Urinary Metabolites of D,L-1,2-Bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol in Mice

NELSON R. TRENNER, JOSEPHINE R. CARLIN, BYRON H. ARISON, and W. J. A. VANDENHEUVEL ˣ

Abstract □ The major urinary metabolite of D,L-1,2-bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol in the mouse was isolated and identified by various chromatographic and spectrometric techniques as the mono-*O*-demethylated phenol. Minor urinary components include the parent drug and a conjugate of the major metabolite.

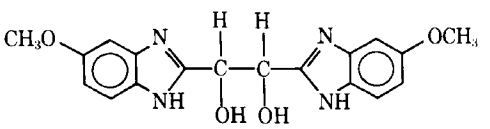
Keyphrases □ D,L-1,2-Bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol—urinary metabolites isolated and identified, mice □ Metabolism—urinary metabolites of D,L-1,2-bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol isolated and identified, mice □ Urinary metabolites—isolation and identification of D,L-1,2-bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol metabolites, mice

The inhibitory effect of D,L-1,2-bis[5-(6)-methoxy-2-benzimidazolyl]-1,2-ethanediol (I) on poliovirus was first demonstrated by Akihama *et al.* (1). More recently, data were presented indicating this compound to be a potent inhibitor of rhinoviruses and its absorption, distribution, and excretion in mice were reported (2). The isolation and identification of the urinary metabolites of I in the mouse are now reported.

EXPERIMENTAL

Urine from 30 mice (20 g, ICR/Ha strain) injected with I (68 mg/kg ip; 2-^{14}C-labeled) was collected for 18–20 hr; the pooled urine contained 45% of the dosed radioactivity. Extraction of an aliquot of the pooled mouse urine with 2 equal volumes of ethyl acetate at pH 6.5 removed 75% of the total urinary radioactivity.

Analytical TLC [silica gel G plates developed with isopropanol–ethyl acetate–ammonium hydroxide (7:9:5)] of this extract gave a zone of R_f 0.57, which accounted for >90% of the extracted radioactivity. A small amount of parent drug (R_f 0.68) was also observed. A reverse isotope dilution assay (3) indicated that it accounts for only 5% of the total radioactivity in the urine. Ion-exchange purification of the metabolite was effected by adsorption on a styrene-type cation-exchange resin[1] (sulfonic acid, ammonium cycle) and elution with 10% ammonium hydroxide in methanol. The effluent contained 21% and the eluate contained 63% of the charged radioactivity. This eluate was further purified by adsorption on a styrene-type anion-exchange resin[2] (quaternary ammonium, acetate cycle). The eluate obtained with 10% acetic acid in methanol contained 51% of the original urinary radioactivity. This material was subjected to preparative TLC with the previously described system. The major zone (R_f 0.57) was eluted from the plate with methanol and was found to contain 36% of the original urinary radioactivity. The TLC eluate was evaporated to dryness and partitioned between ethyl acetate and water; 92% of the radioactivity passed into the organic solvent. Removal of the ethyl acetate *in vacuo* yielded 2.2 mg of a solid, II, which possessed a specific activity 70% that of the parent drug.



I

NMR spectroscopy[3] was carried out in deuteromethanol. Mass spectra[4] were obtained *via* direct probe using a trap current of 60 μamp, an ionizing potential of 70 ev, an accelerating potential of 3.5 kv, and a source temperature of 270°. Derivatization with bis(trimethylsilyl)acetamide[5] (III) (4) directly in a probe tube (Procedure A) involved treatment of the sample (~5 μg) with 5 μl III; the tube was immediately (<30 sec) inserted into the vacuum lock, and the reagent was pumped off through the vacuum system of the spectrometer. The more vigorous derivatization (Procedure B) involved heating the sample (5–10 μg) in III (20 μl) in a tightly capped centrifuge tube for 20 min at 70° and then placing an aliquot of the solution in a probe tube for analysis.

RESULTS AND DISCUSSION

Comparison of the TLC mobilities of I (R_f 0.68) and II (R_f 0.57) suggested a metabolic transformation resulting in an increase in polarity. Furthermore, the metabolite zone (but not the zone of the parent drug) gave a positive color reaction when sprayed with diazotized sulfanilic acid and base, indicating the presence of a phenolic group. The UV spectrum (methanol) of the metabolite was similar to that of I, except that it exhibited a pH dependence not displayed by the drug itself, supporting the possibility that II is phenolic in nature.

NMR spectroscopy of the metabolite disclosed the presence of two nonequivalent 1,2,4-trisubstituted aromatic patterns. This clearly reflected a loss in symmetry compared with the parent molecule and suggested a structural change involving one site. This position was readily identified by the observation that the methoxyl signal (τ 6.19 relative to tetramethylsilane) was reduced to one-half of its original area. The fact that the protons in the modified ring showed only a small downfield displacement led to the proposal that the new substituent was a hydroxyl group.

Mass spectrometry (direct probe) of the metabolite yielded a rather uninformative spectrum; the radioactivity had not volatilized from the probe tube even at a temperature of >200°. This may have been caused by adsorption of the metabolite on residual silica gel from the TLC plate used for final purification (5). Mass spectrometry of the metabolite following treatment (Procedure A) with III proceeded smoothly, and a volatile compound IIa (probe temperature 200°) possessing a molecular ion of 556 amu was observed (Fig. 1). Similar treatment of the parent drug (mol. wt. 354) yielded a trimethylsilyl derivative with a molecular ion of 498 amu; this compound is the di-*O*-trimethylsilyl ether [354 + (2 × 72) =

[1] AG-50W-X2, BioRad Laboratories.
[2] AG-1-X8, BioRad Laboratories.
[3] Varian HA 100 instrument.
[4] LKB model 9000 instrument.
[5] Supelco.

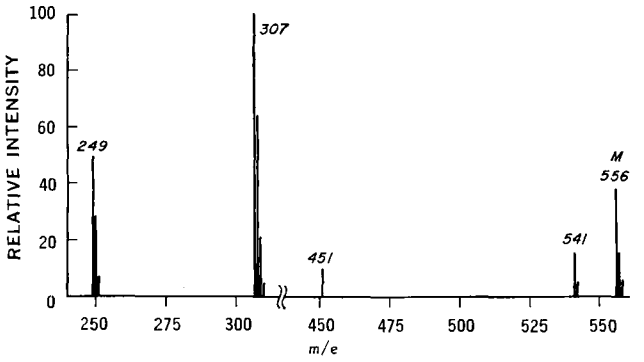
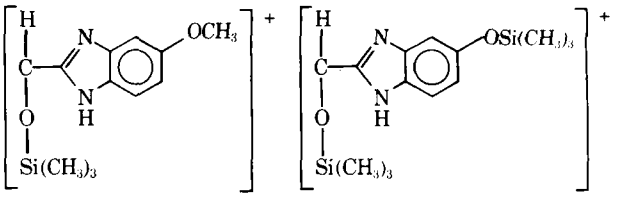


Figure 1—*Mass spectrum of the partially trimethylsilylated (Procedure A) major urinary metabolite.*

498] because imidazole NH groups are not readily derivatized under these mild reaction conditions. The base peak in the spectrum of the parent drug is at *m/e* 249 and results from symmetrical scission of the molecule to form ion IV. Although an intense signal is also observed at *m/e* 249 in the spectrum of II*a*, the base peak is found at *m/e* 307 and can be ascribed to the related ion V.

Derivatization of I with III using Procedure B forms the tetratrimethylsilyl drug with a molecular ion of 642 amu and a base peak at *m/e* 321. Under these more vigorous reaction conditions the metabolite is transformed to a compound (II*b*) with a molecular ion of 700 amu and intense signals at *m/e* 321 and 379 (base peak), which can be ascribed to ions possessing Structures IV and V, respectively, in which the imidazole NH has been transformed to N—$Si(CH_3)_3$. The mass spectral data from both derivatives clearly confirm the unsymmetrical nature of the metabolite and are compatible with the proposal that II is the mono-*O*-demethylated phenol (mol. wt. 340).

The metabolic transformation observed is perhaps not unexpected because the *O*-demethylation of codeine to form morphine is well known (6, 7) and a more recent report involves *O*-demethylation of aflatoxin B_1 (8). One might expect, however, that di-*O*-demethylation would occur with I. A very minor component (<1%) of the ethyl acetate urinary extract with an R_f of 0.44 was detected but not characterized. A considerable quantity of highly polar drug-related material is present in the mouse urine; 25% of the radioactivity remains in the aqueous phase following exhaustive extraction with ethyl acetate. The water-soluble material possesses an R_f of 0.1. Hydrolysis (1 *N* HCl at 100° for 1 hr) followed by neutralization and extraction with ethyl acetate resulted in nearly one-half of the radioactivity passing into the organic solvent. The extracted material possessed TLC behavior compatible with that of II and gave the same positive phenol test, suggesting that much of the water-soluble material is a conjugate of II. (In the case of monkeys dosed with I, all urinary radioactivity is extractable with ethyl acetate. TLC of the extract yields only one radioactive zone,



IV: *m/e* 249 V: *m/e* 307

with mobility and color test corresponding exactly to II.)

No evidence has been found to suggest that the glycol bridge of I undergoes metabolic cleavage. The metabolism of this drug in the mouse, on the basis of urinary metabolites, is essentially mono-*O*-demethylation followed, to a slight extent, by conjugation of the phenolic product.

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[x] To whom inquiries should be directed.