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Biopharmaceutical Studies on Guaiacol Glycerol Ether and Related Compounds. III.[1] Metabolites of Guaiacol Glycerol Ether and Its Mononicotinate. (1)

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Metabolites of guaiacol glycerol ether and its mononicotinate in rabbit urine were investigated. As the metabolites of guaiacol glycerol ether, β-(2-methoxyphenoxy) lactic acid, (2-methoxyphenoxy) acetic acid, β-(4-hydroxy-2-methoxyphenoxy) lactic acid, and (4-hydroxy-2-methoxyphenoxy) acetic acid (trace) were separated with unchanged GGE, and identified with the authentic samples by thin-layer chromatography.

Two of the metabolites, β-(4-hydroxy-2-methoxyphenoxy)lactic acid and (4-hydroxy-2-methoxyphenoxy) acetic acid showed hypocholesteremic action on hypercholesteremia caused by intravenous injection of Triton to rats.

As metabolites of guaiacol glycerol ether in rabbit liver and kidney *in vitro*, only β-(2-methoxyphenoxy)lactic acid was detected with the unchanged drug by thin-layer chromatography.

The preceding paper[3] reported the action of guaiacol glycerol ether (GGE) and guaiacol glycerol ether mononicotinate (GGE-MN) on hypercholesteremia caused by intravenous administration of Triton[4] (oxyethylated tertiary octylphenol formaldehyde polymer), and additional results of observations on pathological tissue preparations of an arteria, spleen, and liver after oral administration of cholesterol and these chemicals to rabbits.

The present work was carried out for the identification of (1) metabolites of GGE and GGE-MN from rabbit urine and (2) metabolites from rabbit liver and kidney *in vitro*, and (3) synthesis of two metabolites of GGE and the action of these chemicals on hypercholesteremia induced by intravenous administration of Triton.

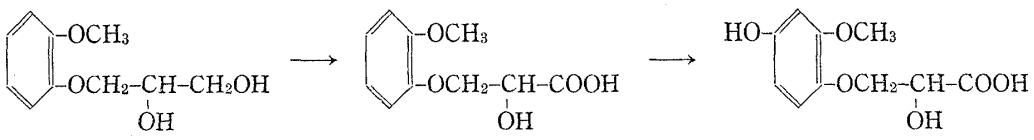
The metabolites of mephenesin may be helpful in finding supposed metabolites of GGE and GGE-MN. Wyngaarden, Woods, and Seevers[5] reported that, in the dog, from 0.1 to 2.0% of the administered dose is excreted as free mephenesin; from 32 to 42% of the administered dose is excreted as conjugated mephenesin in 24 hr, possibly as the glucuronide. The metabolic degradation of mephenesin to β-(2-methylphenoxy)lactic acid and to the second compound, β-(2-methyl-4-hydroxyphenoxy)lactic acid,[6] which is responsible for the appearance of a positive Ehrlich's diazo reaction in the urine of patients receiving the drug, has been reported. Fifteen per cent of the administered drug was isolated as the former compound and 3.3% or more as the latter. Riley[7] also revealed that, of an oral dose of 2 g of mephenesine, 28% was excreted in the urine in 24 hr as β-(2-methyl phenoxy) lactic acid; 54% of a 4 g was similarly eliminated, no appreciable quantity of free mephenesin was excreted in the urine of humans, and no significant amount of the drug was degraded to 2-(methylphenoxy)

1) This paper constitutes Part XXXVI of a series entitled "Studies on Absorption and Excretion of Drugs" by S. Naito.
2) Location: *Yamashina, Misasagi, Higashiyama-ku, Kyoto.*
3) Part II: S. Naito and M. Mizutani, *Chem. Pharm. Bull.* (Tokyo), **17**, 822 (1969).
4) Trade Mark of Rohm & Hass Co., Philadelphia, supplied by Winthrop Laboratories, New York.
5) J.B. Wyngaarden, L.A. Woods, and M.H. Seevers, *Proc. Soc. Exptl. Biol. Med.*, **66**, 256 (1947).
6) R.F. Riley and F.M. Berger, *Arch. Biochem.*, **20**, 159 (1949).
7) R.F. Riley, *J. Pharmacol.*, **99**, 329 (1950).

acetic acid or to *o*-cresol in man or in rats. Aside from these *in vivo* observations, *in vitro* metabolism of this compound has been reported by Maass, Carey, Hamilton, and Heming[8] who showed that mephenesin was oxidatively metabolized *in vitro* by rat and mouse liver slices to β-(2-methylphenoxy)lactic acid, and mephenesin metabolism was markedly inhibited by increasing substrate concentration, but was not altered by the presence of increasing concentration of the product, β-(2-methylphenoxy)lactic acid, in the medium.

On the other hand, Hynie, Hátle, and König[9] tried thin-layer chromatographic separation of GGE from urine, but no identification was made on the spots obtained. Večerková and Novotnā[10] examined GGE in urine by paper chromatography and assumed that two of the metabolites might be β-(2-methoxyphenoxy)- and/or β-(4-hydroxy-2-methoxyphenoxy)lactic acid (Chart 1-a), but did not identify them at all.

a) By Večerková and Novotná



b) From the present work

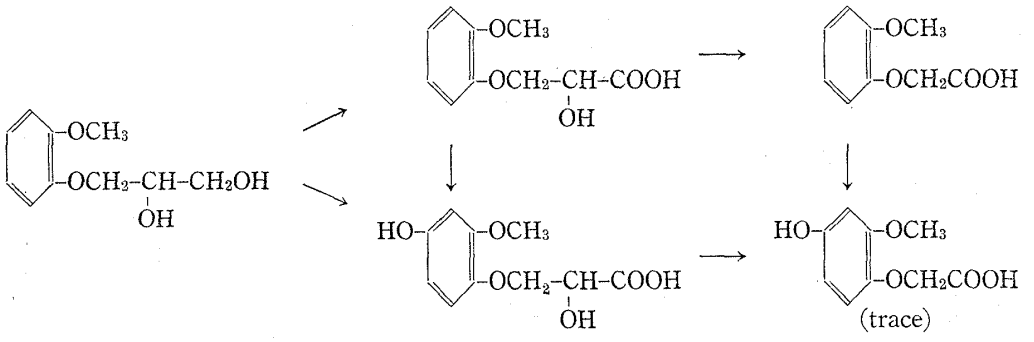


Chart 1. Assumed Metabolic Pathways of Guaiacol Glycerol Ether

The hydrolysate of the glucuronide fraction from urine of rabbits administered GGE–MN was submitted to thin-layer chromatography. The *Rf* values obtained were compared with those of unchanged GGE–MN, GGE, β-(4-hydroxy-2-methoxyphenoxy)lactic acid, β-(2-methoxyphenoxy) lactic acid, (2-methoxyphenoxy) acetic acid, nicotinamide, nicotinic acid, nicotinoylglycine, N^1-methyl-2-pyridone-5-carboxamide, nicotinamide N-oxide, and N^1-methylnicotinamide, and it was assumed that β-(2-methoxyphenoxy)lactic acid must be one of the components of the glucuronide (Table I, Sec. 1). When a mixture of the hydrolysate and β-(2-methoxyphenoxy)lactic acid was spotted, no other spots except the latter substance were observed on the thin-layer chromatogram, but when a mixture of the hydrolysate and other compounds was spotted, other spots appeared.

From the result of thin-layer chromatography, GGE, β-(2-methoxyphenoxy)lactic acid, (2-methoxyphenoxy)acetic acid, nicotinic acid, nicotinamide, N^1-methyl-2-pyridone-5-carboxamide, nicotinoylglycine, nicotinamide N–oxide, and N^1-methylnicotinamide were assumed to be the constituents of the metabolites of GGE–MN in rabbit urine, and unchanged GGE–MN was not detected, which suggested that GGE–MN is metabolized completly in rabbit (Table I, Sec. II). Since so many components of the metabolites of GGE–MN were developed on one glass plate, some misjudgement may have been made.

8) A.R. Maass, P.L. Carey, R.E. Hamilton, and A.E. Heming, *Proc. Soc. Exptl. Biol. Med.*, **103**, 154 (1960).
9) I. Hynie, K. Hātle, and J. König, *Českoslv. Farm.*, **14**, 481 (1965).
10) J. Večerkovā and E. Novotnā, *Českoslov. Farm.*, **14**, 462 (1965).

Table I. Thin-Layer Chromatography

Sect. No.	Material	Solvents	Developed distance in cm	Color developer	Color of spot[a]	*Rf* (assumed sub.[b])
I	hydrolysate of glucuronide fraction (GGE-MN was given) in rabbit urine	benzene–MeOH–$CHCl_3$ (4:3:4)	16	3% $K_2Cr_2O_7$ in H_2SO_4	b	0.43 (L)
II	metabolites of GGE-MN in rabbit urine	benzene–MeOH–$CHCl_3$ (4:3:4)	17	3% $K_2Cr_2O_7$ in H_2SO_4	b	0.80 (G)
					b	0.30 (L)
					b	0.45 (A)
				Dragendorff reagent	b	0.80 (G)
					1-b	0.30 (L)
					1-b	0.45 (A)
					p	0.35 (N)
					p	0.66 (NA)
					p	0.62 (NPC)
					p	0.05 (NG)
					p	0.38 (NO)
					p	0.09 (NNA)
				Ehrlich reagent	r-v	0.80 (G)
					r-v	0.30 (L)
					r-v	0.45 (A)
III	metabolites of GGE in rabbit urine	benzene–MeOH–xylene–AcOH (45:5:5:2)	17	3% $K_2Cr_2O_7$ in H_2SO_4	v	0.32 (A)
					v	0.28 (G)
					v	0.21 (L)
					y	0.13 (HA)
					y-g	0.06 (HL)
IV	metabolites of GGE in rabbit urine	benzene–EtOH–AcOH (50:5:3)	17	3% $K_2Cr_2O_7$ in H_2SO_4	v	0.40 (A)
					v	0.35 (G)
					v	0.24 (L)
					y-g	0.12 (HA)
					y-g	0.03 (HL)
V	metabolites of GGE in rabbit urine	toluene–MeOH–AcOH (45:5:2)	17	3% $K_2Cr_2O_7$ in H_2SO_4	v	0.30 (A)
					v	0.26 (G)
					v	0.18 (L)
					y-g	0.11 (HA)
					y-g	0.05 (HL)
VI	homogenate of rabbit organs[c] with GGE *in vitro*	benzene–dioxan–AcOH (45:20:2)	14	3% $K_2Cr_2O_7$ in H_2SO_4	v	0.40 (G)
					v	0.14 (L)

adsrbent: kieselgel G, 0.25 mm in thickness

a) b: brown 1-b: light brown p: pink r-v: reddish violet v: violet y-g: yellowish green

b) L: β-(2-methoxyphenoxy)lactic acid
G: guaiacol glycerol ether
A: (2-methoxyphenoxy)acetic acid
N: nicotinic acid
NA: nicotinamide
NPC: N^1-methyl-2-pyridone-5-carboxamide
NG: Nicotinoyl glycine
NO: nicotinamide N-oxide
NNA: N^1-methylnicotinamide
HA: (4-hydroxy-2-methoxyphenoxy)acetic acid
HL: β-(4-hydroxy-2-methoxyphenoxy)lactic acid
All of these assumed substances agreed with authentic samples in the colors of their spots and *Rf* values.

c) rabbit organs: liver or kidney

Accordingly, detailed examination was carried out on the urinary metabolites of GGE in rabbits (Chart 1-b). β-(4-Hydroxy-2-methoxyphenoxy)lactic acid, β-(2-methoxyphenoxy)-lactic acid, (2-methoxyphenoxy)acetic acid, and unchanged GGE were identified with the authentic samples[3] by thin-layer chromatography using several different solvent systems (Table I, Sec. III—V). The spot corresponding to (4-hydroxy-2-methoxyphenoxy)acetic acid was extremely faint, and its presence must be trace or questionable.

Two of the four metabolites of GGE, that is, β-(4-hydroxy-2-methoxyphenoxy)lactic acid and (4-hydroxy-2-methoxyphenoxy)acetic acid, were then synthesized by the method of Ludwig, Luts, and West.[11] As in the previous work,[3] effect of these two chemicals on

11) B.J. Ludwig, H. Luts, and W.A. West, *J. Am. Chem. Soc.*, **77**, 5751 (1955).

hypercholesteremia produced by intravenous injection of Triton were determined under the same conditions (Table II). From the results thereby obtained, the following statistical conclusion may be drawn.

Table II. Effect of Metabolites of Guaiacol Glycerol Ether on Triton-induced Hypercholesteremia in Rats

Group[a]	Dose (mg mole/kg)	Serum total cholesterol (mg%)	Inhibition[b] (%)
Normal		61±11[c]	
Control		387±31	
β-(4-Hydroxy-2-methoxyphenoxy)lactic acid	0.66	313±28	23
Normal		65±6	
Control		328±28	
(4-Hydroxy-2-methoxyphenoxy)acetic acid	0.66	267±30	23

a) Each group consisted of 10 rats which were bled 18 hr after intravenous injection of 400 mg/kg of Triton, followed immediately by intraperitoneal injection of each chemical except the control group.

b) $\text{inhibition}(\%)=\frac{\text{control level}-\text{treated level}}{\text{control level}-\text{normal level}}\times 100$

c) standard deviation

a) Difference between the control group and the group given 0.66 mg mole/kg of β-(4-hydroxy-2-methoxyphenoxy) lactic acid is significant at 99% of fiducial limit.

b) Difference between the control group and the group given 0.66 mg mole/kg of (4-hydroxy-2-methoxyphenoxy)acetic acid is significant at 95% of fiducial limit.

Thus, the hypocholesteremic effect of GGE–MN at 0.66 mg mole/kg dose might be due not only to nicotinic acid metabolites but also to GGE metabolites, β-(4-hydroxy-2-methoxyphenoxy)lactic acid and (4-hydroxy-2-methoxyphenoxy)acetic acid.

Aside from the *in vivo* observations, *in vitro* metabolism of GGE has not been reported. Metabolism of GGE by rabbit liver and kidney homogenates was studied and the results of thin-layer chromatography (Table I, Sec. VI) showed that GGE is partially metabolized only to β-(2-methoxyphenoxy)lactic acid, and 4-hydroxy metabolites described earlier could not be observed as in the case of *in vitro* metabolism of mephenesin.[8]

In the present work, assay of very small amounts of each metabolite of GGE was so complicated that no quantitative investigation was made, but this should be done at the next step of this series.

Experimental

Glucuronide in Urine of Rabbit given GGE-MN——Four rabbits (2.5 kg in weight) were each given 1.5 g of GGE-MN with H_2O by a stomach tube. In the next 5 hr, 500 ml of urine was collected which was acidified with a few drops of glacial AcOH and treated with saturated normal lead acetate until no further precipitate was formed. The mixture was filtered, the filtrate was neutralized with NH_3, and treated with excess of saturated basic lead acetated solution. The precipitate was collected by centrifugation, and washed twice by centrifugation with H_2O. The lead salt was suspended in H_2O and decomposed with H_2S. The filtrate separated from PbS was concentrated *in vacuo* at 45—50°. The glucuronide separated as a crystalline solid was dried. The residue dried was then washed with EtOH and acetone (yield, 90 mg).

The glucuronide (40 mg) thus obtained was hydrolyzed with 0.3 ml of 5N HCl at 37±2° for 1 hr, and adjusted to pH 5 with 5N Na_2CO_3 solution. This solution was submitted to thin-layer chromatography (Table I Sec. I).

Metabolites of GGE in Rabbit Urine——The frozen residue (200 mg) of urine of rabbits, each of which was given 1 g of GGE, was dissolved in 1 ml of 5N HCl, the solution was incubated at 37±2° for 1 hr, and extracted twice with 10 ml each of ether. After evaporation of ether, the residue was dissolved in 1 ml of EtOH and this solution was used as the metabolite solution of GGE in rabbit urine.

To make sure, water layer after extraction with ether was extracted twice with 10 ml of ether after adjusting the medium to pH 9 by adding 5N $NaHCO_3$, and ether was evaporated. The residue obtained was acidified with 5N HCl and added with 1 ml of EtOH. This EtOH solution was submitted to thin-layer

chromatography. but any recognizable spots were not observed. Therefore this EtOH solution was not used for examination of the metabolites of GGE.

β-(4-Hydroxy-2-methoxyphenoxy)lactic Acid——Dihydropyran (16.8 g, 0.2 mole) was added to a well-stirred mixture of 14.0 g (0.1 mole) of methoxyhydroquinone[12] in 100 ml of dioxan containing 3 drops of conc. HCl. External cooling was used to maintain the reaction mixture at 25—30°. The mixture was then allowed to react without cooling for 3 hr during which period the temperature rose to about 60°. Ether was added and the alkalisoluble portion was extracted with 10% NaOH solution. The alkaline solution was neutralized with CO_2, diluted with H_2O, and extracted with CCl_4. Concentration of this solution under reduced pressure gave a deposit of 8 g of colored product.

4-Hydroxy-3-methoxyphenyl 2-tetrahydropyranyl ether (4.48 g, 0.02 mole) thus obtained was dissolved in 12 ml of 10% NaOH solution, β-chlorolactic acid (1.25 g, 0.01 mole) was added, and the mixture was refluxed for 3 hr. The solution was cooled, saturated with CO_2, and the ether soluble fraction was removed by extraction. The aqueous portion containing the mixed diether was acidified with HCl, saturated with Na_2SO_4, and extracted with ether. The solution was dried and ether was evaporated. After washing the residue with $CHCl_3$ and two recrystallization from nitropropane 0.38 g of purified product, mp 149—152°, was obtained. *Anal.* Calcd. for $C_{10}H_{12}O_6$: C, 52.63; H, 5.26. Found: C, 52.58; H, 5.35.

(4-Hydroxy-2-methoxyphenoxy)acetic Acid——This compound was prepared by a procedure quite similar to that described above using monochloroacetic acid, affording 0.3 g of colorless needles (from H_2O), mp 83—85°. *Anal.* Calcd. for $C_9H_{10}O_5$: C, 54.55; H, 5.05. Found: C 54.68; H, 5.14.

Triton Hypercholesteremia——Male rats of Wistar strain weighing about 120 g were used. All the animals were fed commercial laboratory chow before and during the experimental period. Each group consisted of 10 rats injected intravenously with 400 mg/kg of Triton in distilled water. Test sample was administered intraperitonealy immediately after the injection of Triton. The animals were killed 18 hr after receiving the Triton injection. At the time of sacrifice, each animal was bled completely, and the bood was reserved for analysis.

Assay of Cholesterol——Cholesterol in blood was determined by the method of Zak.[13]

Metabolites of GGE in Rabbit Liver and Kidney Homogenates *in Vitro*——Approximately 10 g in fresh weight of liver or kidney from adult male rabbit was homogenized with 20 ml of Krebs–Ringer bicarbonate solution ($CaCl_2$ 1.3 g, KCl 1.1 g, $NaHCO_3$ 1.3 g, NaCl 41.8 g made to 500 ml with distilled water, pH 7.4), 10 mg of GGE was added, and mixed well. The mixture was incubated at 37±2° for 5 hr. The reaction was stopped by centrifugation after the addition of 20 ml of EtOH to the mixture. The supernatant was used as a sample for thin-layer chromatography (Table I, Sec. VI). Rabbit liver or kidney homogenate without any drug was treated by the same procedure as above to make the control for thin-layer chromatography.

Acknowledgement The authors are much indebted to Dr. K. Okamoto, Kyoto Pharmaceutical Ind. Ltd., for his generous gift of GGE-MN.

12) This compound was synthsized from vanillin by the method described in "Yuki Gosei" ed. Z. Horii, Kyoritsu Shuppan Co., Tokyo, 1954, p. 31.
13) B. Zak, *Am. J. Clin. Pathol.*, 27, 583 (1957).