Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A[1]

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Summary. Contrary to prior indications, the glycosidic sweeteners stevioside and rebaudioside A are degraded to the diterpenoid aglycone steviol by rat intestinal microflora in vitro. Additional studies with steviol-17-[^{14}C] show almost total absorption from the rat lower bowel following intracecal administration.

Because of the sweetness of its leaves, the small Paraguayan shrub *Stevia rebaudiana* Bertoni, has been the subject of scientific interest for half a century. In 1931 Bridel and Lavieille[2] obtained a pure crystalline glycoside (stevioside) from the dried leaves and found it to be 300 times as sweet as sucrose. They also reported the enzymatic hydrolysis of stevioside to 3 moles of D-glucose and 1 mole of an acidic, tasteless aglycone (steviol). The structure of the aglycone was later elucidated by Mosettig et al.[3], while the configurations and linkage positions of the D-glucoses were fixed by Wood et al.[4,5]. Japanese chemists recently isolated rebaudioside A from *S. rebaudiana*, found it to be a combination of steviol with 4 moles of D-glucose, and observed taste properties superior to stevioside[6]. The structures and proper names of the 2 sweeteners and the aglycone are given in the figure.

An early literature report[7] suggesting that stevioside was excreted intact after oral administration to a rooster combined with our own interest[8] in high-molecular-weight sweeteners exhibiting limited intestinal absorption prompted us to conduct a study of the metabolic stability of stevioside and rebaudioside A.

Materials and methods. a) Using previously reported methodology[9,10], both sweeteners were tested by anaerobic incubation with microbial whole-cell suspensions from rat cecum, and aerobic incubation with sonic cell-free extracts (CFE) prepared from rat cecal contents. Degradation products were quantitated by HPLC in the former, and by a hexokinase/glucose-6-phosphate dehydrogenase assay in the latter.

b) In order to determine the intestinal absorption of steviol released in the lower bowel, steviol-17-[^{14}C] of sp.act.

Table 1. Degradation of diterpene glycoside sweeteners by whole cells of rat cecal bacteria *in vitro*

Substrate	Incubation period (days)	Fraction*	% Recovered as steviol**	Total % of theoretical recovered
Stevioside (2.5 mg/ml)	2	Supernatant	71	107
		Pellet	36	
Rebaudioside A (3.0 mg/ml)	2	Supernatant	60	65
		Pellet	5	
	4	Supernatant	74	83
		Pellet	9	
	6	Supernatant	51	108
		Pellet	57	
Steviol (0.2 mg/ml)	2	Supernatant	80	100
		Pellet	20	
	4	Supernatant	80	87
		Pellet	7	
	6	Supernatant	80	105
		Pellet	25	

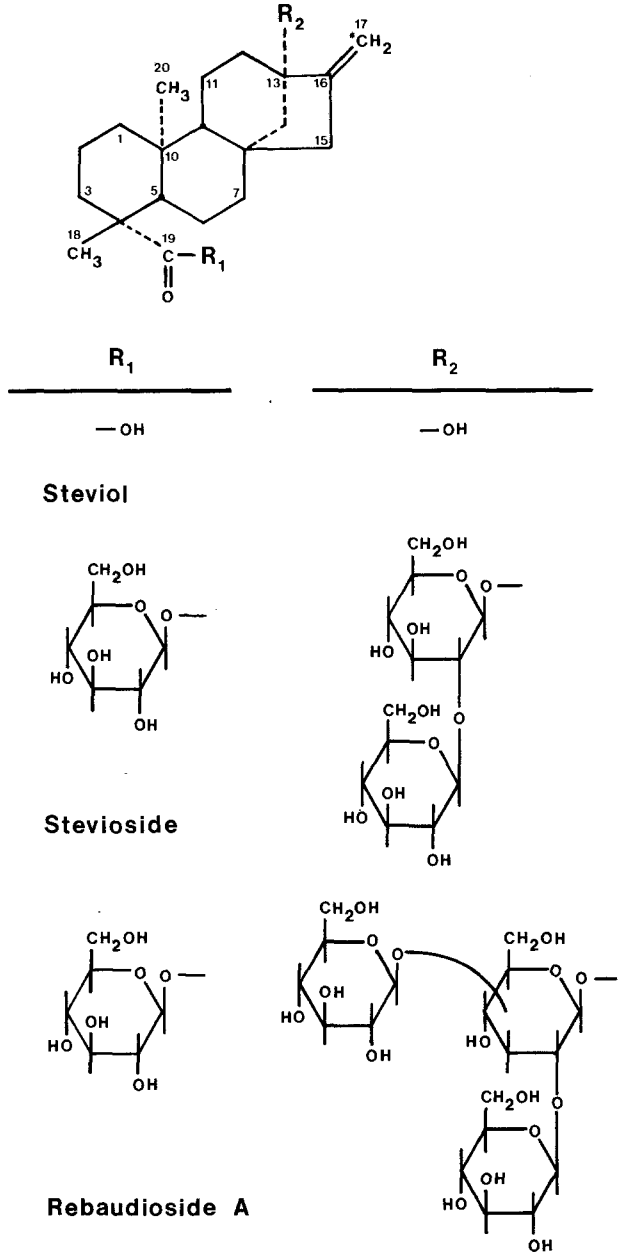
* Cecal bacterial suspensions were prepared by removing the cecal contents from a freshly sacrificed Sprague-Dawley rat and suspending 0.1 g fresh weight/ml in anaerobic Krebs-Ringer 0.25 M phosphate buffer (pH 7.4) containing 0.25 mg dithiothreitol/ml. The suspensions were gassed with oxygen-free N_2–CO_2 (9:1) and passed through Pyrex® wool. 1-ml aliquots of the anaerobic cecal bacterial cell suspensions containing 0.4 mg/ml α-D-glucose were mixed with 1.0 ml of the substrate solution (final concentrations given above), gassed, tightly stoppered and incubated at 37 °C for periods up to 6 days. The bacterial cells were sedimented by centrifugation and the supernatant and pellet fractions analyzed separately. ** Steviol was determined by isocratic high-pressure liquid chromatography (HPLC) with an apparatus equipped with a monochromator operating at 200 nm. Quantitation was based on peak height using a 30 cm × 4 mm inner diameter reverse-phase μ Bondapak C-18 column (Waters Associates) eluted with 50% acetonitrile-50% pH 4.8 0.03 M KH_2PO_4 aqueous,buffer (retention time 7 min).

Table 2. Recovery of radioactivity after administration of steviol-17-[^{14}C] to intact, bile duct ligated or bile duct cannulated rats

Group	Radioactivity (% administered dose) Urine	Feces	Organs	Gut content	CO_2	Bile	Cage	Total
Intact oral[a]	1.48 ± 0.36	96.36 ± 7.07	0.03 ± 0.01	0.22 ± 0.17	0.10 ± 0.01[b]	–	0.08 ± 0.04	98.26 ± 6.98
Ligated oral[c]	96.00 ± 0.28	3.31 ± 1.66	0.05 ± 0.01	0.17 ± 0.17	–	–	0.83 ± 0.09	100.4 ± 1.3
Ligated intracecal[e]	94.3 ± 3.88	6.03 ± 4.28	0.01 ± 0.003	0.03 ± 0.02	0.02 ± 0.001	–	1.16 ± 0.76	101.9 ± 4.4
Cannulated intracecal[d]	–	–	–	–	–	105.5 ± 8.45	–	–

[a] $\bar{X} \pm$ SD, N = 3, normal intact rats dosed orally with 1 ml (1.7 µCi, 0.7 mg) of steviol-17-[^{14}C] in an 0.5% klucel suspension. [b] $\bar{X} \pm$ SD, N = 2, normal intact rats dosed with steviol-17-[^{14}C], as above. [c] $\bar{X} \pm$ SD, N = 2, bile duct ligated rats dosed orally with 1 ml (0.92 µCi, 0.37 mg) of steviol-17-[^{14}C] in an 0.5% klucel suspension. [d] $\bar{X} \pm$ SD, N = 2, bile duct cannulated rats dosed intracecally with 1 ml (1.7 µCi, 0.7 mg) of steviol-17-[^{14}C] in an 0.5% klucel suspension. [e] $\bar{X} \pm$ SD, N = 5, bile duct ligated rats dosed intracecally with 1 ml (2.63 µCi, 0.92 mg) of steviol-17-[^{14}C] in an 0.5% klucel suspension.

2.85 μCi/mg was prepared from steviol acetate via ozonolysis, Wittig reaction with the ylid derived from methyl-[^{14}C]-triphenyl-phosphonium iodide, and deacetylation. The techniques employed for determining intestinal absorption, tissue distribution, and excretion were described earlier[11]. The objective was to simulate the occurrence of steviol in the intestinal tract following ingestion of the glycosides. Thus, the ethanolic dose, dispersed in aqueous hydroxypropyl cellulose, was injected intracecally into rats. The animals, bile duct ligated[12] prior to dosage, were placed in metabolism cages for collection of urine and feces. 2 animals (with metabolism cages) were placed in chambers for collection of respired $^{14}CO_2$. Steviol-17-[^{14}C] was also administered by intracecal injection to 2 bile duct cannulated rats[15], from which bile was collected for 72 h after dosing, and orally by gavage to intact and bile duct ligated rats, maintained as described for the bile duct ligated rats, above.



The structures of steviol (13-hydroxykaur-16-en-19-oic acid), stevioside (19-O-β-glucopyranosyl-13-O[β-glucopyranosyl(1-2)]-β-glucopyranosylsteviol), and rebaudioside A (19-O-β-glucopyranosyl-13-O-[β-glucopyranosyl(1-2)-β-glucopyranosyl(1-3)]-β-glucopyranosylsteviol).

Results and discussion. Table 1 shows that stevioside was completely transformed into steviol by whole-cell suspensions within 2 days, while similar conversion of rebaudioside A required 6 days. Similar hydrolyses by CFE (not shown) proved much slower with steviol being generated from stevioside and rebaudioside A in yields of only 50% and 2%, respectively, after 7 days.

The results indicate that both stevioside and rebaudioside A would be degraded to steviol by microbial action in the mammalian lower bowel. The rebaudioside A to steviol degradation rate is in the range of 0.4–0.8 mg converted/h/g cecal contents. The flora of the rat cecum and the lower human bowel are, quantitatively and qualitatively, quite similar[13,14]. The large bowel of man contains about 10^{11} microbes/g fresh weight and has an average total weight of 1 kg. This suggests that the human bowel could convert over 0.4 g rebaudioside A to steviol per h.

Table 2 shows that steviol-17-[^{14}C] is virtually completely absorbed from the lower bowel of the rat after oral or intracecal administration. The radioactivity was largely excreted in the urine of bile duct ligated animals ($94.3 \pm 3.9\%$ of dose, $N=5$) and in the bile of bile duct cannulated and intact animals ($105.5 \pm 8.4\%$ of dose, biliary excretion, $N=2$; $96.4 \pm 0.4\%$ of dose, fecal excretion, $N=3$).

Very little $^{14}CO_2$ (0.02% of dose) was observed, indicating the stability of the exocyclic methylene label.

In sum, stevioside and rebaudioside A are degraded to the aglycone steviol by rat intestinal microflora in vitro. A similar degradation can be predicted to occur in man. Studies with steviol-17-[^{14}C] show the aglycone to be readily absorbed from the lower bowel of the rat. Analogous absorption from the human large bowel seems probable.

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