Metabolic Studies with the Nonnutritive Sweetener Cycloheptylsulfamate

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Abstract □ The nonnutritive sweetener cycloheptylsulfamate was administered orally to rabbits and rats. The urine of each species was separately collected for 3 days and examined for the metabolites cycloheptylamine, cycloheptanone, and cycloheptanol and for cycloheptylsulfamate. A previously tested GLC method was adapted for the determination of the metabolites. Cycloheptylsulfamate was assayed by hydrolysis and subsequent measurement of the absorbance of the product formed (λ_{max} = 489 nm) by the liberated amine with *p*-benzoquinone. The conversions to the metabolites were 0.276, 0.390, and 0.170%, respectively, in rabbits and 0.064, 0.022, and 0.017%, respectively, in rats.

Keyphrases □ Cycloheptylsulfamate—metabolism, rabbits and rats □ Metabolism—cycloheptylsulfamate, rabbits and rats □ Excretion, urinary—cycloheptylsulfamate and metabolites, rabbits and rats □ Sweeteners—cycloheptylsulfamate, metabolism, rabbits and rats

Cyclamates (sodium and calcium salts of cyclohexylsulfamic acid) have been the subject of considerable investigation over the past few years. This research involved metabolic studies in humans (1–5) and other species (6–12) to determine the metabolites and toxicity of the cyclamates. Further work involved the search for the metabolic site of cyclamate breakdown and the possible microorganisms involved (13–19).

It also seemed worthwhile to evaluate other sulfamates that have a reduced ring and are sweet. Since enzymatic action has been implicated in the breakdown of cyclamate, other sweet sulfamates might be resistant to enzyme action and not be metabolized. Metabolic experiments involving cyclopentylsulfamate were carried out previously (20). Since cycloheptylsulfamate is also sweet (21, 22), in fact, almost as sweet as cyclamate sodium (23), it was tested in rabbits and rats. The results of these metabolic experiments are presented in this paper.

EXPERIMENTAL

Reagents and Chemicals—Sodium cycloheptylsulfamate was synthesized by the reaction of cycloheptylamine in dry chloroform with chlorosulfonic acid (24) and recrystallized twice from ethanol. It was administered orally as an approximately 1% aqueous solution to rats and as an approximately 1.7% aqueous solution to rabbits at doses of 1250 and 349 mg/kg, respectively.

Anal.—Calc. for $C_7H_{14}NNaO_3S$: C, 39.07; H, 6.51; N, 6.51. Found: C, 38.89; H, 6.43; N, 6.56.

Cycloheptylamide[1], cycloheptanone[1], cycloheptanol[1], and dichloromethane[2] were redistilled before use. *p*-Benzoquinone was sublimed. *n*-Dodecane[3], sulfosalicylic acid[2] (Analar), chloroform, ethanol, and 1,4-dioxane (reagent grade) were used as obtained.

Metabolism Experiments—Female New Zealand White rabbits, 3.1–3.6 kg, were kept on solid food and water in metabolism cages[4]. Female Wistar albino rats, approximately 400 g, were kept on solid food and water in smaller metabolism cages[5].

GLC and Visible Spectrophotometry—Cycloheptylamine, cycloheptanol, and cycloheptanone excreted in the urine of both rats and rabbits receiving cycloheptylsulfamate were determined by the GLC method described previously (20) except for the following changes. The column temperature was 130° and the gas flow rates were: nitrogen (carrier), 75 ml/min; hydrogen, 60 ml/min; and overall, 500 ml/min. Under these conditions, the retention times of *n*-dodecane, cycloheptylamine, cycloheptanone, and cycloheptanol were 4.6, 6.3, 8.4, and 13 min, respectively (Fig. 1).

For the purpose of estimating the percent recovery, varying amounts of amine, ketone, and alcohol were determined (Table I). The unmetabolized cycloheptylsulfamate excreted in the urine was determined by visible spectrophotometry (20). Samples of control urine of both species were monitored using both detection methods.

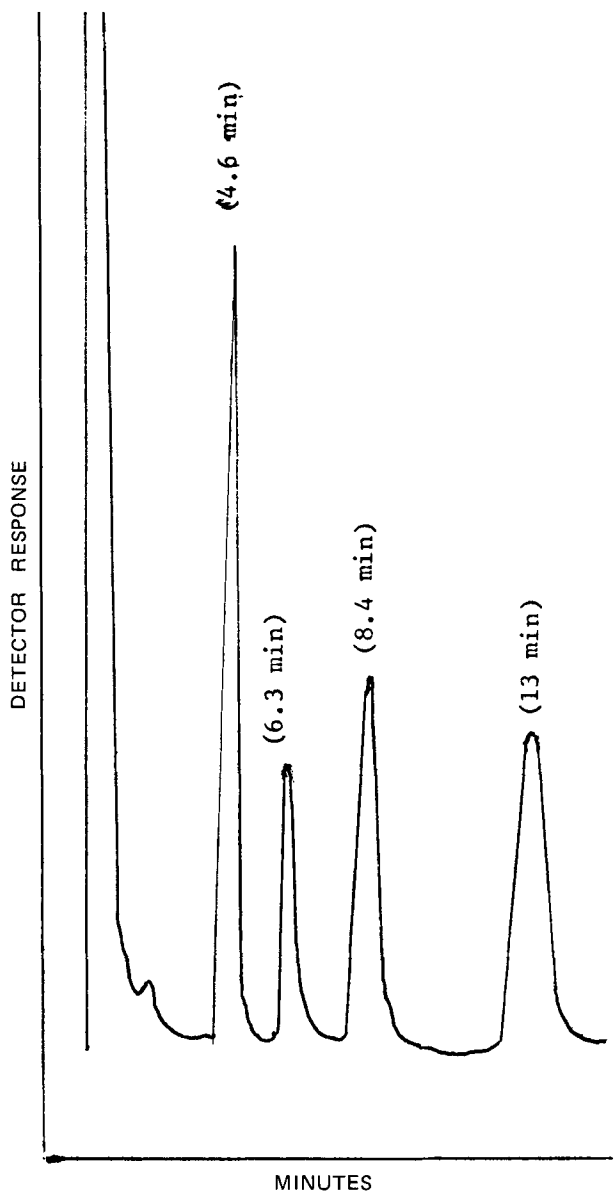

Figure 1—*Gas chromatogram of the urine metabolites of cycloheptylsulfamate (left to right):* n-*dodecane, cycloheptylamine, cycloheptanone, and cycloheptanol.*

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

Table I—Percent Recovery of Cycloheptylamine, Cycloheptanone, and Cycloheptanol from Urine

Amine		Ketone		Alcohol	
μg	%	μg	%	μg	%
60.9	92.69	67.8	90.41	67.5	96.17
121.3	90.90	135.6	98.01	135.0	96.03
151.3	101.33	175.3	87.84	166.2	93.86
225.0	105.87	254.3	101.08	249.0	106.49
303.6	94.90	338.7	97.77	333.0	111.11
456.1	109.25	508.1	115.55	499.8	115.55
874.8	104.2	950.7	99.97	947.8	—[a]
874.8	99.4	950.7	96.97	947.8	97.6
1020.6	103.4	1109.2	98.44	1105.8	84.5
1020.6	98.7	1109.2	99.52	1105.8	—[a]
1166.4	94.6	1267.6	95.42	1263.2	94.9
1166.4	94.6	1267.6	104.91	1263.2	—[a]
Mean ± *SE*	99.15 ± 4.75		98.82 ± 4.48		99.57 ± 7.64

[a] Not determined.

Table II—Metabolism of Sodium Cycloheptylsulfamate in Rabbits

	Parent Drug[a]		Cycloheptanol[b]		Cycloheptanone[b]		Cycloheptylamine[b]		Total Metabolites,
Animal	mg	%	mg	%	mg	%	mg	%	%
1	1141.5	92.72	0.553	0.084	0.779	0.121	2.214	0.342	0.547
2	671.6	54.55	0.186	0.028	0.542	0.084	0.341	0.052	0.167
3	395.4	34.26	0.350	0.057	0.812	0.135	0.225	0.037	0.229
4	1167.3	89.44	4.160	0.599	9.664	1.418	6.016	0.875	2.887
5	656.2	60.92	0.469	0.082	1.105	0.196	0.435	0.076	0.354
Mean ± *SE*	806.4 ± 278.4	66.37 ± 19.76	1.143 ± 1.206	0.170 ± 0.171	2.58 ± 2.95	0.390 ± 0.415	0.289 ± 0.277	0.276 ± 0.265	0.836 ± 0.819

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

Table III—Metabolism of Sodium Cycloheptylsulfamate in Rats

	Parent Drug[a]		Cycloheptanol[b]		Cycloheptanone[b]		Cycloheptylamine[b]		Total Metabolites,
Animal	mg	%	mg	%	mg	%	mg	%	%
1	56.38	11.27	None	—	None	—	0.034	0.012	0.012
2	43.58	8.71	None	—	None	—	Trace	—	—
3	160.12	32.02	0.046	0.017	0.073	0.028	0.519	0.197	0.242
4	322.07	64.41	None	—	Trace	—	0.066	0.025	0.025
5	294.60	58.92	None	—	0.042	0.016	0.061	0.023	0.039
Mean ± *SE*	175.35 ± 106.38	35.06 ± 21.27	0.046	0.017	0.057 ± 0.015	0.022 ± 0.006	0.136 ± 0.126	0.064 ± 0.06	0.079 ± 0.07

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

RESULTS AND DISCUSSION

The results from the analysis of the urine of rabbits and rats for unchanged cycloheptylsulfamate and various metabolites are given in Tables II and III, respectively. The average percentage for unchanged cycloheptylsulfamate excreted in the urine for rats was 35%; for rabbits, it was 66%. In metabolic studies with cyclopentylsulfamate (20), rats excreted 15% unchanged cyclopentylsulfamate in the urine and rabbits excreted 56%. U-^{14}C-Cyclamate sodium also was extensively excreted in the urine by rabbits, whereas the average level of excretion for rats was 40% in the urine (2). In rats that had received a level of cyclamate similar to that used in the present study, about 17% cyclamate was excreted in the urine and about 70% in the feces (25). Thus, the main excretory pathways for these administered sulfamates apparently are the urine for rabbits and the feces for rats.

The average conversion of cycloheptylsulfamate by rabbits to cycloheptylamine, cycloheptanone, and cycloheptanol was 0.276, 0.390, and 0.170%, respectively (Table II). Comparison of these results with those for cyclopentylsulfamate (20) showed that they were slightly higher. The conversion results of Ichibagase *et al.* (14) and Suenaga *et al.* (16) for cyclamate also were lower than the present results for cycloheptylsulfamate in rabbits. Renwick and Williams (2) also found a lower conversion in rabbits for cyclamate.

In the five rats studied, the level of cycloheptylamine found (Table III) was similar to the level of cyclopentylamine found (20) and higher than the level of cyclohexylamine obtained (14, 16) in metabolic studies with cyclopentylsulfamate and cyclamate, respectively. Only one rat excreted cycloheptanol in urine, and only two rats excreted cycloheptanone (Table III).

It appears that cycloheptyl- and cyclopentylsulfamates are cleaved to the same extent in rats, but in rabbits the conversion of cycloheptylsulfamate is higher. Metabolic studies with other sweet sulfamates are necessary to assess the breakdown pattern, if any, and the effect of prolonged administration on metabolite levels.

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Cytotoxic Agents from *Michelia champaca* and *Talauma ovata*: Parthenolide and Costunolide

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Abstract □ The ethanol extract of *Michelia champaca* and the petroleum ether extract of *Talauma ovata* showed activity toward the human epidermoid carcinoma of the nasopharynx test system. The active constituents were sesquiterpene lactones, identified as parthenolide ($C_{15}H_{20}O_3$) and costunolide ($C_{15}H_{20}O_2$). Their identities were proven by elemental analyses, PMR, IR, and mass spectral data, melting-point and mixed melting-point determinations, and comparisons with authentic samples and spectra.

Keyphrases □ Parthenolide—isolated from ethanol extract of bark of *Michelia champaca*, cytotoxic activity evaluated □ Costunolide—isolated from ethanol extract of bark of *Michelia champaca* and petroleum ether extract of roots of *Talauma ovata*, cytotoxic activity evaluated □ *Michelia champaca*—parthenolide and costunolide isolated from ethanol extract of bark, cytotoxic activity evaluated □ *Talauma ovata*—costunolide isolated from petroleum ether extract of roots, cytotoxic activity evaluated □ Cytotoxic activity—plant constituents parthenolide and costunolide evaluated

As a result of the continuing search for plants having tumor inhibitory constituents, it was found that the ethanol extract of the stem bark of *Michelia champaca* L.[1] (Magnoliaceae) and the petroleum ether extract of the roots of *Talauma ovata* A. St. Hil.[2] (Magnoliaceae) showed cytotoxic activity toward the human epidermoid carcinoma of the nasopharynx test system[3] (KB).

DISCUSSION

The cytotoxic agents were found to be parthenolide (from *M. champaca*) and costunolide (from *M. champaca* and *T. ovata*). Parthenolide previously was isolated from several Magnoliaceae plants, including *Magnolia grandiflora* (1) and *M. champaca* (2), and also from *Chrysanthemum parthenium* (Asteraceae) (3). Costunolide has been found mainly in Asteraceae plants, *e.g.*, the "costus root," *Saussurea lappa* (4). Identification of parthenolide and costunolide was achieved by IR, PMR, and mass spectral data and by comparison with authentic spectra and samples.

Parthenolide demonstrated an activity of 2.3 μg/ml, and costunolide showed an activity of 2.8 μg/ml. Activity in the KB test system is defined as $ED_{50} \leq 20$ μg/ml (5).

EXPERIMENTAL[4]

Isolation Procedure—The ground stem of *M. champaca* (7 kg) was extracted exhaustively with ethanol in a Lloyd-type extractor. The air-dried residue was partitioned between chloroform and water. The air-dried chloroform phase (11.8 g) was extracted twice with benzene (500 ml), and the benzene-soluble fraction (8 g) was subjected to silica gel 60 (200 g) column (4 × 46 cm) chromatography. The column was eluted with benzene (1000 ml), chloroform (1000 ml), chloroform–methanol [(95:5) six fractions, 500 ml each], acetone, and methanol.

The dried and ground roots of *T. ovata* (1.0 kg) were extracted by maceration with 3 liters of petroleum ether (bp 30–60°) overnight. After filtration and removal of the solvent from the extract, crude crystals of costunolide formed.

Isolation of Parthenolide—Parthenolide was isolated from the first chloroform–methanol (95:5) fraction by preparative TLC using benzene–dichloromethane–ethyl acetate (3:6:1). Crystallization from ether resulted in colorless plates, mp 115° [lit. (2) mp 115°]. The PMR spectrum was identical to the previously reported spectrum (2). The IR

[1] The plant was collected in India in March 1974. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, Md. A reference specimen was deposited in that herbarium.
[2] The plant was collected in Brazil in June 1974. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, Md. A reference specimen was deposited in that herbarium.
[3] Of the Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md.
[4] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrophotometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.