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Metabolic Studies of the Nonnutritive Sweeteners Cyclopentylmethylsulfamate and Cyclopentylsulfamate: Determination of Metabolites in Rat Urine

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Abstract □ The *nonnutritive* sweetener sodium cyclopentylmethylsulfamate was fed to Wistar albino rats. The urine was collected for 3 days, combined, and examined (GLC) for the metabolites cyclopentylmethylamine and cyclopentylmethanol. The percent conversion to these metabolites was 0.077 and 0.0102, respectively. The percent conversion to cyclopentylmethylamine was the lowest conversion to amine observed when compared to the metabolism of three other sweet sulfamates, cyclopentylsulfamate, cycloheptylsulfamate, and cyclooctylsulfamate, previously administered to rats. The average excretion of unmetabolized sulfamate was 15.4%. Sodium cyclopentylsulfamate was fed to rats over 9 days, and an analysis was carried out for the metabolites cyclopentylamine, cyclopentanone, and cyclopentanol. A decrease in the level of metabolites occurred after the first 3 days.

Keyphrases □ Cyclopentylsulfamate and cyclopentylmethylsulfamate—metabolism in rats □ Metabolism—various sulfamates in rats □ Sulfamates, various—metabolism in rats compared □ Sweeteners, nonnutritive—various sulfamates, metabolism in rats compared

The artificial sweetener cyclamate (*N*-cyclohexylsulfamate) has been the subject of many investigations, including the feeding of the compound to different animal species to study its distribution and metabolism (1–4). Until 1966, it was generally considered that the compound, whether administered orally, intravenously, or intraperitoneally, was excreted entirely unchanged. However, in 1966, Kojima and Ichibagase (5) found cyclohexylamine in the urine of dogs and humans who had been fed cyclamate. Various toxicological studies (6–9) then showed that certain animals fed cyclamate developed lesions, chromceromal breaks, and tumors of the urinary bladder. The presence of cyclohexylamine as a metabolite and the possibility of cyclamate being a carcinogen resulted in the sweetener being banned in 1969 in the United States and Britain.

The purposes of this investigation were to study the level of excretion of sweet-tasting sulfamates related to cyclamate, to determine whether metabolic breakdown occurs, and, if so, to compare the levels of breakdown with the levels reported for cyclamate. Cycloheptylsulfamate and cyclopentylsulfamate were administered to rats and rabbits, and the results were reported previously (10, 11). The recent surprising finding (12) that sodium cyclopentylmethylsulfamate is sweet and the suggestion that its amine metabolite may be less carcinogenic than, for example, cyclohexylamine prompted study of this compound.

This paper describes the results of *in vivo* administration of cyclopentylmethylsulfamate to rats and the extended feeding of cyclopentylsulfamate to rats.

EXPERIMENTAL

Reagents and Chemicals—Cyclopentylmethylamine, bp 140–143°, was prepared by the following route: cyclopentanone[1] → cyclopentanol (13) → cyclopentylbromide (14) → cyclopentylcyanide (15) → cyclopentylmethylamine (16). This amine was sulfamated by the method of Audrieth and Sveda (1) and recrystallized (twice) from ethanol.

Anal.—Calc. for $C_6H_{12}NNaO_3S$: C, 35.82; H, 5.97; N, 6.96. Found: C, 35.85; H, 5.45; N, 6.81.

Sodium cyclopentylsulfamate was prepared as previously described (10). Cyclopentylmethylamine, cyclopentylamine[1], cyclopentylmethanol[1], cyclopentanol[1], cyclopentanone[1], and dichloromethane[2] were redistilled before use. *p*-Benzoquinone was sublimed; *n*-dodecane[3], analytical reagent grade sulfosalicylic acid[4], chloroform, ethanol, and 1,4-dioxane (reagent grade) were used as obtained.

Feeding Experiments—Female Wistar albino rats, ~300 g, were kept on solid food and water in metabolism cages[5]. Prior to feeding, the urine of each rat was collected and monitored using both detection methods (described later), and the rats were then starved of water for 24 hr. Sodium cyclopentylmethylsulfamate (1450 mg/kg) was administered orally in aqueous solution (25–30 ml of water). The extended feeding of sodium cyclopentylsulfamate was carried out over 9 days as follows: 200 mg in 20 ml of water was fed for the first 5 days, sulfamate was not given for the 6th and 7th days, and feeding was resumed for the 8th and 9th days.

The urine of the rats fed cyclopentylmethylsulfamate was collected

[1] Aldrich Chemical Co.
[2] May and Baker.
[3] British Drug Houses.
[4] AnalaR grade, May and Baker.
[5] NKP cages, Kent, England.

for 3 days after feeding. The urine samples were refrigerated, and the samples were bulked and analyzed at the end of 3 days. The urine of the rats fed cyclopentylsulfamate was collected and refrigerated each day for 9 days. The samples were then analyzed as described below.

GLC Analysis and Standardization—Cyclopentylmethylamine and cyclopentylmethanol were determined by a GLC procedure described previously (10). The conditions for this separation were: column temperature, 120°; carrier (nitrogen) flow rate, 60 ml/min; and internal standard, *n*-dodecane. Under these conditions, the retention times of cyclopentylmethylamine, *n*-dodecane, and cyclopentylmethanol were 4.8, 7.8, and 12.4 min, respectively (Fig. 1).

A standard curve was prepared by injecting microliter portions of the amine and the alcohol into 5-ml samples of urine. Details of the extraction from urine and the injection of samples were similar to those previously described for cyclopentylsulfamate metabolites (10). Urine samples taken before and after feeding were similarly analyzed. To estimate the percent recovery, varying amounts of amine and alcohol were determined by the established procedure (10); the results are given in Table I. GLC analysis of the metabolites of cyclopentylsulfamate and percent recovery data were reported previously (10).

Visible Spectrophotometic Analysis and Standardization—The details for the analysis of cyclopentylsulfamate were reported previously[6] (10). A similar procedure was used for cyclopentylmethylsulfamate, except that the hydrolysis in dioxane solution took 4.5 hr at 95° for completion. A calibration graph was prepared by injecting microliter quantities of cyclopentylmethylamine into 25-ml samples of chloroform. The amine was subsequently reacted with *p*-benzoquinone according to the published procedure (10). The plot of absorbance *versus* concentration was linear over the 20–100-μg/ml range.

Stability of Cyclopentylmethylsulfamate and Cyclopentylsulfamate during Storage in Urine and Sampling Procedure for GLC—The possibility of sulfamate conversion to one or more of the metabolites while refrigerated in urine or during the sampling procedure was checked by adding the two sulfamates individually to rat urine at levels of 5, 10, and 15 mg/ml. The urine samples were then refrigerated for 3 days. After basification and extraction with dichloromethane, GLC analysis showed that neither sulfamate had been converted to any of its metabolites.

RESULTS AND DISCUSSION

By using different sweet sulfamates, the effect of structural modification of the cyclamate nucleus on the stability of the compound in the body was studied. By elucidating the stability of various sulfamates *in vivo*, it may be possible to predict and prepare a sweet sulfamate resistant to metabolic breakdown and, therefore, less likely to cause cancer.

Table II summarizes the results of feeding cyclopentylmethylsulfamate to five rats at a level of 1450 mg/kg. Three rats excreted cyclopentylmethylamine and cyclopentylmethanol. No attempt was made to search for other metabolites, since it is clear from the work on cyclamate that the amine, alcohol, and ketone (where it exists) are the principal metabolites. The average conversion of cyclopentylmethylsulfamate to its corresponding amine was 0.011%.

Previously (17), the conversion of cyclamate to cyclohexylamine, cyclohexanone, and cyclohexanol in rats was reported to be 0.0068, 0.0005, and 0.0003%, respectively. These data point to the fact that cyclopentylmethylsulfamate is cleaved to a greater extent than its cyclohexyl analog in rats. In another study (17) with rats, the average conversion to cyclamate was 0.006%, again showing the greater stability of the cyclamate compound over that of cyclopentylmethylsulfamate.

The results of feeding cyclopentylsulfamate (10) and cycloheptylsulfamate (11) to rats and rabbits were also reported. In the rat, the percentage conversion of cyclopentylsulfamate and cycloheptylsulfamate to cyclopentylamine and cycloheptylamine was 0.57 and 0.064, respectively[7]. These figures are significantly greater than those found previously (17) for the conversion of cyclamate to cyclohexylamine and suggest that cyclamate is more stable than cyclopentylsulfamate and cycloheptylsulfamate. The results for cyclopentylmethylsulfamate are presented in Table II; it was more stable than cyclopentylsulfamate, cycloheptylsulfamate, and cyclooctylsulfamate in the rat but was metabolized more easily than cyclamate.

Table II also shows the percentage unmetabolized sulfamate recovered in the urine for the five rats; the average recovery of cyclopentylmethylsulfamate was 15.4%. Previous studies showed that the percent of cyclopentylsulfamate (10) and cycloheptylsulfamate (11) recovered in the urine was 15.17 and 35.06, respectively. This result could indicate that the main excretory pathway for sulfamates in rats is through the feces, with the level of absorption from the intestine being small. Evidence for this possibility was provided by the study of Derse and Daun (18); in rats administered cyclamate, about 17% of it was excreted in the urine and about 70% was excreted in the feces. Bickel *et al.* (19) administered cyclamate at a level of 2500 mg/kg to rats and observed that 32% was excreted in the urine. The present results for other sweet sulfamates support the idea that the main excretory pathway for sulfamates is *via* the feces.

Cyclopentylsulfamate was also fed to rats over 9 days to ascertain if a pattern of metabolic breakdown was observable; the results are shown in Table III. In some rats, metabolic breakdown of the sulfamate occurred to cyclopentylamine, cyclopentanone, and cyclopentanol. However, no trend or increase was observed in the level of metabolites due to continued administration of the compound for 5 days. The rats did not receive a dose

Table I—Percent Recovery of Cyclopentylmethylamine and Cyclopentylmethanol from Urine

Amine		Alcohol	
μg	%	μg	%
348.0	97.41	1111.2	91.79
348.0	95.68	740.8	90.71
174.0	103.4	370.4	100.43
104.0	104.5	296.3	106.31
69.6	110.3	222.2	101.26
Mean ± *SE*	102.2 ± 4.58		98.10 ± 5.48

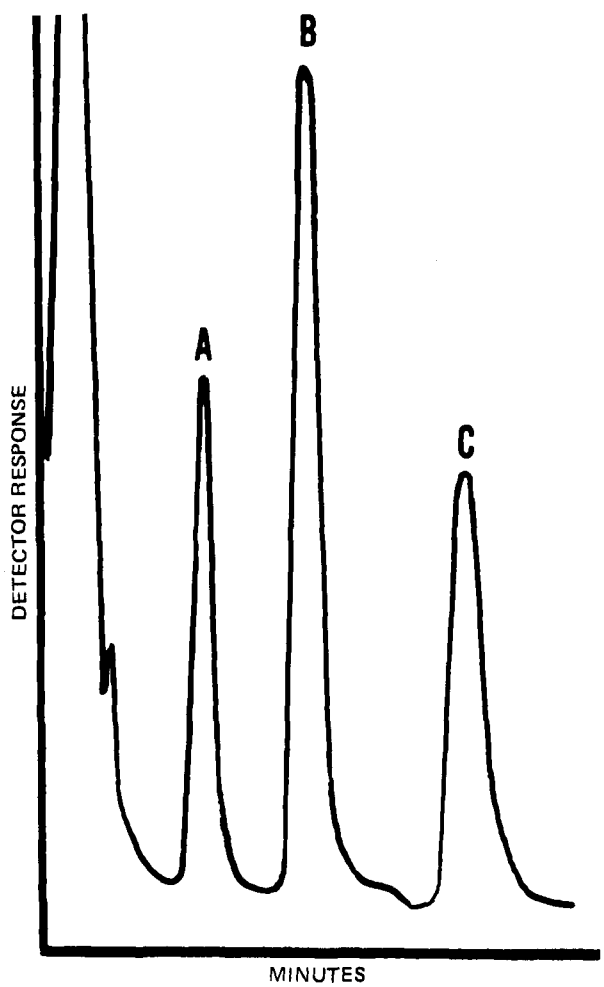

Figure 1—*Gas chromatogram of the urine metabolites of cyclopentylmethylsulfamate. Key: A, cyclopentylmethylamine (4.8 min); B, n-dodecane (7.8 min) (internal standard); and C, cyclopentylmethanol (12.4 min).*

[6] Perkin-Elmer 124 double-beam spectrophotometers.

[7] In similar feeding experiments, cyclooctylsulfamate was converted to the extent of 0.21% on the average in rats.

Table II—Metabolism of Cyclopentylmethylsulfamate in Rats

Animal	Recovered Sulfamate, Visible Spectrophotometry		Metabolites, GLC				Total Metabolites, %
			Cyclopentylmethylamine		Cyclopentylmethanol		
	mg	%	mg	%	mg	%	
1	160.3	32.0	0.0347	0.013	0.037	0.015	0.028
2	35.7	7.1	0.0178	0.007	0.017	0.006	0.013
3	43.5	8.6	0.0384	0.015	0.040	0.016	0.031
4	100.5	20.1	None	—	None	—	—
5	47.4	9.4	None	—	None	—	—
Mean ± *SE*	77.4 ± 42.3	15.4 ± 8.4	0.029 ± 0.009	0.011 ± 0.004	0.031 ± 0.009	0.012 ± 0.004	0.024 ± 0.007

Table III—Metabolism of Cyclopentylsulfamate after Prolonged Administration to Rats (200 mg/Animal)

Rat	Metabolite	1[a]	2	3	4	5	6	7	8	9
	Cyclopentylamine	14[b] (0.015)	—[c]	34 (0.037)	23 (0.025)	37 (0.040)	T[d]	47 (0.052)	T	T
1	Cyclopentanone	T	T	8 (0.009)	T	T	—	—	—	—
	Cyclopentanol	81 (0.089)	T	6 (0.006)	T	6 (0.006)	4 (0.04)	—	—	—
	Cyclopentylamine	52 (0.05)	24 (0.05)	15 (0.011)	T	T	T	10 (0.011)	T	T
2	Cyclopentanone	—	T	T	T	—	—	—	—	—
	Cyclopentanol	16 (0.017	T	—	T	—	T	—	—	—
	Cyclopentylamine	12 (0.013)	T	28 (0.03)	T	T	T	10 (0.11)	T	T
3	Cyclopentanone	T	11 (0.012)	T	8 (0.009)	—	—	T	T	—
	Cyclopentanol	T	6 (0.007)	—	T	—	T	T	T	—
	Cyclopentylamine	39 (0.042)	42 (0.046)	25 (0.027)	—	T	T	T	T	T
4	Cyclopentanone	T	T	T	4 (0.004)	T	T	T	—	—
	Cyclopentanol	T	T	1 (0.001)	T	26 (0.028)	—	—	—	—
	Cyclopentylamine	56 (0.061)	21 (0.023)	14 (0.015)	—	38 (0.042)	13 (0.014)	17 (0.018)	T	T
5	Cyclopentanone	—	T	—	5 (0.006)	T	—	—	—	—
	Cyclopentanol	—	—	16 (0.017)	48 (0.052)	—	48 (0.052)	—	—	—

[a] Days are numbered consecutively. [b] Amount excreted (micrograms) and, in parentheses, percent conversion. [c] Indicates that no metabolites were observed. [d] The conversion was too low to be measurable.

of sulfamate on Days 6 and 7 but were again administered the compound on Days 8 and 9. No increase in the level of metabolites was observed, and in all cases the levels dropped below those found for the first 3 days.

Cyclopentylamine was the principal metabolite excreted by the five rats. The average conversion to the amine for the results given in Table III is 0.027%. Furthermore, Rat 5 excreted cyclopentylamine regularly for 6 days before the level finally dropped. The other metabolites, cyclopentanone and cyclopentanol, were excreted in an irregular pattern for the five animals. The average conversion of cyclopentylsulfamate in rats to the ketone and alcohol was 0.008 and 0.026%, respectively. These *in vivo* studies will be continued with other sweet sulfamates.

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