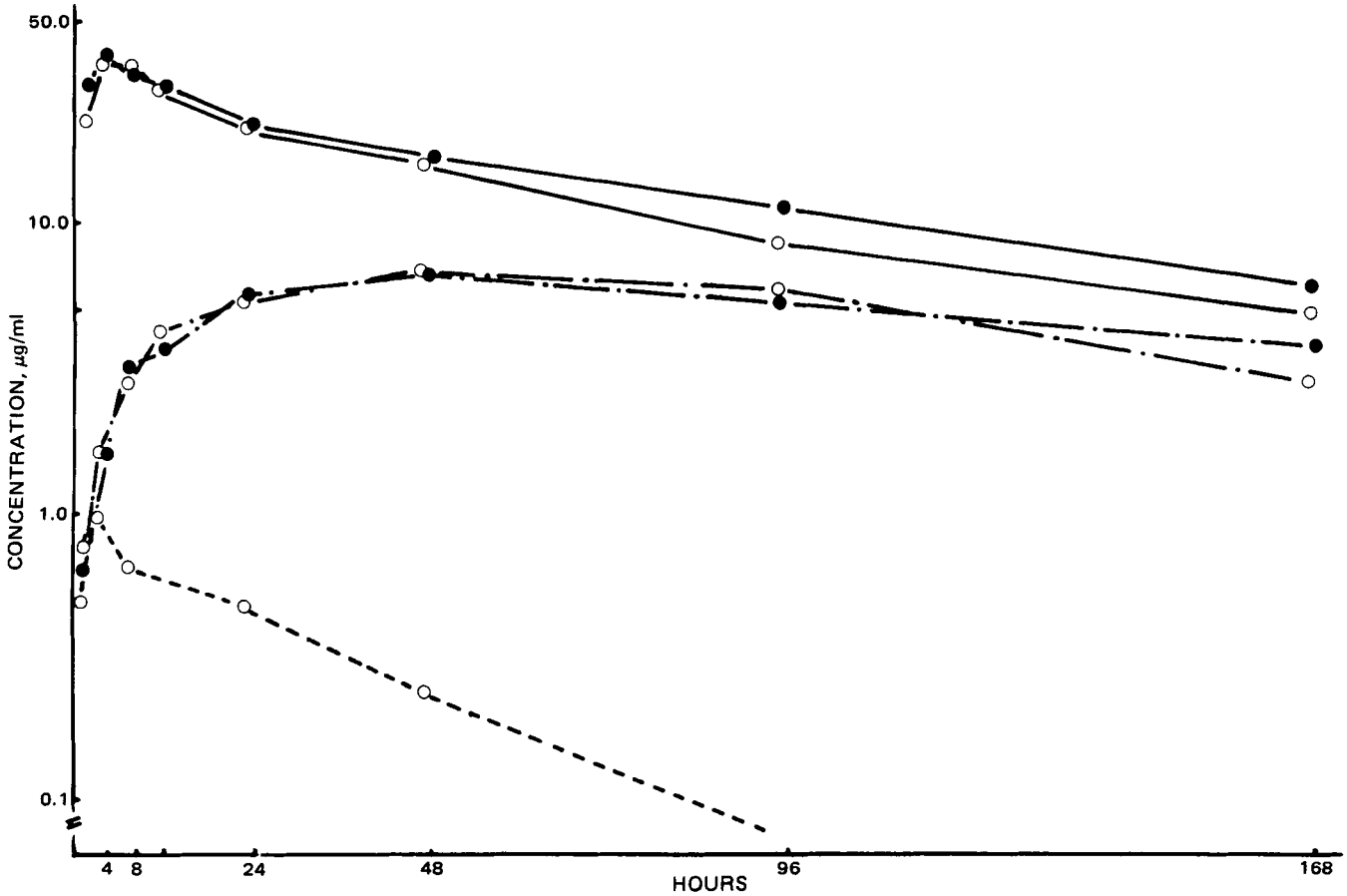



**Figure 1**—*Mean plasma concentration of phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone after oral administration of suxibuzone or phenylbutazone. Key: —, phenylbutazone; -·-, oxyphenbutazone; ---, γ-hydroxyphenylbutazone; ○, suxibuzone administration, ●, phenylbutazone administration.*

SS 1: chloroform–methanol–formic acid (100:50:5)
SS 2: ethyl acetate–butanone–formic acid–water (50:30:10:10)
SS 3: chloroform–methanol–formic acid (100:40:5)
SS 4: chloroform–methanol–formic acid (100:20:5)
SS 5: chloroform–methanol–acetone–formic acid (100:20:20:5)

**Spectrometric Measurements**—Direct inlet mass spectra were measured under the following conditions: ionizing energy, 75 eV; ionizing current, 200 μA; and acceleration voltage, 7 kV. GC–mass spectrometry was performed with a mass spectrometer under similar conditions, except that the ionizing energy was 24 eV and by GC under the conditions described above. Field desorption–mass spectrometry was carried out using a carbon emitter; acceleration voltage, 7 kV; and cathode voltage, −6 kV.

UV spectra were measured in methanol solution. NMR spectra were recorded in deuterated methanol with tetramethylsilane as internal standard.

**Treatment of Subjects and Collection of Samples**—Six male volunteers (weight, 54–68 kg; age, 27–35 years), who had had no other medication for at least 2 weeks before the experiments, were randomly divided into two groups and given a single 426-mg (3 capsules) oral dose of suxibuzone[8] or an equimolar amount, 300 mg (3 tablets), of phenylbutazone[9]. Four weeks after the first administration, the test was repeated on the two groups but interchanging the drug.

Blood was withdrawn at intervals during the first 7 days after drug administration and mixed with heparin to rapidly separate the plasma. Urine was collected quantitatively for the first 7 days, and feces were collected for the first 2 days. The excreta were kept frozen until analyzed.

**Identification and Quantitative Analysis of Metabolites in Plasma**—Plasma (6 ml) taken from blood of three volunteers 2 and 4 hr after drug administration was used for identification of metabolites. The sample was diluted with 9 ml of saline, adjusted to pH 2 with 2 *N* HCl, and extracted twice with 6 volumes of benzene–cyclohexane (1:1 v/v). The organic phase was divided into two portions, which were evaporated under nitrogen gas.

One residue was dissolved in a small amount of methanol and chromatographed on a reversed-phase column, using a mobile phase of a linear gradient of methanol in 0.05 *M* $KH_2PO_4$(0–100% methanol, 8%/min; flow rate, 2.0 ml/min) on a high-pressure liquid chromatograph[10] (4).

The other residue was dissolved in 100 μl of a freshly prepared solution of anhydrous chloroform and *N,O*-bis(trimethylsilyl)trifluoroacetamide[11] (I) (1:4 v/v) and stood for 1 hr at room temperature. The resulting trimethylsilylated derivatives were then analyzed by GC[12] and GC–mass spectrometry[13].

Plasma samples (2 ml) separated 2, 4, 8, 12, 24, 48, 96, and 168 hr after drug administration, were analyzed quantitatively by GC using tetraphenylethylene as an internal standard. The recoveries of suxibuzone, phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone added to plasma at concentrations of 5–20 μg/ml were all more than 90%. The detection limits were 0.1 μg/ml for suxibuzone, and 0.05 μg/ml for other metabolites.

**Identification of Free Metabolites in Urine**—Samples of 5–10 ml of pooled urine were acidified and extracted with benzene–cyclohexane and the remaining aqueous phase was then extracted twice with 6 volumes of methylene chloride. The organic phases were then treated as described for plasma and the metabolites were identified by HPLC, GC, and GC–mass spectrometry.

**Identification of Conjugated Metabolites in Urine**—Pooled urine samples (500–1000 ml) were adjusted to pH 2 and extracted with benzene–cyclohexane to eliminate free metabolites. The aqueous phase was then saturated with sodium chloride and extracted twice with 6 volumes of ethyl acetate, and the organic layer was evaporated to dryness. The

[8] Lot 8D 77, Taiho Pharmaceutical Co.
[9] Butazolidin, Lot 1520 ATOH FF, Japan-Ciba-Geigy.
[10] Model LC-2, Shimadzu, Kyoto, Japan.
[11] Pierce Chemical Co.
[12] Model GC-4CM, Shimadzu, Kyoto, Japan.
[13] Model JMC-01SG-2 connected with a JGC-20K Gas Chromatography, JEOL, Tokyo, Japan.

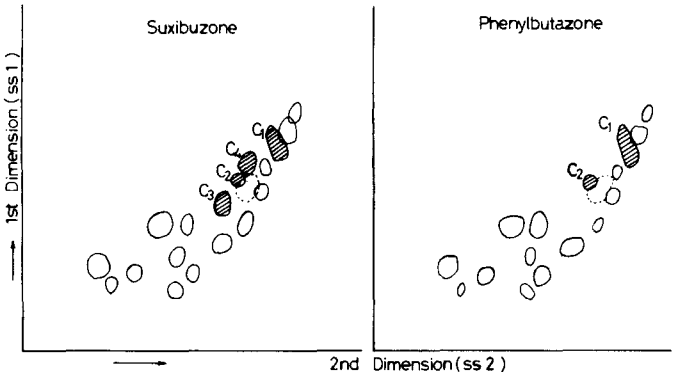



**Figure 2**—*Typical examples of thin-layer chromatograms of glucuronides in urine after suxibuzone or phenylbutazone administration.*

residue was dissolved in distilled water (containing 1% v/v methanol) and the glucuronide fraction was obtained by the method of Kamil *et al.* (5). The glucuronide fraction was analyzed by TLC on silica gel. Samples were spotted on the gel and developed in two dimensions with solvent systems SS 1 and SS 2. The material visualized by reaction with naphthoresorcinol (blue) or anisaldehyde (green-gray), which were not seen on chromatography of control urine, were scraped off, dissolved in 5 ml of 0.067 (1/15) *M* acetate buffer (pH 5.5), and incubated with 1000 U of β-glucuronidase[14] at 37° for 3 hr. The samples were then treated in a way similar to that described for identification of free metabolites.

The four kinds of glucuronides (II, III, IV, V) were isolated and identified as follows. The 0–6 hr urine samples from three volunteers were

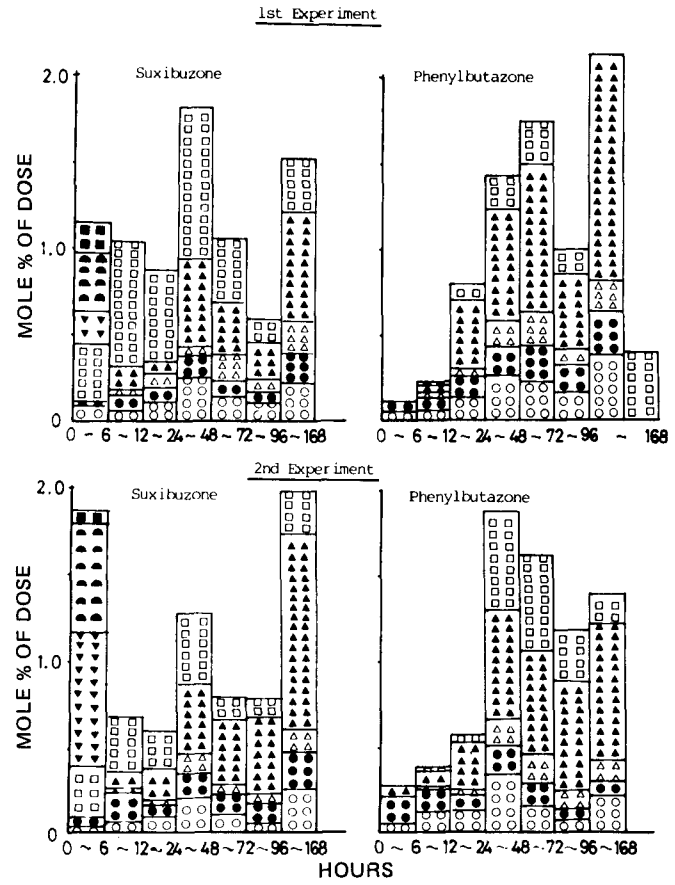



**Figure 3**—*Urinary excretion of unchanged drugs and their metabolites after oral administration of suxibuzone or phenylbutazone. Key:* ○, *phenylbutazone;* ●, *phenylbutazone glucuronide;* △, *oxyphenbutazone;* ▲, *oxyphenbutazone glucuronide;* □, *γ-hydroxyphenylbutazone;* ■, *suxibuzone glucuronide;* ◓, *4-hydroxymethylphenylbutazone glucuronide;* ▼, *4-hydroxymethyloxyphenbutazone glucuronide.*

[14] Type I, Bacterial, Sigma Chemical Co.

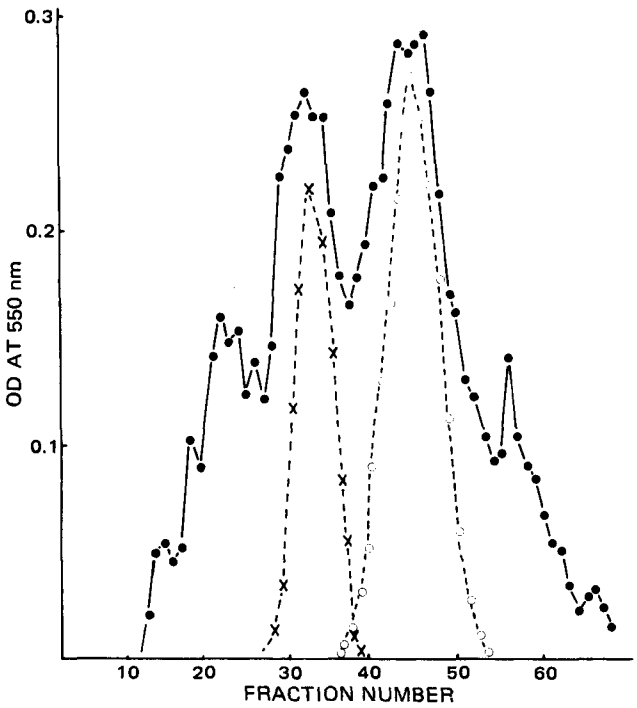



**Figure 4**—*Elution pattern of II and III on Dowex-1. Key:* ●, *orcinol reaction, OD at 550 nm;* X, *II;* ○, *III, TLC(SS 2), OD at 254 nm.*

combined and used for isolation of II and III; 12–24 hr urine samples were used for isolation of IV. In addition, bile from rats treated orally with phenylbutazone was used as a source of V to obtain a sufficient amount for spectrometric measurement. Each urine sample (1000 ml) was adjusted to pH 2 with concentrated HCl and extracted twice with 6 volumes of methylene chloride. The aqueous phase was applied to an Amberlite XAD-2 column, and the fraction eluted with 20–80% methanol was purified by the method of Kamil *et al.* (5). The glucuronide mixture thus obtained was separated on a Dowex-1 column with 60% methanol in 0.01 *N* HCl and by preparative TLC with SS 2.

The isolated glucuronides were then subjected to spectrometric measurements. For mass spectrometry, the glucuronides were converted to their methyl and methyl acetyl derivatives as follows. The glucuronide fraction was dissolved in a small amount of methanol (~500 μl), mixed with 5 volumes of ethereal diazomethane, and kept for 20 min at room temperature. The methylated glucuronides were dissolved in anhydrous pyridine (500 μl), mixed with an equal volume of anhydrous acetic acid, stood overnight, and then precipitated by adding distilled water.

Bile was collected by cannula from the bile duct of rats (male Wistar, 200–250 g) over a 24-hr period after receiving 200 mg/kg phenylbutazone by stomach tube. Fifty milliliters of the bile was diluted with 8 volumes of a saline solution. The aqueous phase remaining after extraction of free metabolites was saturated with sodium chloride and extracted twice with 6 volumes of ethyl acetate. The extract was evaporated under reduced pressure, and the residue was dissolved in a small amount of methanol and subjected to preparative TLC with SS 2. The fraction that released phenylbutazone on β-glucuronidase treatment was eluted with methanol and purified further with HPLC on a reversed-phase column using 40% methanol (flow rate, 1.0 ml/min) as a mobile phase. The samples obtained were characterized by UV[15], mass spectrometry, and NMR[16] spectra.

**Quantitative Analysis of Metabolites in Urine**—Samples (5 ml) of urine collected 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, and 96–168 hr after drug administration were used to assay for metabolites. Each urine sample (pH 2) was extracted with 10 ml of 0.1 *M* phosphate buffer (pH 7.0). The aqueous phase was acidified and extracted twice with 20 ml of methylene chloride and the extracts were treated in the same way as plasma extracts. This procedure eliminated interfering peaks in the chromatogram.

For determination of the amounts of conjugated metabolites, the aqueous phase obtained after extraction with benzene–cyclohexane was used. The extracts with ethyl acetate were treated with a mixture of 3000

[15] Model 124, Hitachi, Tokyo, Japan.
[16] Model MH-400, Bruker.

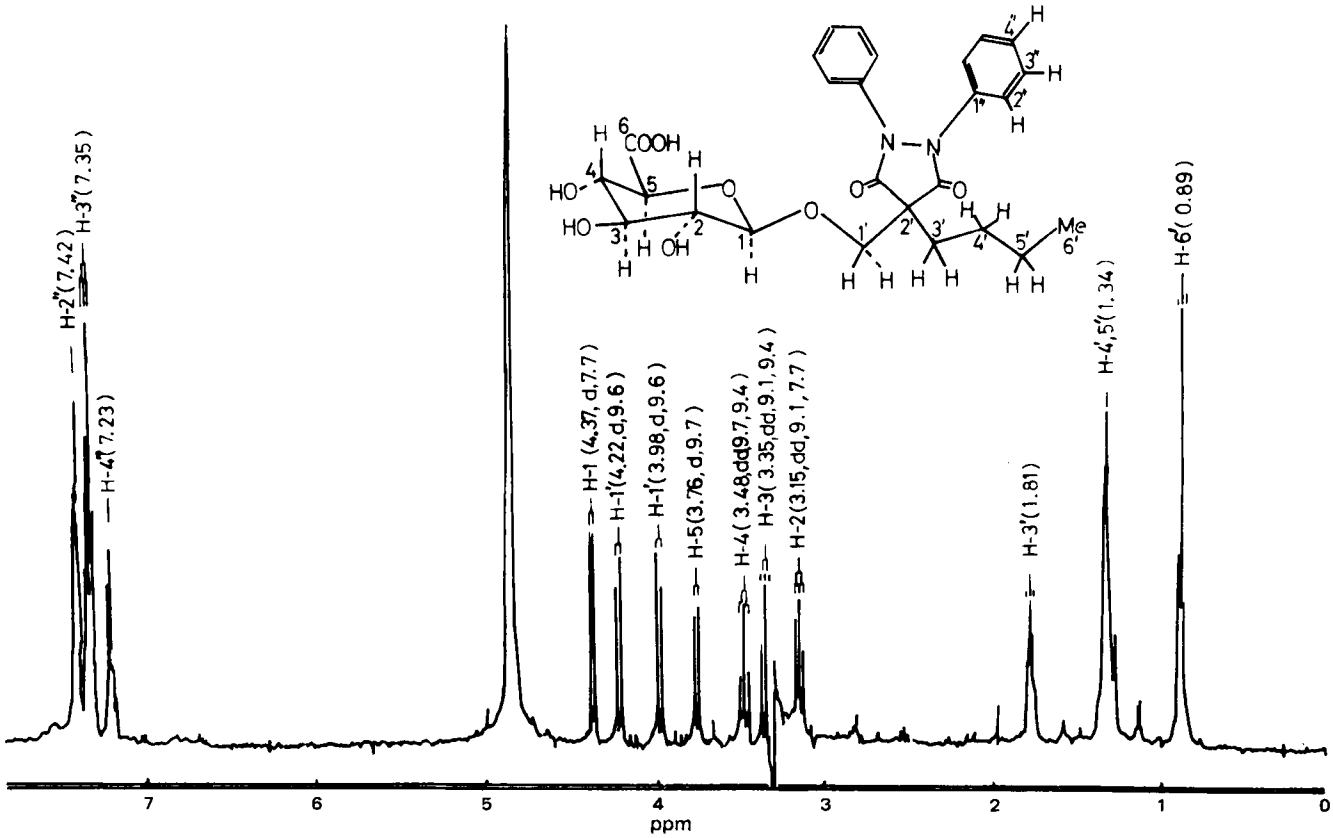



**Figure 5**—*NMR spectrum of II.*

U of β-glucuronidase and 10 U of sulfatase[17] in acetate buffer (pH 5.5) at 37° for 3 hr, and the concentrations of the respective aglycones released were determined by GC as described previously.

The recoveries of synthetic reference compounds in the range of 10–40 μg/ml by these extraction procedures were all >90%.

**Quantitative Analysis of Suxibuzone and Phenylbutazone in Feces**—The daily feces were homogenized with 3 volumes of water, adjusted to pH 2, and extracted with 5 volumes of benzene-cyclohexane. The concentrations of suxibuzone and phenylbutazone were determined by GC by procedures similar to those described for urine samples.

## RESULTS

**Identification of Metabolites in Plasma**—The metabolites in plasma were first analyzed by HPLC. Three specific metabolites with retention times of 10.5, 10.0, and 9.4 min were observed in plasma after suxibuzone administration. These metabolites were identified as phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone by comparing their retention times to those of authentic samples. No unchanged suxibuzone or 4-hydroxymethylphenylbutazone, a possible metabolite of suxibuzone, was detected. The plasma extract was also analyzed by GC and GC-mass spectrometry after trimethylsilylation of the metabolites. Three metabolites with retention times of 4.0, 7.9, and 6.8 min, corresponding to those of trimethylsilylated phenylbutazone, trimethylsilylated oxyphenbutazone, and trimethylsilylated γ-hydrophenylbutazone, respectively, were observed. These metabolites were confirmed further by GC-mass spectrometry. The trimethylsilylated derivatives had characteristic fragments at $m/z$ 380($M^+$), 337, 308, and 246 like trimethylsilylated phenylbutazone, at 468, ($M^+$), 425, 396, 334, and 246 like trimethylsilylated oxyphenbutazone, and at 468($M^+$), 376, 351, 338, and 246 like trimethylsilylated γ-hydroxyphenylbutazone, respectively. No conjugates were detected in the residual aqueous phase of plasma remaining after extraction with benzene–cyclohexane. Thus, the main metabolites in human plasma after suxibuzone administration were confirmed to be phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone. These metabolites were the same as those observed in plasma after phenylbutazone administration.

**Quantitative Analysis of Metabolites in Plasma**—The time courses of changes in the mean plasma phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone concentrations after administration of suxibuzone and phenylbutazone are shown in Fig. 1. The plasma phenylbutazone concentration was higher than that of other metabolites after administration of either suxibuzone or phenylbutazone. The mean plasma phenylbutazone concentration reached a maximum of 35.96 and 38.51 μg/ml as soon as 4 hr after administration of suxibuzone and phenylbutazone, respectively, and then decreased slowly between 8 and 168 hr, corresponding to elimination half-lives of 61.8 ± 5.0 (*SE*) hr on suxibuzone administration and 72.3 ± 3.6 hr on phenylbutazone administration. These values are comparable to that of 70.9 ± 5 hr reported by Levi *et al.* (6).

Change in the plasma oxyphenbutazone concentration exhibited similar time courses after administration of suxibuzone and phenylbutazone; it reached a maximum of ~7 μg/ml after 48 hr and decreased to 3–4 μg/ml within 168 hr.

On the other hand, the time course of change in the plasma γ-hydroxyphenylbutazone concentration after suxibuzone and phenylbutazone administrations were somewhat different; namely, it remained <0.1 μg/ml for at least 168 hr after phenylbutazone administration, but reached a maximum of ~1 μg/ml at 4 hr after suxibuzone administration. The difference between its concentrations 2–24 hr after administration of phenylbutazone and suxibuzone was statistically significant ($p < 0.05$).

These results show that suxibuzone was rapidly decomposed to phenylbutazone and that the plasma levels of phenylbutazone and oxyphenbutazone after suxibuzone administration were as high as those after phenylbutazone administration.

**Identification of Metabolites in Urine**—As in plasma, phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone were extracted from urine with benzene–cyclohexane. A metabolite extracted with methylene chloride and derivatized with I exhibited a specific peak with a retention time of 13.0 min on GC. This metabolite was determined as p-γ-dihydroxyphenylbutazone by GC–mass spectrometry. The mass spectrum of this trimethylsilylated derivative had characteristic fragment ions at $m/z$ 556($M^+$), 426, 334, 246, 181, 93, and 77, like those reported previously (7). No unchanged suxibuzone or 4-hydroxymethylphenylbutazone was detected in the urine and the specifically determined metabolites were the same as those found in the urine of subjects treated with phenylbutazone.

**Enzymatic Identification of Conjugated Metabolites**—An enzy-

[17] Type V, Sigma Chemical Co.

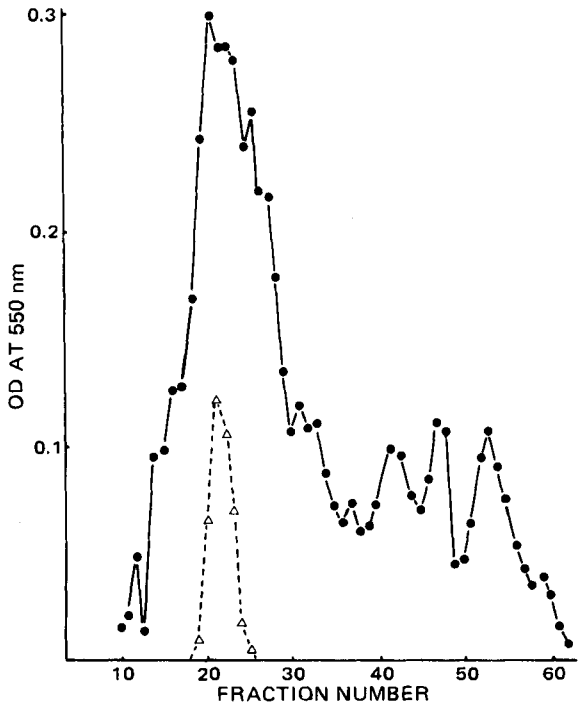



**Figure 6**—*Elution pattern of IV on Dowex-1. Key: ●, orcinol reaction, OD at 550 nm; △, IV, TLC(SS 2), OD at 254 nm.*

matic method was used to identify conjugated metabolites. The glucuronide mixture was separated by two-dimensional TLC, and the spots of material located by color reactions were analyzed by GC–mass spectrometry after hydrolysis with β-glucuronidase and derivatization with I. Typical thin layer chromatograms of glucuronides in urine after administration of suxibuzone and phenylbutazone are shown in Fig. 2. Analysis of each spot on the chromatogram after suxibuzone administration by GC showed that spot $C_1$ corresponded to trimethylsilylated phenylbutazone, spots $C_2$ and $C_3$ to trimethylsilylated oxyphenbutazone, and spot $C_4$ to trimethylsilylated suxibuzone and trimethylsilylated phenylbutazone, respectively. Chromatograms of urine samples after phenylbutazone administration gave spots $C_1$ and $C_2$ which corresponded to trimethylsilylated phenylbutazone and trimethylsilylated oxyphenbutazone, respectively. When the trimethylsilylated suxibuzone derivative of spot $C_4$ was analyzed by GC–mass spectrometry, the mass fragments coincided with those of the authentic compound; *m/z* 510($M^+$), 485, 395, 380, 264, 183, 173, 105, 93, and 77. This confirms that spot $C_4$ contains suxibuzone glucuronide. Spots $C_2$ and $C_3$ both contained trimethylsilylated oxyphenbutazone. Spot $C_3$ was found only in the 0–6 hr urine sample after suxibuzone administration, whereas spot $C_2$ was observed after both suxibuzone and phenylbutazone and gradually became larger with increase in the plasma concentration and urinary excretion of oxyphenbutazone (Figs. 1 and 3). Hence, spot $C_2$ is considered to correspond to oxyphenbutazone glucuronide. Similarly, spot $C_1$ was expected to be phenylbutazone glucuronide (V) and the glucuronides (III,II) corresponding to spots $C_3$ and $C_4$ appeared only after suxibuzone administration.

**Structures of Metabolites II and III**—Compounds II and III were isolated on a preparative scale by the procedure shown in Fig. 4. Parts of both metabolites were converted to their methyl (II/1,III/1) and methyl acetyl (II/2,III/2) derivatives. The mass and NMR spectra (Fig. 5) show that II and III are 4-hydroxymethylphenylbutazone glucuronide and 4-hydroxymethyloxyphenbutazone glucuronide, respectively; *i.e.*, conjugates in which the oxymethyl residue at the C-4-position of the pyrazolidine ring is attached to glucuronic acid *via* an O—C bond. In the mass spectra of the methyl derivatives II/1 and III/1, the presence of glucuronyl residue was indicated by molecular ions at *m/z* 528 and 558, respectively, together with corresponding aglycone fragment ions at *m/z* 338 and 368. (Under the conditions used, III was dimethylated at the carboxyl group of glucuronic acid and at the hydroxyl group of one of the phenyl rings.) This was confirmed by the mass spectra of the methyl acetyl derivatives II/2 and III/2; namely, molecular ions were observed at *m/z* 654 and 684, respectively, indicating triacetylation at the three hydroxyl groups of glucuronic acid. The existence of an oxymethyl residue was deduced from subsequent fragmentation of *m/z* 338 to 308 (loss of $CH_2{=}O$) for II/1 and

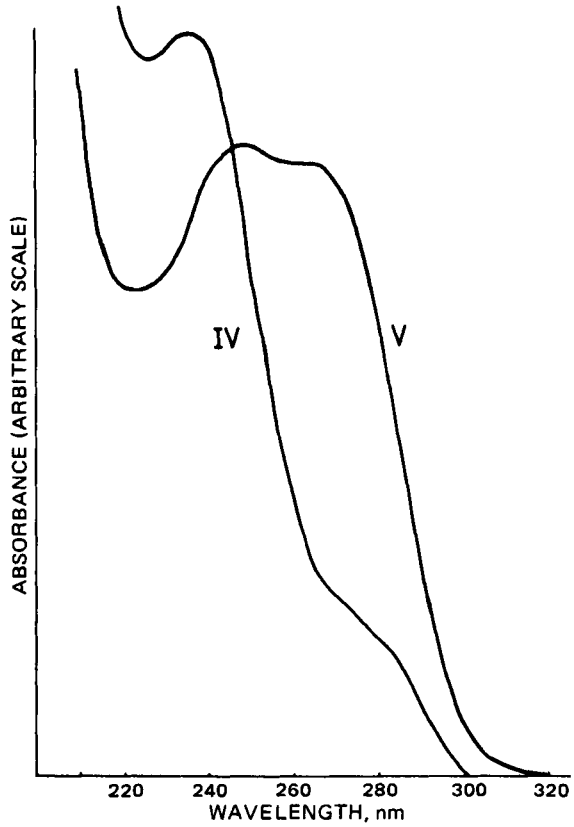
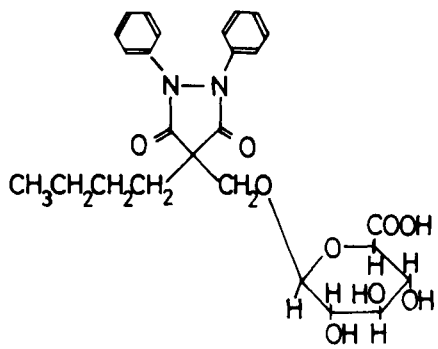


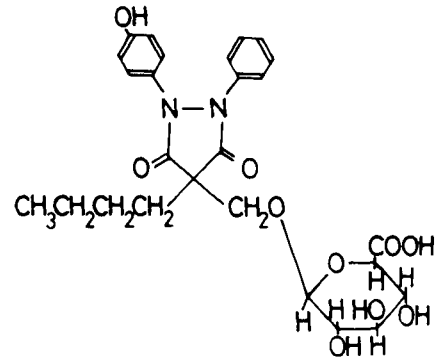

**Figure 7**—*UV spectra of IV and V.*

of *m/z* 368 to 338 for III/1. Furthermore, the ion set of *m/z* 338, 308, 264, and 183 observed for II/1 coincided with that of authentic 4-hydroxymethylphenylbutazone. The fragment ions at *m/z* 368, 338, 294, and 213 observed for III/1 were each shifted by *m/z* 30 relative to the corresponding fragment ions generated from 4-hydroxymethylphenylbutazone. This suggests the involvement of a methoxy groups in one of the phenyl rings (*e.g.*, $CH_3OC_6H_4N^+{=}NC_6H_5$ for the fragment ion at *m/z*

II




III



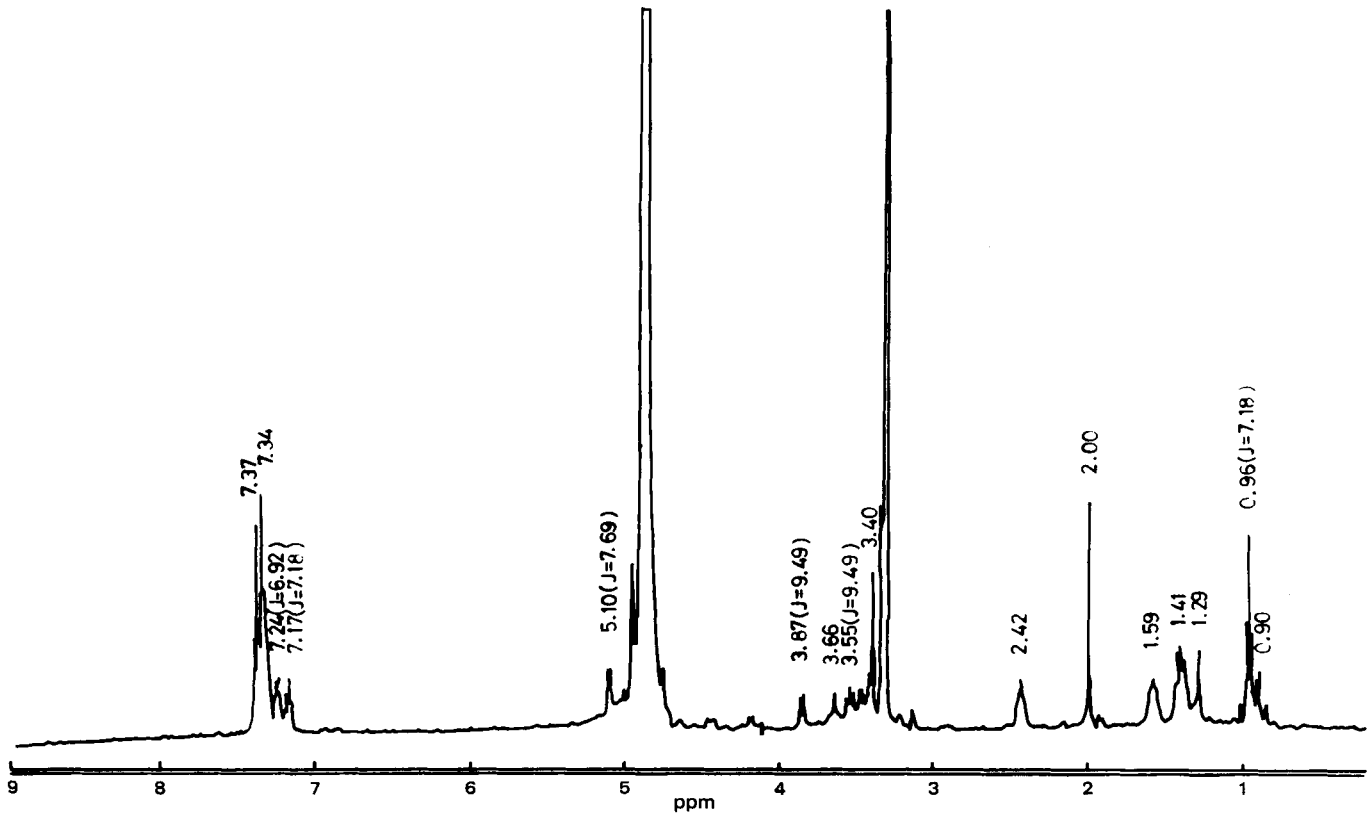



**Figure 8**—*NMR spectrum of V.*

213) and consequently suggests that the hydroxyl group in one of the phenyl rings is not attached to glucuronic acid. Confirmation of the assignments of the positions of glucuronidation in II and III were obtained from NMR spectra. The 400-MHz spectrum of II is shown in Fig. 5. Of significance for the assignment of glucuronidation at the oxygen of oxymethyl group is the appearance of an anomeric proton of glucuronic acid at $\delta$4.37 ppm and of methylene protons of the oxymethyl group at $\delta$4.22 and $\delta$3.98 ppm, both of which are separated into a doublet with a geminal coupling constant of 9.6 Hz. The anomeric proton of glucuronic acid in III appeared at $\delta$4.36 ppm, which is very close to that in II. The doublet signals ($J$ = 9.0 Hz) of aromatic protons at $\delta$6.72 and $\delta$7.22 ppm established the existence of the hydroxyl group at the *para*-position of one of the phenyl rings in III. The proposed structures of II and III are shown.

The aglycones of 4-hydroxymethylphenylbutazone and 4-hydroxymethyloxyphenbutazone obtained by hydrolysis of II and III seemed to be converted to phenylbutazone (or trimethylsilylated phenylbutazone) and oxyphenbutazone (or trimethylsilylated oxyphenbutazone), respectively, during the process of hydrolysis with $\beta$-glucuronidase and/or trimethylsilylation with I under the conditions used. Hence, the spot $C_4$ was concluded to be that of a mixture of suxibuzone glucuronide and II, while spot $C_3$ was concluded to be that of III.

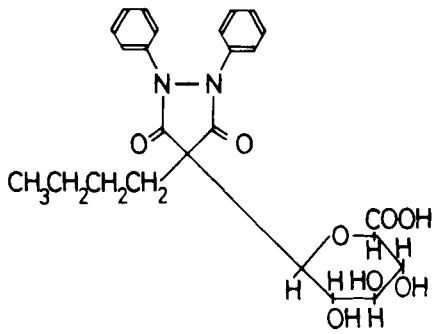
**Structures of IV and V**—The existence of C-4-phenylbutazone glucuronide in humans; *i.e.*, a conjugate in which the pyrazolidine ring is directly attached to glucuronic acid *via* a C—C bond and which is not hydrolyzed by $\beta$-glucuronidase, was demonstrated previously (8). On the other hand, the existence in humans and animals of a phenylbutazone glucuronide that can be hydrolyzed by $\beta$-glucuronidase (9, 10) was reported. Since there is little of this hydrolyzable phenylbutazone glucuronide in human urine, the structure of this compound(V) was first elucidated using bile of rats treated with phenylbutazone and then the identity of the material in rats and humans confirmed by TLC. C-4-phenylbutazone glucuronide (IV) was isolated from human urine.

The elution pattern of IV is shown in Fig. 6. Compound IV was identified as C-4-phenylbutazone glucuronide by mass spectrometry and UV studies (Fig. 7). The presence of glucuronic acid in the methyl ester of

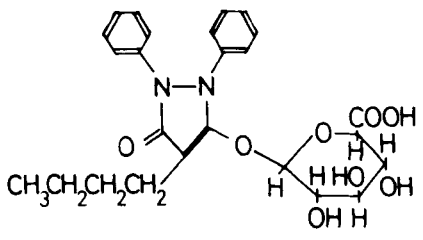
**Table I—Fecal Excretion of Suxibuzone and Phenylbutazone after Oral Administration**

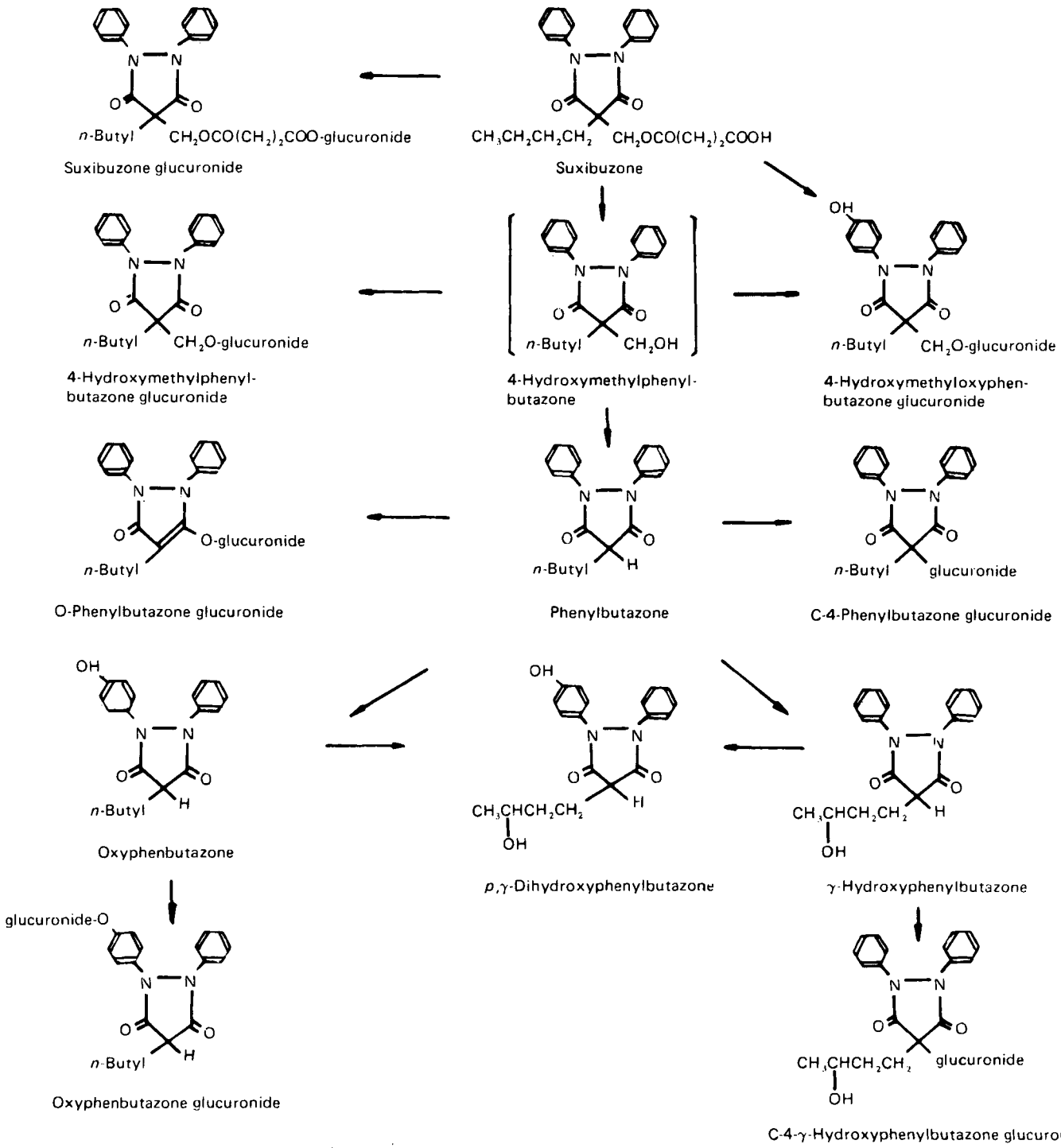
| Subject Number | | mole % of dose 0–24 hr | 24–48 hr |
|---|---|---|---|
| | Suxibuzone Administration | | |
| 1 | Suxibuzone | 0.0 | 0.0 |
| | Phenylbutazone | 0.2 | 0.2 |
| 2 | Suxibuzone | 0.0 | 0.0 |
| | Phenylbutazone | 0.5 | 0.3 |
| 3 | Suxibuzone | 0.0 | 0.0 |
| | Phenylbutazone | 0.2 | 0.2 |
| | Phenylbutazone Administration | | |
| 1 | Phenylbutazone | 0.2> | 0.2> |
| 2 | Phenylbutazone | 0.2> | 0.2> |
| 3 | Phenylbutazone | 0.4 | 0.5 |

IV




V






*Scheme I—Metabolic pathway of suxibuzone in humans.*

IV was evidenced by a molecular ion at $m/z$ 498 and a corresponding aglycone fragment ion at $m/z$ 308. The UV spectrum of IV revealed the presence of diphenyldioxopyrazolidine ring with an intact 1,3-dicarbonyl system exhibiting a UV absorbance maximum ($\lambda_{max}$) at 236 nm. These data coincide well with those reported previously (8).

Since V was labile under the conditions used for derivatization with diazomethane and with I, the molecular ion was detected by field desorption–mass spectrometry. The field desorption–mass spectrum of V showed two ion peaks of a molecular ion at $m/z$ 484 and a fragment ion at $m/z$ 308 corresponding to its aglycone. Positive evidence for an O—C attachment of glucuronic acid was obtained from the UV and NMR spectra of V (Figs. 7 and 8). The UV spectrum indicated the presence of an enolic dioxopyrazolidine ring exhibiting a UV absorption at $\lambda_{max}$ = 250 and ~265 nm instead of at $\lambda_{max}$ = 236 nm for IV with an intact 1,3-dicarbonyl system. The NMR spectrum established an O—C attachment of glucuronic acid more directly by exhibiting downfield shifts of an anomeric proton of the glucuronic acid at δ5.10 ppm ($J$ = 7.69 Hz) and of allylic protons of the $n$-butyl side chain at δ2.42 ppm. From these results it was concluded that V is an $O$-phenylbutazone glucuronide. The proposed structures of IV and V are as shown.

When co-chromatographed by two-dimensional TLC with solvent systems SS 1 and SS 2, IV and V migrated together giving a single spot. Hence, spot $C_1$ in Fig. 2 is considered to contain both $O$-phenylbutazone glucuronide and IV. With solvent system SS 5, however, IV and V were separated by TLC exhibiting $R_f$ values of 0.46 and 0.51, respectively. The methanol extracts of spot $C_1$ after suxibuzone or phenylbutazone administration were chromatographed with solvent system SS 5 using IV and V as reference compounds. The band corresponding to that of V was eluted with methanol and treated with β-glucuronidase and the release of phenylbutazone was confirmed by GC. The band corresponding to that of IV did not release phenylbutazone on β-glucuronidase treatment.

**Quantitative Analysis of the Metabolites in Urine**—Figure 3 shows

the molar percentages of doses of suxibuzone and phenylbutazone excreted as various metabolites in the urine in different times. (Since 4-hydroxymethylphenylbutazone was obtained by hydrolysis of its conjugate with β-glucuronidase and was observed as phenylbutazone on GC, the ratio of II to *O*-phenylbutazone glucuronide by TLC separation was determined first. The amount of each metabolite was then calculated from the ratio and the total amount observed as phenylbutazone. The amount of III and oxyphenbutazone glucuronide were determined by similar treatments.) As seen in Fig. 3, the composition of metabolites excreted in the first 6-hr urine samples after administration of suxibuzone and phenylbutazone were different. The 0–6 hr urine after suxibuzone administration mostly contained the metabolites specific to suxibuzone administration; namely, suxibuzone glucuronide, II, and III. The amounts of these metabolites excreted were 0.12% of the dose of suxibuzone for suxibuzone glucuronide, 0.45% for II, and 0.44% for III. The amounts of these metabolites were negligible in urine samples collected at later times after suxibuzone administration.

Only the urinary excretion of γ-hydroxyphenylbutazone differed after administrations of suxibuzone and phenylbutazone; those of phenylbutazone, oxyphenbutazone, and their conjugates were similar. The percentages of the urinary excretion of phenylbutazone and *O*-phenylbutazone glucuronide during 168 hr after drug administration were 0.93 and 0.79%, respectively, of the dose of suxibuzone, and 1.20 and 0.95%, respectively, of the dose of phenylbutazone. Similarly, in the 0–168 hr samples, oxyphenbutazone and its conjugate amounted to 0.49 and 2.73%, respectively, of the dose of suxibuzone, and 0.63 and 3.42%, respectively, of the dose of phenylbutazone. On the other hand, the excretion of γ-hydroxyphenylbutazone in the first 24-hr urine samples was 2.44% of the dose of suxibuzone. This was 15 times that after phenylbutazone administration and seems to reflect the concentrations in the plasma. At more than 24 hr after administration of suxibuzone and phenylbutazone, the excretions of γ-hydroxyphenylbutazone were similar.

The cumulative urinary excretion of the metabolites measured amounted to ~8% of the dose within 168 hr after administration of either suxibuzone or phenylbutazone.

**Excretion in Feces**—The fecal excretions of suxibuzone and phenylbutazone are summarized in Table I. The percentages of excreted phenylbutazone were <1% of the dose, after both suxibuzone and phenylbutazone administration. No unchanged suxibuzone was detected in the feces; therefore, the absorptions of both drugs appear to be complete.

## DISCUSSION

On the basis of the present results on suxibuzone and phenylbutazone in humans and findings on phenylbutazone by other groups (7, 8, 11–15), a scheme for the biotransformation of suxibuzone is shown in Scheme 1.

Analysis of urinary metabolites elucidated possible pathways of biotransformation from suxibuzone to phenylbutazone. No unchanged suxibuzone was observed in the plasma, but the excretion of suxibuzone glucuronide in the urine suggests that part of the oral dose of suxibuzone is absorbed in an unchanged form and excreted as the glucuronide. Detection of II suggests that suxibuzone was hydrolyzed by esterases (9) to give 4-hydroxymethylphenylbutazone, which is considered to be labile and to decompose to phenylbutazone spontaneously unless otherwise conjugated with glucuronic acid at the oxymethyl side chain. The excretion of III indicates the existence of a pathway in which unchanged suxibuzone or an intermediary between suxibuzone and phenylbutazone (*e.g.*, 4-hydroxymethylphenylbutazone) undergoes oxidation at the *para*-position of one of the phenyl rings. Consequently, this means that part of the unchanged suxibuzone absorbed can yield oxyphenbutazone without being metabolized to phenylbutazone. If this type of pathway exists for producing γ-hydroxyphenylbutazone (*e.g. via* a 4-hydroxymethyl-γ-hydroxyphenylbutazone), it could explain the significantly higher plasma level of γ-hydroxyphenylbutazone during a 24-hr period after suxibuzone administration. [The higher plasma level of γ-hydroxyphenylbutazone after suxibuzone administration may contribute to the lower plasma level of uric acid than that after phenylbutazone administration (10), since γ-hydroxyphenylbutazone was reported to enhance the excretion of uric acid (16).] The excretions of these metabolites specific to suxibuzone in the first 6 hr urine may be the cause of the slightly (though not significantly) lower plasma level of phenylbutazone after suxibuzone administration.

Although V hydrolysed by β-glucuronidase in rat or human urine could not be detected previously (7, 8), a hydrolyzable *O*-phenylbutazone glucuronide was isolated, the existence of which has already been suggested (9, 10). Hence, phenylbutazone undergoes two different types of glucuronidation and is excreted in the urine.

Most ester-type drugs are considered to be hydrolyzed by esterases bound to the intestinal mucosa, so the form of the drug which enters the portal venous blood will be greatly affected by the activities of these esterases (17–19). This also seems to be the case for suxibuzone, judging from the rate of hydrolysis of suxibuzone determined using homogenates of intestine of various animals and from the metabolites detected in plasma and urine (9). For example, in dogs, in which the intestine have very low esterase activity, unchanged suxibuzone was detected in the plasma; but in rats, in which the intestine has high esterase activities, no suxibuzone was detected in the plasma and no suxibuzone glucuronide or II were found in the urine. In humans the esterase activity of the intestine is reported to be relatively high (18, 19), and no unchanged suxibuzone was detected in the plasma or feces. Moreover, the changes in concentration of metabolites in the plasma exhibited almost the same time courses after the administration of suxibuzone and phenylbutazone. These observations imply that most of the suxibuzone administered orally is hydrolyzed to phenylbutazone by intestinal esterases before transfer to the portal venous blood.

The results obtained in the present study indicate that suxibuzone is a prodrug of phenylbutazone in humans. The metabolic fate and pharmacokinetic properties of suxibuzone ensure that this drug is as effective as phenylbutazone. The reason why suxibuzone is less ulcerogenic than phenylbutazone is considered to be that suxibuzone remaining in GI tracts is not as harmful as phenylbutazone to GI mucosa, since the unchanged suxibuzone does not inhibit the function of mitochondria and prostaglandin synthetases (unpublished data).

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