# Identification of Urinary Metabolites of *p*-Phenoxyphenol Methanesulfonate in Rat and Man

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**Abstract** □ The urinary metabolites of *p*-phenoxyphenol methanesulfonate (I) in rat and man were characterized. The drug was extensively metabolized in both species and excreted mainly in the form of conjugated phenolic compounds. The major metabolite of I in both species was identified as the conjugated monohydroxylated compound, *p*-(4-hydroxyphenoxy)phenol methanesulfonate (II). Three minor metabolites were also characterized and tentatively identified as: (*a*) a dihydroxylated derivative, probably *p*-(3,4-dihydroxyphenoxy)phenol methanesulfonate (III); (*b*) a hydroxy and methoxy derivative, probably *p*-(4-hydroxy-3-methoxyphenoxy)phenol methanesulfonate (IV); and (*c*) a dihydroxy and methoxy derivative (V). The exact positions of the aromatic substituents in these three compounds were not rigorously established. Compounds II and IV were found in both free and conjugated forms, but Compounds III and V were found in the conjugated form only. Qualitatively, the metabolites of I found in rat and human urine were similar. The hypolipidemic activity of II was equivalent to that of the parent compound.

**Keyphrases** □ *p*-Phenoxyphenol methanesulfonate—identification of urinary metabolites, rat, man □ Urinary metabolites—*p*-phenoxyphenol methanesulfonate, rat, man

*p*-Phenoxyphenol methanesulfonate[1] (I, $C_{13}H_{12}O_4S$, mol. wt. 264) has been shown to be an active hypocholesterolemic agent in both surfactant[2]-treated and dietary cholesterol and cholic acid-fed rats. Its pharmacological properties were reported previously (1). To study the absorption, metabolism, and excretion of this compound in animals and man, a GLC method for measuring the intact drug was developed. Little, if any, unchanged drug was found in the urine of I-treated rats. Analysis of the urine specimens, using TLC techniques, indicated that the compound was metabolized to several products. Therefore, studies were initiated to: (*a*) identify the major urinary metabolites of this compound in rats and man, and (*b*) determine the biological activities of the major metabolites in rat and man.

## EXPERIMENTAL

**Dosage and Collection of Samples** *Rat*—Micronized I was suspended in 0.25% methylcellulose in sterile water with a glass Teflon homogenizer. The suspension was administered orally to five Sprague–Dawley rats at a dose of 100 mg./kg. Urine samples were collected quantitatively for 24 hr. postadministration. A second group of five rats, dosed with the vehicle only, served as controls.

*Man*—Five subjects each received orally a single 1-g. dose of I in hard filled capsules. Urine specimens were collected quantitatively 24 hr. postadministration. Urine specimens collected prior to drug administration were used as controls.

**Method**—All urine specimens were extracted and analyzed with a previously described GLC–mass spectrometric procedure (2). The pooled urine samples were acidified to pH 3.5 with acetic acid and extracted six times with chloroform. The chloroform layers were separated and concentrated under reduced pressure. The extracted urine was heated at 45° for 1 hr. under reduced pressure to remove the dissolved chloroform and then hydrolyzed with a mixture of β-glucuronidase and aryl sulfatase[3] in 0.1 *M* sodium acetate buffer at pH 5.0 for 16 hr. at 37°. The hydrolyzed urine samples were then extracted five times with chloroform. The chloroform layers were separated, combined, and evaporated to dryness. Control urine was processed in the same manner.

The extracted residues from the unhydrolyzed and hydrolyzed urine specimens were chromatographed on silica gel G-254 thin-layer plates, using chloroform–methanol (95:5) as the developing solvent. The chromatograms were examined under UV light, and zones that appeared on the plates with the drug-treated urine extract but not on the plates of the control were scraped and eluted with chloroform–methanol (4:1). The purified metabolite was evaporated to dryness and silylated with bis(trimethylsilyl)acetamide before injection into a gas chromatograph–mass spectrometer[4], equipped with a 1.21-m. (4-ft.) column of 1% SE-30 on 80–100-mesh Gas Chrom Q. The oven was operated isothermally at 200° and the ionization potential was set at 70 ev. Carrier gas (helium) flow was kept at 60 ml./min.

## RESULTS

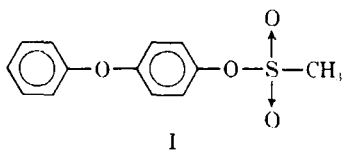
A gas chromatogram of the crude extract from β-glucuronidase–aryl sulfatase-hydrolyzed human urine is shown in Fig. 1. Three peaks, with retention times of 1.55, 2.4, and 2.85 min., were found in the drug-treated urine extract but were absent in the control. The small peak found at the retention time of 3.9 min. was not related to the drug. Unchanged I, which should have a retention time of 0.66 min., was not detectable in this analysis.

Figure 2 shows a thin-layer chromatogram of the same extract. Again three components are present in the urine extract from drug-treated human subjects but not in the control. Each zone was scraped off the plate, eluted with solvent, derivatized with bis(trimethylsilyl)-acetamide, and injected into the gas chromatograph–mass spectrometer. Four drug-related compounds were identified. Two metabolites were found in the more polar zone ($R_f$ 0.49). The identification of these compounds is described in the following sections.

***p*-(4-Hydroxyphenoxy)phenol Methanesulfonate**—The *p*-hydroxylated metabolite of I (Compound II) separated readily from partially purified urine extracts as white flaky crystals. The isolated material, m.p. 128–129°, appeared 99% pure as judged by GLC. Elemental analysis indicated that the compound had an empirical formula of $C_{13}H_{12}O_5S$.

*Anal.*—Calc. for $C_{13}H_{12}O_5S$: C, 55.71; H, 4.29; S, 11.43. Found: C, 55.82; H, 4.58; S, 11.35.

The UV spectrum was similar to the parent drug except the weaker bands (271 and 277 nm. in the unchanged drug) were shifted 5 nm. to the longer wavelengths. Addition of base (0.1 *N* NaOH) to the ethanolic solution shifted the strong absorption band (230 nm. in the unchanged drug) 12 nm. to the long wavelength side (242 nm. in the metabolite), indicating the presence of a monohydric phenol (3). In the IR spectrum of the metabolite, the 695 cm.$^{-1}$ band of the unchanged drug (monosubstituted phenyl) was absent, and strong absorption bands were observed at 820 (*p*-substituted phenyl), 1195 (phenol C—O vibration), and 3460 (OH vibration) cm.$^{-1}$, indicating good agreement with the structural assignment. The NMR spectrum



I

[1] U-22,105.
[2] Triton, oxyethylated tertiary octylphenol formaldehyde polymer.
[3] Sigma type H-2 β-glucuronidase No. G-0876.
[4] LKB-9000.

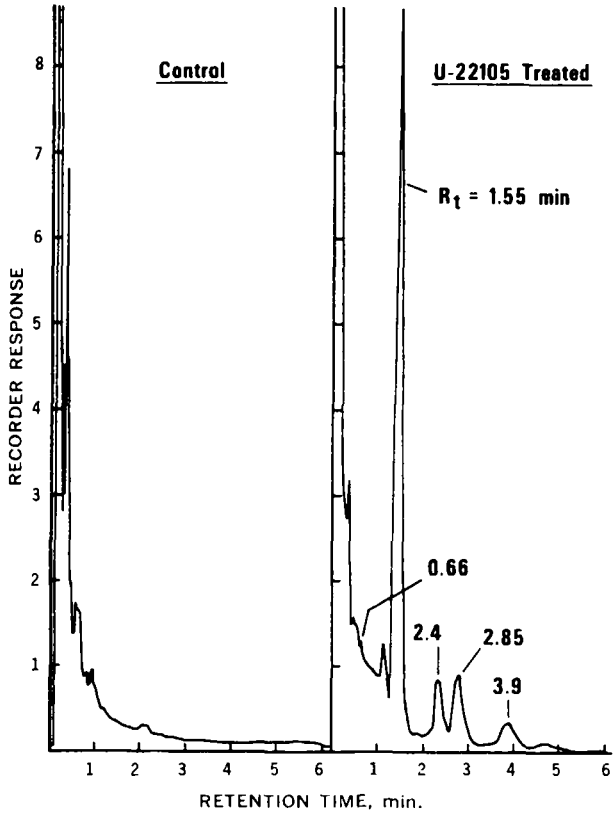



**Figure 1**—*Gas–liquid chromatograms of extracts from β-glucuronidase–aryl sulfatase-hydrolyzed human urine.*

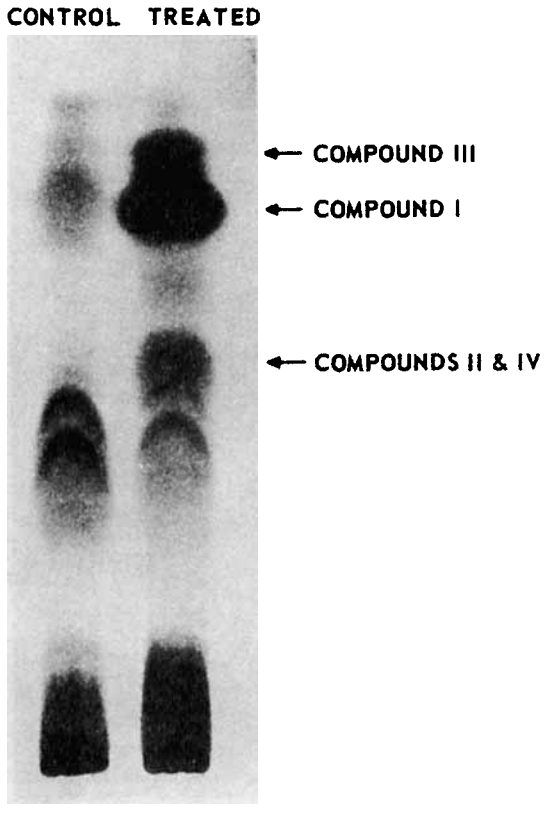
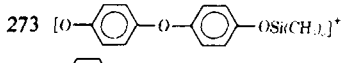
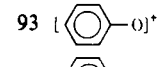



**Figure 2**—*Thin-layer chromatograms of extracts from β-glucuronidase–aryl sulfatase-hydrolyzed human urine.*

showed two sets of *AA'BB'* multiplets, indicating that the hydroxyl substitution was *para*. Furthermore, the isolated material gave a strong positive response to bipyridyl ferric chloride reagent (4), which is characteristic of phenolic compounds. Conclusive identification of the isolated material was obtained from mass spectral analyses. The isolated Compound II, Fig. 3*a*, showed a strong mass ion at 280 (molecular ion) and fragmentation ions at 201, 173, 145, 117, 93, and 65. The loss of methanesulfonate (M − 79 = 201) was characteristic of all compounds related to I. Subsequent loss of mass 28 (CO) was characteristic of phenols. The assignment of major fragments is summarized in Table I. The trimethylsilylated derivative of I (Fig. 3*b*) showed a similar type spectrum, except all mass peaks were found 72 mass units higher. Therefore, it was concluded that this compound was the *p*-hydroxylated metabolite of I.

**Table I**—Common Mass Peaks Appearing in *p*-Phenoxyphenol Methanesulfonate-Related Compounds

| *m/e* | Structure | Comments |
|---|---|---|
| M − 79 | Loss of $-SO_2CH_3$ | Appears in all spectra |
| M − 28 | Loss of CO | Appears in all spectra |
| M − 15 | Loss of $CH_3$ | Appears in all trimethylsilyl ethers and all phenyl methyl ethers |
| M − 30 | Loss of HCHO | Appears in all phenyl methyl ethers |
| M − 88 | Loss of $C_3H_8OSi$ | Appears in trimethylsilyl ethers of dihydric phenols |
| 273 |  | Appears in all trimethylsilyl ethers |
| 93 |  | Appears in all free phenolic compounds |
| 77 |  | Appears in all spectra |
| 65 |  | Appears in all spectra |

Compound II was subsequently synthesized by reacting 4,4'-oxydiphenol with methanesulfonyl chloride in pyridine at 0°. The reaction mixture was poured into a mixture of ice water and extracted with chloroform. Compound II was isolated from the extract by silica gel column chromatography. The authentic material had identical chromatographic and spectroscopic (UV, IR, NMR, and mass) characteristics to the isolated material. The hypolipidemic activity of II was tested in the surfactant[2]-induced hypercholesterolemic rats and was found to be equivalent to that of the parent I.

In the urine of both species, the major portion of II existed as conjugates. A trace amount was also found in the free form.

**Dihydroxy Metabolite**—Compound III was a minor metabolite found only in conjugated form. The amount present in the urine was insufficient for isolation. This compound gave a brownish-red spot on the thin-layer plate when sprayed with bipyridyl ferric chloride reagent, indicating that it was a phenol. The mass spectrum of the bis(trimethylsilyl) derivative is shown in Fig. 4. The molecular ion was observed at *m/e* 440, and strong fragmentation ions were observed at 361 (M − 79) and 273 [M − 79 − $(CH_3)_3SiO$]. Subsequent loss of 28 mass units (CO) was not as prominent as in II. The positions of the two hydroxyl groups were difficult to assign. However, since the predominant product produced by the enzymatic hydroxylation of *p*-substituted phenol is catechol (6), the structure of III was probably *p*-(3,4-dihydroxyphenoxy)phenol methanesulfonate.

**Hydroxy Methoxy Metabolite**—Compound IV was found both free and conjugated in the urine of rat and man. It was isolated by preparative TLC from the mother liquor remaining after crystallization of I. Attempts to crystallize IV were unsuccessful. The mass spectrum of IV (Fig. 5*a*) showed a strong molecular ion at mass 310. Loss of methanesulfonate (M − 79) and subsequent loss of 28 mass units (CO) identified IV as a drug-related compound. In the mass spectrum of the trimethylsilyl derivative (Fig. 5*b*), prominent ions were observed at 382 ($M^+$) and 303 (M − 79). In both spectra, loss of 30 ($CH_2O$) or 31 ($CH_3O$) mass units from the molecular ion or the M − 79 ion indicated the presence of a phenyl methyl ether linkage (8). The IR spectrum of IV was quite similar to that of II. Strong bands, observed at 3460 $cm.^{-1}$, were assigned to the phenolic OH. The aromatic system and the methanesulfonate group of the parent

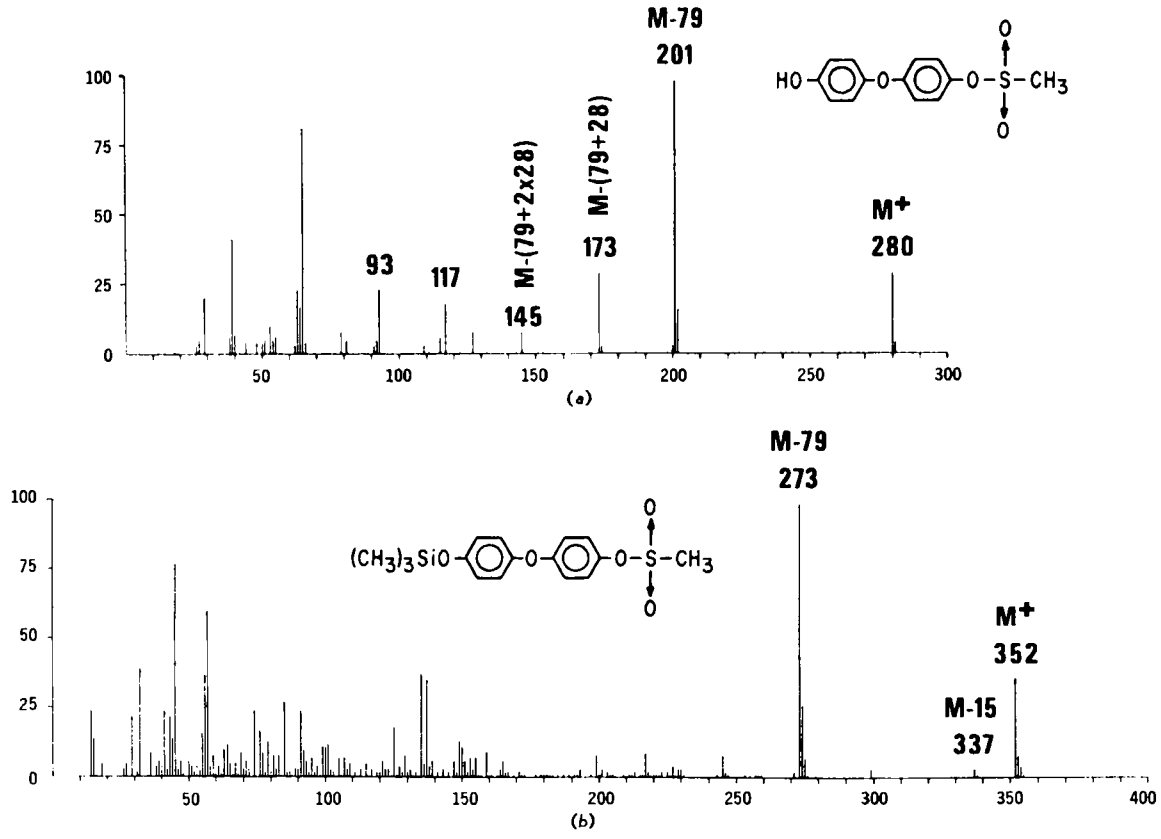



**Figure 3**—*Mass spectra of Compound II. Key:* (a), *free phenol; and* (b), *trimethylsilyl derivative.*

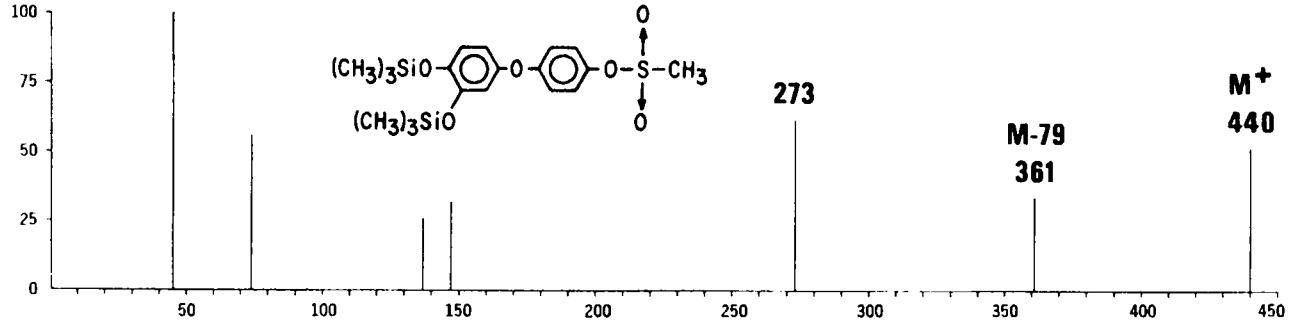



**Figure 4**—*Mass spectrum of bis(trimethylsilyl) derivative of Compound III.*

compound were still present. Neither the presence of methoxy nor the position of aromatic substitution could be conclusively established by IR spectroscopy.

NMR spectroscopy indicated the presence of a methoxy group *ortho* or *meta* to a *para*-substituted hydroxy group on the phenoxy ring. The methoxy group showed a singlet at $\delta$ 3.78 and shifted only one of the aromatic protons (*meta* to the methoxy) upfield to $\delta$ 6.9. An unperturbed *AA'BB'* multiplet centered at $\delta$ 7.21 indicated that the phenol ring was still intact. Since the predominant product from enzymatic hydroxylation of phenol is catechol (9) and since catechol-*O*-methyltransferase will not methylate resorcinol (8), it is unlikely that the methoxy group would substitute *meta* to the free *para*-substituted hydroxy group on the phenoxy ring. Therefore, IV is tentatively assigned a 4-hydroxy-3-methoxyphenoxyphenol molecular structure.

**Dihydroxy Methoxy Metabolite**—Compound V, a minor metabolite of I, could not be isolated in pure form. Evidence for its presence was obtained solely from GLC–mass spectrometric analysis of crude urine extracts. It was observed in the urine of both species only in conjugated form. After silylation with bis(trimethylsilyl)-acetamide, a strong mass ion at *m/e* 470 (Fig. 6) was observed corresponding to the disilylated derivative of Compound V. Loss of methanesulfonate (M − 79) and carbon monoxide (M − 28) indicated the presence of a drug-related compound. The presence of methoxy group was confirmed by the loss of 30 ($CH_2O$) mass units.

## DISCUSSION

These results indicate that orally administered I followed typical metabolic pathways for aromatic compounds (9) and produced a group of phenolic compounds and their conjugates (Scheme I). The major routes of metabolism were: (*a*) hydroxylation of the phenoxy ring, and (*b*) conjugation of the resulting phenols with glucuronic acid or sulfate. A small portion of the hydroxylated I was further metabolized to yield the corresponding catechol, which underwent *O*-methylation to the methoxyphenol derivatives. Essentially all of the metabolites were excreted as either glucuronic acid or sulfate conjugates.

Since the NMR spectra of IV could not be factored with certainty because of the overlapping of the two highly coupled sets of aromatic hydrogens, plus the fact that not enough quantities of III and V were isolated for detailed NMR study, the exact positions of the aromatic substituents in III–V were not rigorously established. However, the limited information obtained from the NMR study of IV provided some clues for further speculation. As described

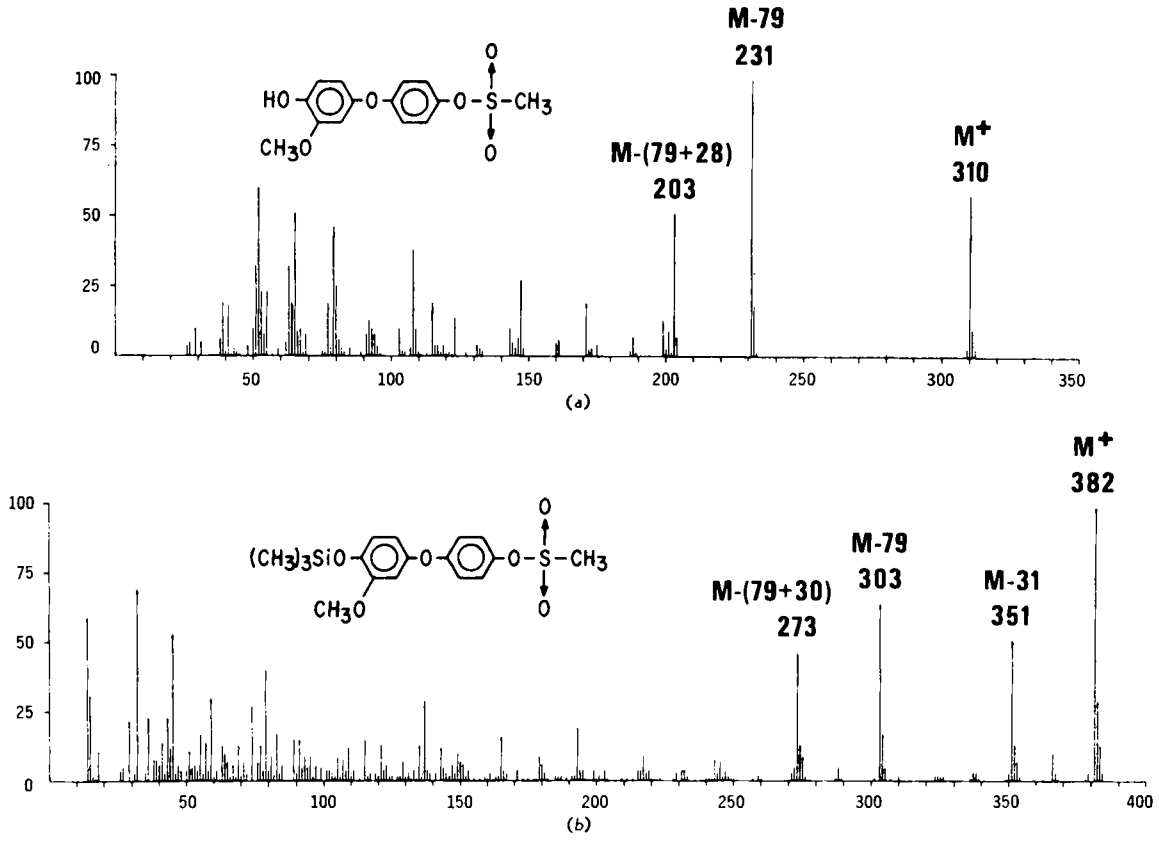
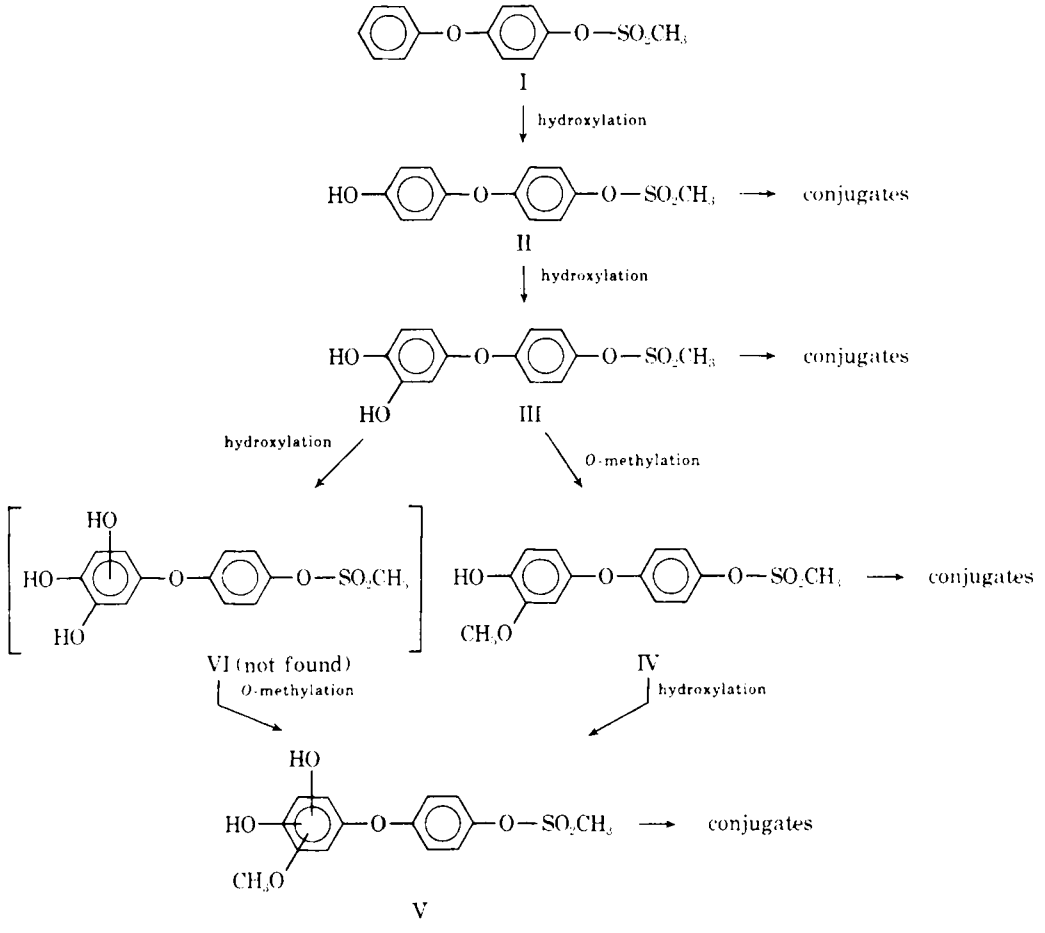



**Figure 5**—*Mass spectra of Compound IV. Key:* (a), *free phenol; and* (b), *trimethylsilyl derivative.*




*Scheme I—Proposed metabolic pathways of I in rat and human*

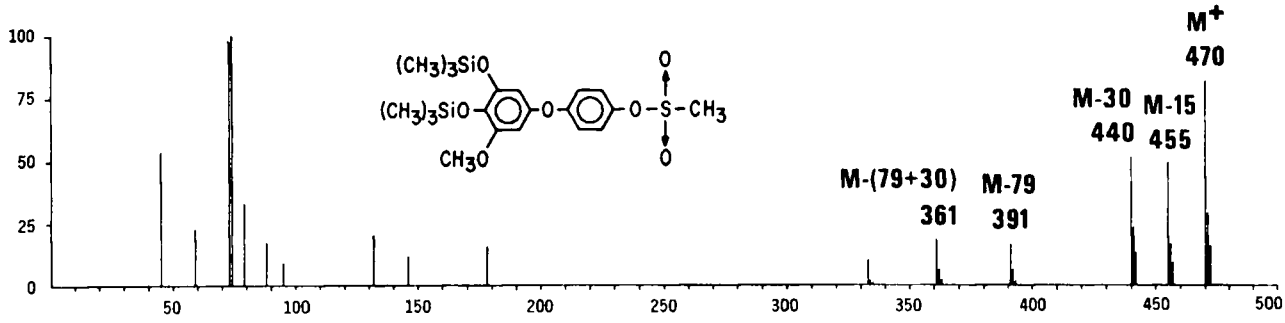



**Figure 6**—*Mass spectrum of bis(trimethylsilyl) derivative of Compound V.*

previously, the most probable structure for IV appeared to be 4-methoxy-3-hydroxyphenoxyphenol methanesulfonate. If this structure is correct, then III, presumably the precursor of IV, should have the structure of 3,4-dihydroxyphenoxyphenol methanesulfonate. The catechol structure would also be in line with the predicted hydroxylation product of II, since it was shown (6) that the principal hydroxylation product of *p*-substituted monophenol is the corresponding catechol. For example, *p*-cresol, *p*-nitrophenol, and *p*-chlorophenol are all hydroxylated to the corresponding catechol derivatives in rabbits (9). The structure of Compound V was even more obscure. It could be the hydroxylation product of IV or generated from III by hydroxylation followed by *O*-methylation. An unequivocal identification must await the synthesis of all possible isomers for comparison.

In view of the absence of unchanged drug in the urine, the rate of metabolism must be much faster than the rate of excretion. Quantitatively, the monohydroxylated compound (I) was the most abundant metabolite in the urine. In the human, the 24-hr. post-administration urinary excretion of I accounted for about 15–30% of the dose.

Since radioisotopically labeled I is not available, the extraction efficiency of drug-related materials from the urine is unknown. The possibility exists that some extremely water-soluble metabolite(s) of the parent drug was not found because of unfavorable partition coefficients between chloroform and water, for example, the trihydroxylated metabolite (VI), which would be the precursor of IV if it resulted from metabolized hydroxylation of III.

## REFERENCES

(1) W. A. Phillips and P. E. Schurr, *Fed. Proc.*, **27**, 439(1968).

(2) F. F. Sun, *J. Pharm. Sci.*, in press.

(3) A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Macmillan, New York, N. Y., 1964, p. 95.

(4) G. M. Barton, *J. Chromatogr.*, **20**, 189(1965).

(5) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p. 116.

(6) D. V. Parke, "The Biochemistry of Foreign Compounds," Pergamon, New York, N. Y., 1968, p. 147.

(7) C. S. Barnes and J. L. Occolowitz, *Aust. J. Chem.*, **16**, 219 (1962).

(8) M. S. Masri, D. J. Robbins, O. H. Emerson, and F. Deeds, *Nature*, **202**, 878(1964).

(9) R. T. Williams, "Detoxication Mechanisms," Wiley, New York, N. Y., 1959, p. 190.

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