The Metabolism of Ethyl Maltol and Maltol in the Dog

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Ethyl maltol (2-ethyl-3-hydroxy-1,4-pyrone), a new flavor enhancing agent ("Veltol-Plus," Pfizer Inc.) was administered to dogs by both the oral and intravenous routes, and the excreta analyzed for unchanged compounds and for metabolites. Parallel intravenous studies were also carried out in the same species with maltol for comparison. Orally administered ethyl maltol was rapidly and extensively absorbed. Elimination was also extensive and rapid, involving conjugation as glucuronide and ethereal sulfate, then excretion in the urine to the extent of 65 to 70% within 24 hr. Rate studies after intravenous dosage indicated that the bulk (86%) of the recovered conjugates were excreted within 6 hr. In the case of maltol, 57% of the intravenously administered dose was recovered in 24 hr; 88% of the total excretion occurred in the first 6 hr.

Maltol (2-methyl-3-hydroxy-1,4-pyrone), a naturally occurring substance, has been marketed as a food flavor enhancing agent for a number of years by several chemical concerns in the United States and Europe ("Veltol," Pfizer Inc.; "Corps Praline," Firmenich, Inc.) (Sanders, 1966). A synthetic homolog, ethyl maltol (this generic name was coined in analogy to ethyl vanillin, a homolog of vanillin), possessing approximately six times the potency of maltol (Stephens and Allingham, 1968), has been on the market ("Veltol-Plus," Pfizer Inc.) since 1967. In connection with the biological safety evaluation of ethyl maltol, Gralla *et al.* (1969) recently reported on its toxicity. As a part of the safety evaluation, we investigated the metabolic fate of ethyl maltol and of its naturally occurring homolog, maltol, in the mammalian organism (dog). Oral studies on ethyl maltol were carried out with prestressed animals (97 days at 200 mg per kg per day dosage); fresh animals were used for intravenous studies on ethyl maltol and maltol.

METHODS

Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems (v/v): (A) Chloroform-formic acid-ethanol 2:2:1 (lower phase); (B) Methyl ethyl ketone-acetone-water-formic acid 80:6:12:2; (C) *n*-Heptane-toluene-acetic acid-water 10:10:17:3; (D) Chloroform-ethyl acetate 3:1, saturated with formamide; and (E) Benzene-acetic acid-water 2:2:1. Detector reagents: (a) 0.2% *p*-nitrobenzenediazonium fluoroborate in 50% aqueous methanol; (b) Silver nitrate-sodium hydroxide (Trevelyan *et al.*, 1950); (c) 0.5% Potassium permanganate in 5*N* sulfuric acid.

Identification and Synthesis of Metabolites. Urine excreted over a 24 hr period following intravenous administration of ethyl maltol (10 mg per kg) was examined by paper chromatography (system D, detector reagent a). No unchanged ethyl maltol (R_f 0.7) was found. However, treatment of the urine with mineral acid (refluxing equal volumes of urine and 6*N* sulfuric acid for 2 hr in oil bath) resulted in the formation of substantial quantities of ethyl maltol. This observation led to the conclusion that ethyl maltol is excreted as one or more conjugates, possibly as ethereal sulfate and/or β-glucuronide. Treatment of the urine with β-glucuronidase (0.2*M* pH 5 sodium acetate-acetic acid buffer, 1 mg β-glucuronidase per ml of urine, incubated 29 hr at 46° C) also regenerated ethyl maltol, indicating the presence of a β-glucuronide. Sequential treatment first with 0.25*N* hydrochloric acid (15 min at 100° C), known to hydrolyze sulfates but not β-glucuronides (Garton *et al.*, 1949; Masamune, 1933; Porteous and Williams, 1949; Sperber, 1948), then with β-glucuronidase or 6*N* sulfuric acid (conditions described above) resulted in incremental regeneration of ethyl maltol in both steps, and thus implicated both sulfate and glucuronide as conjugates. Final confirmation of the metabolic route was obtained by identification of the urine components of treated animals with synthetic metabolites. Urine constituents were separated by paper chromatography (system B, detector reagents b for glucuronide, c for sulfate). The zones, which showed identical R_f values with synthetic standards (see below), were cut out and eluted with water. After acid hydrolysis the presence of ethyl maltol was demonstrated by paper chromatography (R_f 0.7, system D, detector reagent a), and by ultraviolet spectroscopy (λ_{max} in 0.01*N* HCl/MeOH: 278 nm; in 0.01*N* NaOH/MeOH: 323 nm).

2-Ethyl-3-hydroxy-1,4-pyrone β-D-glucopyranosiduronate was synthesized by the following route. D-Glucuronolactone was converted to methyl tetra-*O*-acetyl-D-glucopyranosiduronate by the method of Bollenback *et al.* (1955). Bromination with hydrogen bromide in acetic acid (Goebel and Babers, 1935) yielded methyl (tri-*O*-acetyl-β-D-glucopyranosyl bromide) uronate, which was condensed with ethyl maltol according to the method of Heyns and Kelch (1953), yielding 2-ethyl-3-hydroxy-1,4-pyrone methyl triacetyl-β-D-glucopyranosiduronate, m.p. 156–157° C, $[\alpha]_D$ − 91.4° (c = 1, $CHCl_3$), λ_{max} 256 nm, log ε 4.0. Anal. calcd for $C_{20}H_{24}O_{12}$: C, 52.63; H, 5.30. Found: C, 52.38; H, 5.30. R_f 0.67 (system C, detector reagent b). This derivative, representing a pure compound, was used to establish the experimental conditions for complete acid hydrolysis.

Hydrolysis with barium hydroxide and acidification with sulfuric acid gave 2-ethyl-3-hydroxy-1,4-pyrone β-D-glucopyranosiduronate, which could not be obtained in a crystalline state; λ_{max} 255 nm, R_f 0.57 (system B, detector reagent b).

The maltol analog was prepared by the same route: 2-methyl-3-hydroxy-1,4-pyrone methyl triacetyl-β-D-glucopyranosiduronate, mp 127–130° C, $[\alpha]_D$ −33° (c = 1, $CHCl_3$), λ_{max} 255 nm, log ε 3.7, R_f 0.65 (system C, detector reagent b).

Potassium 2-ethyl-3-hydroxy-1,4-pyrone sulfate was synthesized by treatment of ethyl maltol with chlorosulfonic acid (Lieberman *et al.*, 1948; Neuberg and Simon, 1925), followed by neutralization with potassium hydroxide. Unreacted starting material was removed by extraction with chloroform, and the product isolated by lyophilization. Mp approx. 225° C (decomp.), λ_{max} 256 nm, color reaction with ferric

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Table I. Urinary and Fecal Excretion of Ethyl Maltol in % of Administered Dose (EMAD) and in % of Ethyl Maltol Excreted (EMEX)

Dog No. and Sex	Day	EMAD Free Ethyl Maltol	EMAD		EMEX		Ratio Sulfate: Glucuronide	EMAD Sulfate + Glucuronide	EMAD Sulfate + Glucuronide[b]	EMAD Fecal
			Sulfate[a]	Glucuronide	Sulfate[a]	Glucuronide				
8656	98	0.07	11.3	42.0	21.2	78.8	0.27	53.3	52.0	0.22
Female	99	0.18	10.6	73.2	12.7	87.3	0.15	83.8	85.4	0.28
Mean		0.13	11.0	57.6	17.0	83.0	0.21	68.6	68.7	0.25
D-58	98	0.17	14.6	45.2	24.4	75.6	0.32	59.8	68.8	4.35
Male	99	0.07	11.2	47.9	18.8	81.2	0.23	59.1	69.0	0.10
Mean		0.12	12.9	46.6	21.6	78.4	0.28	59.5	68.9	2.23
Overall Mean		0.13	12.0	52.1	19.3	80.7	0.22	64.0	69.6	1.24

[a] Includes free ethyl maltol. [b] Determined as sum.

chloride negative. R_f 0 (systems A, D, and E, detector reagent c). Acid hydrolysis yielded ethyl maltol, (UV, paper chromatography).

The maltol analog was prepared the same way and showed the same properties.

Hydrolysis of the Metabolites and Colorimetric Determination of the 2-Alkyl 3-Hydroxy-1,4-pyrones Formed. In order to be amenable for colorimetric determination as their ferric complexes, ethyl maltol and maltol were regenerated from the metabolites by graded acid hydrolysis. Heating the ethereal sulfates in 0.25*N* sulfuric acid for 15 min at 100° C caused their complete hydrolysis, whereas the glucuronides proved to be stable under these conditions. Hydrolysis of the glucuronides required refluxing in 3*N* sulfuric acid for 2 hr.

Ethyl maltol and maltol were determined colorimetrically as their ferric complexes at 520 nm. (Reagent: 1% ferric ammonium sulfate in 0.36*M* sulfuric acid. Concentrations were read from a standard curve linear in the range of 0.5 to 5 mg%.)

Isolation of Ethyl Maltol and Maltol from Urine. The ethyl maltol and maltol formed by hydrolysis of the metabolites was extracted five times from the acid solution with chloroform. After replacement of the chloroform by 40% aqueous methanol the pyrones were determined as described above. Since chloroform extracts of normal urine heated with an equal volume of 6*N* sulfuric acid undergo a slight color change in the presence of ferric ions, the intensity of this background color was determined experimentally and applied as a correction. Mean value of 23 determinations. Absorbance at 520 nm 0.015 (range: 0.005 to 0.028). The color inherent to excess reagent was found to be negligible (transmission *vs.* water: 99%).

Synthetic metabolites were added to normal dog urine, which was then put through the described two step hydrolysis, and extracted with chloroform after each step.

Recoveries:	Metabolite	Ethyl Maltol	Maltol
	Sulfate	83%	83.5%
	Glucuronide	90.5%	85%

These recovery factors, as well as the background color factor were included in the calculations of ethyl maltol and maltol excreted.

Metabolism of Orally Adminstered Ethyl Maltol. The urinary and fecal excretion of ethyl maltol was determined in chronic feeding study dogs over a 48 hr period. Two dogs (beagles), one male and one female, were given single oral doses of 200 mg per kg per day on two successive days. Both animals had been prestressed for 97 days prior to this study. They were maintained in metabolism cages, fed once daily, and water was given *ad libitum*. The excreta were collected for 24 hr after each dose. All analytical determinations were performed in duplicate.

Urinary Excretion. Determination of Free Ethyl Maltol. Background and recovery. Four normal urine samples (20 ml each) were adjusted to pH 5.0, extracted with five times 20 ml of chloroform, the extract was evaporated *in vacuo* at 45° C, the residue dissolved in 5 ml of methanol, 1 ml of ferric ammonium sulfate reagent added, and diluted to 10 ml with water. The absorbance was measured at 520 nm against a reagent blank. The mean value for background was found to be 0.1. Recovery (normal urine fortified with ethyl maltol) amounted to 98%. Free ethyl maltol was determined by extraction of urine samples of dosed animals as described above.

Determination of Total Conjugates (Sulfate and Glucuronide). 20 ml of urine of each dog of each of the two subsequent 24 hr collections were hydrolyzed by refluxing with equal volumes of 6*N* sulfuric acid for 2 hr. Extractions and assays were performed as described.

Separate Determination of Sulfate and Glucuronide. An aliquot of the urine samples was mixed with the necessary amount of 2*N* sulfuric acid to give a 0.25*N* acid solution. The mixture was heated in a steam bath for 15 min, cooled, and extracted with five times 20 ml of chloroform. The combined chloroform extracts were processed and assayed as described. The aqueous phase from the chloroform extraction was mixed with an equal volume of 6*N* sulfuric acid and refluxed for 2 hr. The hydrolyzate was processed as described above.

Fecal Excretion. The dog feces was mixed with four parts of water and homogenized on a paint shaker. Aliquots were withdrawn, weighed, and processed as urine samples. Fecal solids were removed by centrifugation after hydrolysis. The background correction for the colorimetric assay was found to amount to 0.0075 per 10 g of feces, and the recovery (fortified normal feces) to 90%.

RESULTS

The results are represented in Table I.

Metabolism of Intravenously Administered Ethyl Maltol and Maltol. This study was performed as a crossover comparison. Four dogs (beagles), two males and two females, were administered single doses of 10 mg per kg of ethyl maltol and maltol intravenously. The animals were maintained in metabolism cages and fed once daily; water was given *ad libitum*. Urine was collected after 3, 6, 24, 48, and 72 hr. In order to insure urine excretion in the 0 to 3 and 3 to 6 hr intervals, 200 ml of water was administered by stomach tube immediately following injection. This study

Table II. Cumulative Urinary Excretion of Ethyl Maltol and Maltol Conjugates in % of Administered Dose

Compound	Dog No.	Sex	Hours 0–3	3–6	6–24	Total 0–24	24–48	48–72	Total 0–72
Ethyl Maltol	D-29	M	39.6	8.2	0.7	48.5	...	...	48.5
	8773	F	51.5	29.2	10.6	91.3	...	...	91.3
	D-30	M	11.5	10.5	4.9	26.9	2.1	...	29.0
	8627	F	12.8	71.6	7.0	91.4	2.1	3.0	96.5
	Average					64.5			66.3
Maltol	D-30	M	20.9	15.9	6.2	43.0	3.3	...	46.3
	8627	F	46.7	26.0	6.5	79.2	1.5	...	80.7
	D-29	M	21.3	15.4	3.8	40.5	...	...	40.5
	8773	F	33.2	30.9	2.5	66.6	...	...	66.6
	Average					57.3			58.5

was started with fresh (not prestressed) dogs. The second administration (crossover) was done 7 days after the first. Crossover dogs No. D-30 and 8627 had received maltol first, and crossover dogs No. D-29 and 8773 had received ethyl maltol first. The cumulative urinary excretion of ethyl maltol and maltol conjugates (sulfate and glucuronide) in percent of the administered dose is represented in Table II.

DISCUSSION AND CONCLUSION

Since no significant amount of ethyl maltol was detected in feces, and since the urinary excretion in the oral study was comparable to that in the intravenous experiment, it is apparent that ethyl maltol is readily absorbed from the gastrointestinal tract. The fact that orally dosed dogs had been on continuous prestress dosage immediately prior to this experiment should not introduce significant error since any slight overlap should be compensated by the fact that only a 24-hr post dose interval was used. This is also substantiated by the intravenous results in which 98% of the average total excretion of conjugates occurs within 24 hr post dosage. It is readily apparent that both the synthetic homolog, ethyl maltol, and the natural methyl compound, maltol, are rapidly and extensively metabolized in the dog and excreted by the conjugation pathway common to phenolic compounds. Within the precision of the methods used herein it can be concluded that the two homologs are metabolically equivalent when injected intravenously.

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Received for review May 25, 1970. Accepted September 14, 1970.