β-(2-Methoxyphenoxy)lactic Acid, the Major Urinary Metabolite of Glyceryl Guaiacolate in Man

W. J. A. VANDENHEUVEL▲, J. L. SMITH, and R. H. SILBER

Abstract □ An acidic metabolite of glyceryl guaiacolate was isolated from human urine by solvent extraction and column chromatography. Combined GLC–mass spectrometry of the trimethylsilyl and trimethylsilyl-d_9 derivatives of the metabolite and mass spectrometry of the underivatized metabolite have demonstrated its structure to be β-(2-methoxyphenoxy)lactic acid.

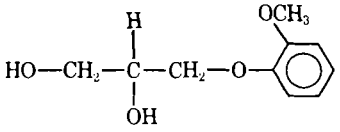
Keyphrases □ Glyceryl guaiacolate—identification of β-(2-methoxyphenoxy)lactic acid as major metabolite, human urine, GLC–mass spectroscopy □ β-(2-Methoxyphenoxy)lactic acid—identification as major metabolite of glyceryl guaiacolate in human urine, GLC–mass spectroscopy □ GLC–mass spectroscopy—identification of β-(2-methoxyphenoxy)lactic acid as major metabolite of glyceryl guaiacolate □ Mass spectroscopy—identification of β-(2-methoxyphenoxy)lactic acid as major metabolite of glyceryl guaiacolate

Glyceryl guaiacolate[1] (I) has been used as an expectorant and for other purposes (1). Blood concentrations of the drug have been determined by GC (1) and chemical (2) procedures, but urinary data are lacking and little is known about its metabolism. In this communication, the identification of the major urinary metabolite of I in man by means of mass spectrometric techniques is reported.

EXPERIMENTAL

Urine from a normal male volunteer who ingested 1 g. of the drug was collected for 3 hr. after the dose. The 0–3-hr. urine contained about 440 mg. of metabolite when assayed by an analytical (colorimetric) procedure[2] employing I as the standard. Extraction into methylene chloride over a pH range of 1–8, followed by an alkaline wash, showed that no unchanged drug was present, but there was an acidic metabolite which could be readily extracted at pH 1–3. No metabolite was extracted at pH 6 or above. The metabolite fraction was obtained by extraction (five times) at pH 1 with an equal volume of solvent; the combined extract contained 420 mg. on the basis of the colorimetric procedure. After the solution was taken to dryness, the residue was subjected to column chromatography[3] with a solvent system of benzene–methanol–glacial acetic acid (80:15:5) to yield a fraction (400 mg.) of about 95% purity, as shown by chemical analysis and determination of dry weight.

In a second experiment, 0–4-hr. urine from the volunteer was extracted 13 times (the final extract contained only 0.2% of the metabolite in the total extract) with an equal volume of methylene chloride at pH 1. The 0–4-hr. urine contained 94% (515 mg.) of the total metabolite excreted in 24 hr. Aliquots of the chromatographically purified metabolite and the 0–4-hr. total metabolite fraction (60% purity) were subjected to mass spectrometry and



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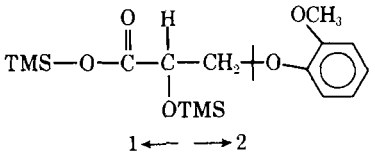


Scheme I—(TMS = trimethylsilyl)

combined GLC–mass spectrometry (as trimethylsilyl derivatives). Both isolates were shown to contain β-(2-methoxyphenoxy)lactic acid; there was no evidence for the presence of detectable amounts of other metabolites.

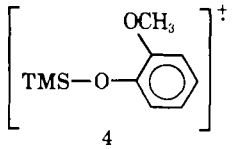
Mass spectra were obtained by normal procedures[4]. The instrument settings were: ionizing potential, 70 ev.; source temperature, 270°; accelerating potential, 3.5 kv.; and trap current, 60 μa. Chromatographic conditions were: 1.2-m. (4-ft.) × 2-mm. i.d. spiral glass column; 3% SE-30 on 100–120-mesh acid-washed and silanized Gas Chrom P; temperature programmed from 110 to 200° at 5°/min.; and helium flow rate, 30 ml./min.

Trimethylsilylation was carried out by dissolving 25 mcg. of the metabolite in 50 μl. of bis(trimethylsilyl)acetamide[5] or bis(trimethylsilyl)acetamide-d_{18}[6] and allowing the solution to stand at room temperature for 30 min.

RESULTS AND DISCUSSION

The metabolite (the 0–3- and 0–4-hr. isolates gave the same results) was subjected to direct probe mass spectrometry and found to exhibit a molecular ion of *m/e* 212, 14 mass units greater than the molecular weight of the parent drug. No peak was observed when the sample was exposed to a variety of GC conditions, but temperature-programmed analysis of a portion of the sample treated with bis(trimethylsilyl)acetamide did give one peak (Fig. 1). Combined GLC–mass spectrometry of the trimethylsilyl derivative indicated a molecular weight of 356 (Fig. 2); the metabolite possesses two functional groups [212 + (2 × 72) = 356]. As expected, the molecular weight of the trimethylsilyl-d_9 derivative prepared by reaction with bis(trimethylsilyl)acetamide-d_{18} (3–5) was 374.

The fragmentation patterns of the trimethylsilyl and trimethylsilyl-d_9 derivatives conclusively demonstrated the structure of the metabolite to be β-(2-methoxyphenoxy)lactic acid. Fragment ion 1, *m/e* 233 (d, 251)[7], must arise by scission of the methylene carbon–oxygen bond as shown in Scheme I.



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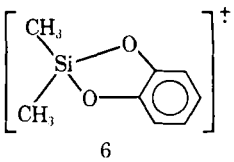

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[1] This compound is also known as guaiacol glyceryl ether.
[2] R. H. Silber, to be published.
[3] Analtech GF silica gel, 250 μ.
[4] With an LKB model 9000 instrument.
[5] Supelco.
[6] Merck Sharp & Dohme of Canada
[7] The *m/e* value of the trimethylsilyl-d_9 derivative.

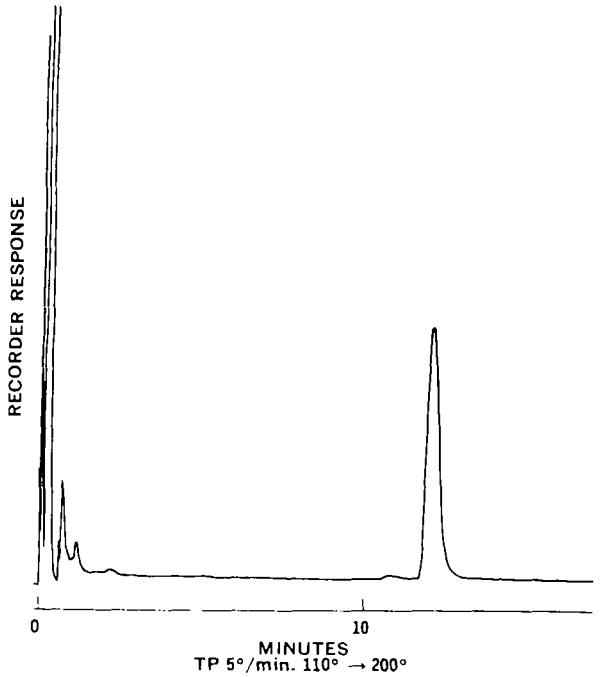


Figure 1—*Gas chromatogram resulting from analysis of the trimethylsilylated, 0-3-hr. chromatographically purified metabolite of I.*

The corresponding fragment ion 2, *m/e* 123 (d, 123), is also observed. Both derivatives yield ion 3, *m/e* 149, arising by loss of carbotrimethylsilyloxy and trimethylsilanol. Another key fragment ion is 4, *m/e* 196 (d, 205), the formation of which must involve C—O bond scission and rearrangement of a trimethylsilyl group (6–8) onto the ether oxygen atom. The fragment ion of *m/e* 181, 5, is 4 minus a trimethylsilyl methyl group, rather than the methoxyl methyl, as required by the *m/e* of the analogous trimethylsilyl-d_9 fragment, 187. The cyclic siloxonium ion, 6, is proposed to account for the fragment of *m/e* 166. The trimethylsilyl-d_9 analog of 6 must be found and is at *m/e* 172. Cyclic ions of similar nature have been reported for the trimethylsilyl derivatives of *ortho*-disubstituted aromatics (9–11).

Reasonable structures for the four most intense fragment ions (*m/e* 124, 100%; *m/e* 109, 82%; *m/e* 137, 55%; and *m/e* 122, 45%) in the mass spectrum of the metabolite are shown in Structures 7–10. Structure 9 results from the loss of the entire side chain with the transfer of a proton back into the ether oxygen atom.

The proposed metabolite structure is required by the mass shifts resulting from the trimethylsilyl deuterium-labeling experiment, and fragment ions satisfying this structure are found in the mass spectrum of the metabolite. The IR and UV spectra of the metabolite are also consistent with this proposal. Naito *et al.* (12) employed TLC techniques to identify β-(2-methoxyphenoxy)lactic acid, β-(4-hydroxy-2-methoxyphenoxy)lactic acid, and (2-methoxyphenoxy)-acetic acid as urinary metabolites (possibly glucuronides) of I in

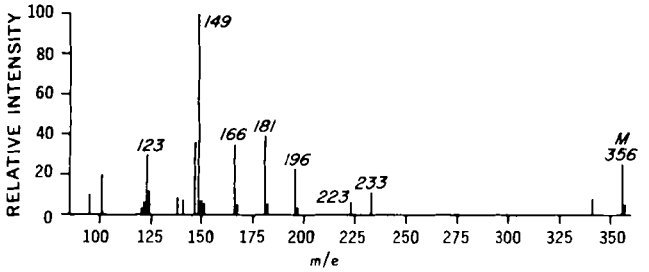
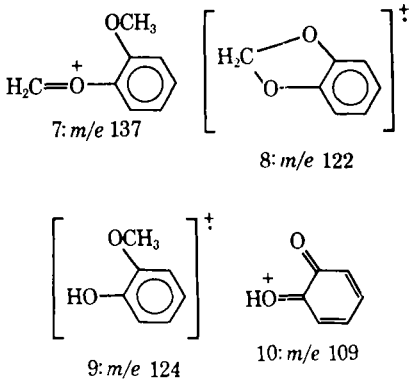


Figure 2—*Mass spectrum of the trimethylsilylated, 0–3-hr. chromatographically purified metabolite of I.*



the rabbit. The metabolism of meprophendiol [3-(2-methoxy-4-propionylphenoxy)-1,2-propanediol] has been reported to involve oxidation of the glyceryl chain to form the corresponding lactic acid and a smaller amount of the acetic acid (13).

Riley (14) identified β-(2-methylphenoxy)lactic acid as a major (15% of the dose) human urinary metabolite of myanesin [3-(*o*-toloxy)-1,2-propanediol]; only 3% of the dose was excreted as β-(2-methyl-4-hydroxyphenoxy)lactic acid, and no significant conversion to (2-methylphenoxy)acetic acid was observed.

The experimental procedure employed in the present study would be expected to result in the isolation of acidic substances such as β-(2-methoxyphenoxy)acetic and β-(4-hydroxy-2-methoxyphenoxy)-lactic acids, as well as β-(2-methoxyphenoxy)lactic acid, but no evidence of their presence was observed in human urine. The unequivocal identification of β-(2-methoxyphenoxy)lactic acid as the major urinary metabolite of glyceryl guaiacolate in the human thus parallels the results of Riley (14) with myanesin.

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