Metabolic Studies with Nonnutritive Sweeteners Cyclooctylsulfamate and 4-Methylcyclohexylsulfamate

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Abstract □ The nonnutritive sweeteners cyclooctylsulfamate and 4-methylcyclohexylsulfamate were fed separately to female Wistar albino rats, and the urine was examined for the possible metabolites cyclooctylamine, cyclooctanone, cyclooctanol, 4-methylcyclohexylamine, 4-methylcyclohexanone, and *cis*- and *trans*-4-methylcyclohexanols. The average percent conversions to cyclooctylamine, cyclooctanone, and cyclooctanol were 0.127, 0.08, and 0.092, respectively. The average percent conversions to 4-methylcyclohexylamine and 4-methylcyclohexanone were 0.007 and 0.0013, respectively. No *cis*- or *trans*-4-methylcyclohexanol metabolites were found. With cyclooctylsulfamate, 42% was recovered unchanged from the urine. Cyclooctyl- and cycloheptylsulfamates were metabolized to a greater extent than cyclopentylsulfamate, which, in turn, was metabolized to a greater extent than cyclohexylsulfamate (cyclamate) and 4-methylcyclohexylsulfamate.

Keyphrases □ Cyclooctylsulfamate—metabolism in rats □ 4-Methylcyclohexylsulfamate—metabolism in rats □ Metabolism—cyclooctylsulfamate and 4-methylcyclohexylsulfamate in rats □ Sweeteners, nonnutritive—cyclooctylsulfamate and 4-methylcyclohexylsulfamate, metabolism in rats

Previously, sodium cyclopentylsulfamate (1), cycloheptylsulfamate (2), and cyclopentylmethylsulfamate (3) were administered to rabbits and/or rats. Cyclopentylsulfamate also was administered over a prolonged period to rats (3). The purpose of these studies was to probe the effect of structural modification of the cyclamate, *i.e.*, cyclohexyl, nucleus on the stability of these compounds in the body. Metabolism to the amine and other metabolites was determined by GLC, and the percent of recovered sulfamate in the urine was determined by hydrolyzing to the corresponding amine, coupling with *p*-benzoquinone, and measuring the absorbance of the colored product.

The remaining sweet, unsubstituted, reduced ring sulfamate not studied previously is cyclooctylsulfamate. Since this compound, first prepared in 1954 (4), is of the same order of sweetness as cyclamate[1], it was included in the *in vivo* feeding experiments. 4-Methylcyclohexylsulfamate also is of comparable sweetness to cyclamate[2]; since no substituted cyclamates have been screened metabolically, it too was included. This paper describes the feeding of these two sulfamates to rats and the subsequent screening of urine for metabolites.

EXPERIMENTAL

Reagents and Chemicals—The sodium salts of cyclooctylsulfamate and 4-methylcyclohexylsulfamate were synthesized by reaction of the appropriate amines in dry chloroform with chlorosulfonic acid according to the method of Audrieth and Sveda (7). Each compound was recrystallized twice from 95% ethanol.

Anal.—Calc. for $C_8H_{16}NNaO_3S \cdot H_2O$: C, 38.86; H, 7.28; N, 5.66. Found: C, 38.72; H, 7.86; N, 5.43. Calc. for $C_7H_{14}NNaO_3S$: C, 39.06; H, 6.5; N, 6.5. Found: C, 39.02; H, 6.46; N, 6.74.

[1] The order of relative sweetness is cyclamate > cycloheptyl > cyclooctyl > cyclopentyl (5, 6).

[2] Unpublished results.

Table I—Percent Recovery of Cyclooctylamine, Cyclooctanone, and Cyclooctanol from Urine

Amine		Ketone		Alcohol	
μg	%	μg	%	μg	%
90.2	80.20	199.9	78.05	97.4	80.84
150.4	85.40	299.8	78.03	162.3	85.82
210.6	88.20	399.8	72.70	227.2	95.94
300.9	97.68	499.8	87.63	324.6	87.6
601.9	98.67	599.7	98.03	649.3	97.92
902.8	79.74	—	—	974.0	92.64
Mean ± *SE*	88.28 ± 6.59		82.88 ± 7.94		90.12 ± 5.37

Cyclooctylamine[3] and dichloromethane[4] were redistilled. Cyclooctanone[5], cyclooctanol[5], 4-methylcyclohexylamine[6], 4-methylcyclohexanone[6], *cis*- and *trans*-4-methylcyclohexanols[6], *n*-dodecane[7], sulfosalicylic acid[8], chloroform, ethanol, and dioxane (reagent grade) were used as obtained. *p*-Benzoquinone[4,7] was sublimed before use.

Feeding Experiments—Female Wistar albino rats, ~300 g, were kept on solid food and water in special rat metabolism cages[9]. Prior to sulfamate feeding, the urine of each rat was collected and monitored for metabolites by GLC. The samples of sodium cyclooctylsulfamate and sodium 4-methylcyclohexylsulfamate to be used for feeding were tested for occluded amines by dissolving 20 mg in water, extracting with dichloromethane, concentrating the extracts to dryness, and resuspending the residues in dichloromethane containing the internal standard, *n*-dodecane. No occluded amine was observed when these samples were chromatographed.

Sodium cyclooctylsulfamate (1450 mg/kg) was administered orally in 25–30 ml of water in calibrated drinking bottles. One rat refused to take the solution until it was diluted twice. Sodium 4-methylcyclohexylsulfamate (960 mg/kg) was administered orally in 20 ml of water.

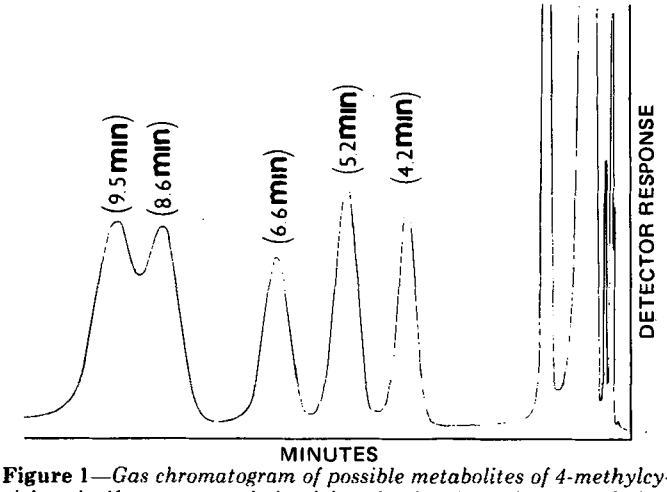

Figure 1—*Gas chromatogram of possible metabolites of 4-methylcyclohexylsulfamate: 4-methylcyclohexylamine (4.2 min), 4-methylcyclohexanone (6.6 min), and* cis- *(8.6 min) and* trans- *(9.5 min) 4-methylcyclohexanols. The internal standard was* n-*dodecane (5.2 min).*

[3] Aldrich Chemical Co.
[4] May & Baker.
[5] Ralph Emanuel.
[6] Fluka.
[7] British Drug Houses.
[8] May & Baker, AnalaR grade.
[9] NKP, Kent, England.

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Table II—Percent Recovery of 4-Methylcyclohexylamine, 4-Methylcyclohexanone, and *cis*- and *trans*-4-Methylcyclohexanols

Amine		Ketone		*cis*-Alcohol		*trans*-Alcohol	
μg	%	μg	%	μg	%	μg	%
0.15	106.7	0.07	105.714	0.03	93.33	0.100	102.000
0.15	108.0	0.5	96.00	0.04	100.00	0.250	91.200
0.2	84.0	0.5	91.50	0.20	96.000	0.912	98.710
0.25	92.0	1.097	101.730	0.917	94.220	—	—
0.44	97.7	1.83	83.680	0.917	100.109	2.740	94.525
0.44	100.0	6.396	92.250	2.75	102.109	6.380	112.850
6.09	111.475	10.97	107.468	6.42	112.149	10.942	98.702
9.57	97.806	10.97	113.843	8.25	99.49	10.942	96.219
13.92	100.862	—	—	11.00	99.91	8.210	94.714
17.4	100.144	—	—	11.00	99.873	8.210	100.868
30.45	100.493	—	—	16.51	101.756	16.410	98.964
Mean ± *SE*	99.925 ± 5.1		99.02 ± 8.16		99.9 ± 3.0		98.875 ± 3.8

Table III—Metabolism of Sodium Cyclooctylsulfamate in Rats

Animal	Parent Drug[a]		Cyclooctanol[b]		Cyclooctanone[b]		Cyclooctylamine[b]		Total Metabolites
	mg	%	mg	%	mg	%	mg	%	
1	256.1	51.2	0.100	0.035	0.082	0.029	0.115	0.041	0.105
2	333.0	66.6	None	—	None	—	None	—	—
3	136.8	27.2	0.487	0.174	0.410	0.147	0.623	0.224	0.545
4	128.3	25.6	0.721	0.250	0.598	0.215	1.026	0.37	0.835
5	35.2	17.6	None	—	None	—	None	—	—
Mean ± *SE*	213.6 ± 81.17	42.6 ± 16.2	0.261 ± 0.169	0.092 ± 0.075	0.22 ± 0.141	0.08 ± 0.051	0.353 ± 0.116	0.127 ± 0.085	0.297 ± 0.196

[a] Determined using visible spectrophotometry. [b] Determined by GLC.

Table IV—Metabolites of Sodium 4-Methylcyclohexylsulfamate[a] in Rats

Animal	4-Methylcyclohexylamine[b]		4-Methylcyclohexanone[b]	
	mg	%	mg	%
1[c]	0.009	0.006	None	—
2	0.005	0.003	None	—
3[c]	0.009	0.006	None	—
4	0.003	0.004	None	—
5[c]	0.008	0.005	0.0012	0.008
6	0.029	0.019	None	—
Mean ± *SE*	0.0106 ± 0.007	0.0073 ± 0.0045	0.0002	0.0013

[a] Rat 4 received only approximately half the amount of sulfamate, *i.e.*, 500 mg/kg. [b] Determined by GLC. [c] Urine was collected for 5 days after feeding, because these rats had excreted small volumes of urine after 3 days.

Table V—Metabolism of Sulfamates in Rats

Cyclic Sulfamate	Percent Metabolites			Reference
	Amine	Ketone	Alcohol	
Pentyl	0.057	0.016	0.008	1
Pentylmethyl	0.007	—	0.07	3
Hexyl	0.03	0.002	0.003	11
Hexyl	0.0063	0.0004	0.0004	12
4-Methylhexyl	0.007	0.0013	—	This work
Heptyl	0.051	0.009	0.003	2
Octyl	0.127	0.08	0.092	This work

Urine was collected for 3 days (for 5 days in some cases) after feeding and was refrigerated as described previously (1). The stability of sodium cyclooctylsulfamate in urine was checked as described previously (1).

GLC and Visible Spectrophotometric Analysis—Cyclooctylamine, cyclooctanone, and cyclooctanol were determined in urine by a reported GLC method (8). The percent sodium cyclooctylsulfamate in urine was determined by coupling, with *p*-benzoquinone, the amine produced on hydrolysis (9). Table I gives the percent recovery from urine of three metabolites of cyclooctylsulfamate.

Four metabolites of 4-methylcyclohexylsulfamate could be determined by GLC from a single injection. The details of the column and the general method were described previously (8, 10). The column temperature was 120°, and the gas flow rates were: nitrogen (carrier), 60 ml/min; hydrogen, 80 ml/min; and overall, 500 ml/min. Under these conditions, the retention times of 4-methylcyclohexylamine, *n*-dodecane, 4-methylcyclohexanone, and *cis*- and *trans*-4-methylcyclohexanols were 4.2, 5.2, 6.6, 8.6, and 9.5, min, respectively (Fig. 1). Table II gives the percent recoveries from urine of these metabolites.

RESULTS AND DISCUSSION

To test the efficiency of the extraction from urine, the percent recovery of varying amounts of the amine, the ketone, and the alcohol was determined by GLC (Tables I and II). The results from the analysis of rat urine for unchanged cyclooctylsulfamate and various metabolites are given in Table III. The average percentage of unchanged cyclooctylsulfamate in rat urine was 42%. Three of the five rats excreted metabolites of cyclooctylsulfamate, and the average percent conversions were 0.127, 0.08, and 0.092 for the amine, the ketone, and the alcohol, respectively (Table III).

Only two metabolites were detected in the urine of rats fed 4-methylcyclohexylsulfamate (Table IV). The percent conversions to the amine and the ketone were 0.0073 and 0.0013, respectively. Only one of the six rats gave the ketone, and none excreted the alcohol. From a single injection, it was possible to determine the amine, the ketone, and the *cis*- and *trans*-alcohol compounds of 4-methylcyclohexylsulfamate (Fig. 1).

Table V lists the average percent conversions to metabolites for the various sulfamates. The most obvious point to emerge from a comparison of the data in Table V is that the conversion of cyclooctylsulfamate to metabolites was somewhat greater than the conversion of cycloheptylsulfamate or cyclopentylsulfamate to metabolites. The conversion of the latter compound into its metabolites was, in turn, greater than the conversions of cyclamate to cyclohexylamine, cyclohexanone, and cyclohexanol. Furthermore, 4-methylcyclohexylsulfamate appeared to be metabolized to about the same extent as cyclamate. Thus, cyclamate and its ring-substituted derivatives apparently are more stable to metabolic cleavage than any of their neighboring reduced ring sulfamates.

REFERENCES

(1) G. A. Benson and W. J. Spillane, *J. Pharm Sci.*, **65**, 1841 (1976).

(2) *Ibid.*, **66**, 881 (1977).

(3) *Ibid.*, **67**, 226 (1978).

(4) F. F. Blicke, H. E. Millson, Jr., and N. J. Doorenbos, *J. Am. Chem. Soc.*, **76**, 2498 (1954).

(5) B. Unterhalt and L. Böschemeyer, *Z. Lebensm.-Unters.-Forsch.*, **145**, 93 (1971).

(6) G. A. Benson and W. J. Spillane, *J. Med. Chem.*, **19**, 869 (1976).

(7) L. F. Audrieth and M. Sveda, *J. Org. Chem.*, **9**, 89 (1944).
(8) G. A. Benson and W. J. Spillane, *J. Chromatogr.*, **136**, 318 (1977).
(9) G. A. Benson and W. J. Spillane, *Anal. Chem.*, **48**, 2149 (1976).
(10) P. H. Derse and R. J. Daun, *J. Assoc. Offic. Anal. Chem.*, **49**, 1090 (1966).
(11) H. Ichibagase, S. Kojima, K. Inoue, and A. Suenaga, *Chem. Pharm. Bull.*, **20**, 175 (1972).
(12) A. Suenaga, S. Kojima, and H. Ichibagase, *ibid.*, **20**, 1357 (1972).

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GLC Determination of Purity of Schiff Bases Bakrine and Saddamine

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Abstract □ GLC methods were developed for the investigation of impurities in bakrine and saddamine. The method used for bakrine was unsuitable for saddamine since two possible saddamine impurities, benzylamine and salicylaldehyde, reacted very readily in solution to form saddamine, thus giving a false low value for the original concentration of these impurities. The method devised for saddamine involved silylation, which greatly reduced the possibility of saddamine formation from its precursors and also enabled the detection of another possible impurity, salicylic acid. The method described has an obvious application to the determination of other Schiff bases.

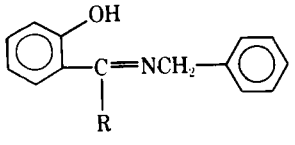
Keyphrases □ Bakrine and impurities—GLC analysis in prepared samples □ Saddamine and impurities—GLC analysis in prepared samples □ Anti-inflammatory agents, potential—bakrine and saddamine, GLC analysis in prepared samples

Bakrine, *N*-[1-(*o*-hydroxyphenyl)ethylidine]benzylamine (I), and saddamine, *N*-[*o*-hydroxyphenylmethylidine]benzylamine (II), are novel Schiff bases (1) with promising anti-inflammatory properties (2). These compounds are currently undergoing clinical trials in Iraq.

During the development of a method for the determination of the purity of bakrine and saddamine samples, spectrophotometric and TLC procedures were shown to lack specificity; GLC was the method of choice because of its speed, sensitivity, and specificity. However, although a solution of bakrine containing expected impurities could be analyzed satisfactorily by direct injection onto the GLC column, a similar procedure for saddamine gave inconsistent results. Therefore, the possibility that saddamine could be reformed from any salicylaldehyde and benzylamine present as impurities in the final product was investigated.

EXPERIMENTAL

Reagents and Materials—Bakrine[1], saddamine[1], and bis(trimeth-



I: R = CH_3
II: R = H

[1] Maybridge Chemical Co., Cornwall, England.

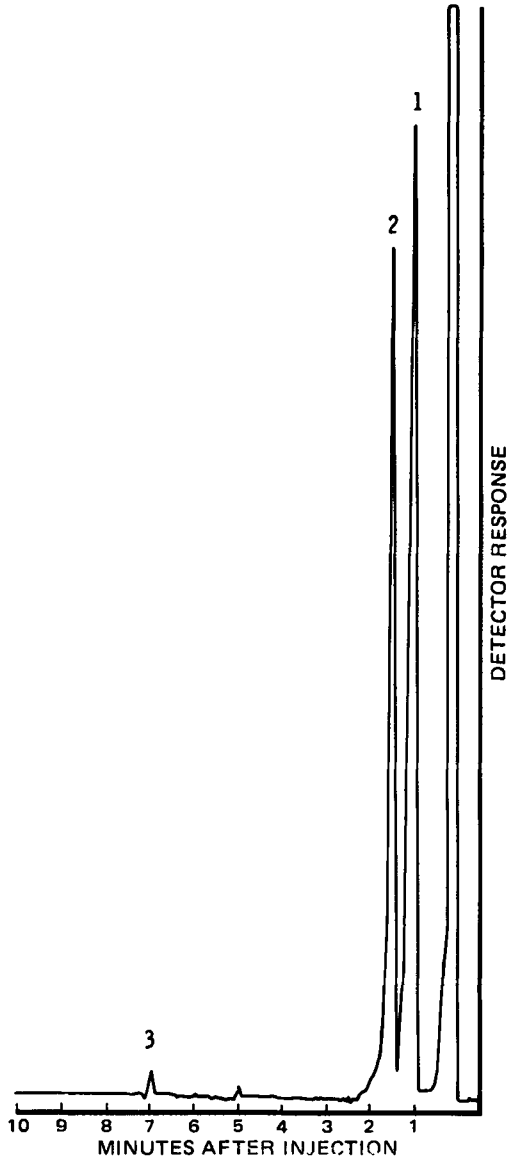


Figure 1—*Gas chromatogram of benzylamine (peak 1) and 2-hydroxyacetophenone (peak 2) in 1,2-dichloroethane. Very little bakrine (peak 3) was formed.*

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