# Determination of the Nonnutritive Sweetener Sodium Cyclopentylsulfamate and Three of Its Metabolites, Cyclopentylamine, Cyclopentanone, and Cyclopentanol, in Urine of Rats and Rabbits

G. ANTHONY BENSON and WILLIAM J. SPILLANE ˣ

**Abstract** □ The nonnutritive sweetener sodium cyclopentylsulfamate was fed to Wistar albino rats and New Zealand White rabbits. Urine was collected for 3 days after feeding, combined, and examined for the metabolites cyclopentylamine, cyclopentanone, and cyclopentanol and for sodium cyclopentylsulfamate. A GLC method allowing the simultaneous analysis (with convenient retention times) for the amine, ketone, and alcoholic metabolites was developed. Sodium cyclopentylsulfamate was assayed by hydrolysis in acidified dioxane–water and subsequent measurement of the absorbance of the product formed ($\lambda_{max}$ = 490 nm) by the liberated amine with *p*-benzoquinone. The average conversion to cyclopentylamine, cyclopentanone, and cyclopentanol was 0.103, 0.171, and 0.054% in the rabbit and 0.057, 0.016, and 0.008% in the rat, respectively.

**Keyphrases** □ Sodium cyclopentylsulfamate and metabolites—GLC analysis, rat and rabbit urine □ GLC—analysis, sodium cyclopentylsulfamate and metabolites, rat and rabbit urine □ Sweeteners—sodium cyclopentylsulfamate, GLC analysis, rat and rabbit urine □ Sulfamates, substituted—sodium cyclopentylsulfamate, GLC analysis, rat and rabbit urine

Despite the ban (since 1970) on the use of cyclamates, *i.e.*, salts of cyclohexylsulfamic acid, as sweetening additives in foods and soft drinks, considerable interest in these compounds exists. Most recent work has concentrated on the toxicity of cyclamates in humans (1–3) and other species (4–9) and on the metabolic sites of cyclamate breakdown in humans and other animals and the enzymes involved (10–14).

Because of the ban and because no toxicological studies have been reported on other sulfamates, it seemed worthwhile to evaluate some sulfamates that are structurally related to the banned cyclamates. This approach appeared to hold promise because: (*a*) many other sulfamates, especially those retaining the reduced ring, are sweet (15–17); (*b*) certain of these sulfamates, *e.g.*, sodium cyclopentylsulfamate, might be less easily metabolized to primary amines than cyclamates were to cyclohexylamine (18) and would, therefore, lack carcinogenic activity; and (*c*) such compounds might be commercially viable, since routes from the less expensive alcohols or ketones to the amines (which are then sulfamated) can be readily envisaged (16, 17, 19). To determine the level of metabolism to amines and also to check for other metabolites, *in vivo* feeding experiments with sodium cyclopentylsulfamate were conducted.

## EXPERIMENTAL

**Reagents and Chemicals**—Sodium cyclopentylsulfamate was synthesized by reaction of the amine in dry chloroform with chlorosulfonic acid according to the method of Audrieth and Sveda (15) and recrystallized (twice) from ethanol.

*Anal.*—Calc. for $C_5H_{10}NNaO_3S$: C, 32.1; H, 5.3; N, 7.4. Found: C, 32.2; H, 5.5; N, 7.4.

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

**Feeding Experiments**—New Zealand White rabbits, female, approximately 2.7 kg, were kept on solid food and water in metabolism cages[5]. Wistar albino rats, female, approximately 300 g, were kept on solid food and water in smaller metabolism cages[6]. Prior to feeding, the urine of both species was collected and monitored using the two detection methods. Sodium cyclopentylsulfamate (370 mg/kg for rabbits, 1330 mg/kg for rats) was administered orally in aqueous solution.

For each species, urine was collected for 3 days after feeding. The urine samples collected from each animal were refrigerated daily. At the end of the 3rd day, the three samples were combined and analyzed as described. The combined samples from each animal were never refrigerated for more than 1 day prior to analysis. The stability of sodium cyclopentylsulfamate in urine was tested by refrigeration of urine containing sodium cyclopentylsulfamate (2.5, 5, and 7.5 mg/ml) for 4–5 days. No amine, ketone, or alcohol could be detected in any solution using the described method.

**GLC Analysis and Standardization**—Cyclopentylamine, cyclopentanone, and cyclopentanol were determined on a gas chroma-

Table I—Percent Recovery of Cyclopentylamine, Cyclopentanone, and Cyclopentanol from Urine

| Amine | | Ketone | | Alcohol | |
|---|---|---|---|---|---|
| μg | % | μg | % | μg | % |
| 860 | 104.89 | 940 | 100.31 | 940 | 96.11 |
| 860 | 96.28 | 940 | 95.26 | 940 | 89.46 |
| 1000 | 104.70 | 1100 | 98.43 | 1100 | 96.46 |
| 1000 | 107.18 | 1100 | 100.86 | 1100 | 95.38 |
| 1140 | 96.80 | 1260 | 99.85 | 1260 | 97.21 |
| 1140 | 99.41 | 1260 | 100.86 | 1260 | — |
| 1290 | 96.27 | 1420 | 97.26 | 1420 | 88.60 |
| 1290 | 100.93 | 1420 | 98.73 | 1420 | 91.13 |
| Mean ± *SE* | 100.80 ± 3.61 | | 98.94 ± 1.52 | | 93.47 ± 2.78 |

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

Table II—Metabolism of Sodium Cyclopentylsulfamate in Rabbits

| Animal Number | Recovered Sulfamate (Visible Spectrophotometry) | | Cyclopentanol (GLC) | | Cyclopentanone (GLC) | | Cyclopentylamine (GLC) | | Total Metabolites, % |
|---|---|---|---|---|---|---|---|---|---|
| | mg | % | mg | % | mg | % | mg | % | |
| 1 | 592.3 | 59.20 | 1.096 | 0.237 | 0.454 | 0.100 | 0.55 | 0.121 | 0.458 |
| 2 | 500.8 | 50.08 | 0.100 | 0.022 | 0.716 | 0.159 | 0.62 | 0.136 | 0.317 |
| 3 | 778.2 | 77.82 | Trace | — | 1.458 | 0.324 | 0.48 | 0.105 | 0.429 |
| 4 | 439.8 | 43.98 | None | — | 0.756 | 0.168 | 0.316 | 0.069 | 0.237 |
| 5 | 499.8 | 49.98 | 0.055 | 0.012 | 0.468 | 0.104 | 0.394 | 0.086 | 0.202 |
| Mean ± *SE* | 562.2 ± 98.5 | 56.2 ± 9.83 | 0.25 ± 0.24 | 0.054 ± 0.051 | 0.77 ± 0.014 | 0.171 ± 0.061 | 0.472 ± 0.09 | 0.103 ± 0.02 | 0.328 ± 0.091 |

Table III—Metabolism of Sodium Cyclopentylsulfamate in Rats

| Animal Number | Recovered Sulfamate (Visible Spectrophotometry) | | Cyclopentanol (GLC) | | Cyclopentanone (GLC) | | Cyclopentylamine (GLC) | | Total Metabolites, % |
|---|---|---|---|---|---|---|---|---|---|
| | mg | % | mg | % | mg | % | mg | % | |
| 1 | 20.34 | 5.09 | None | — | 0.031 | 0.017 | 0.083 | 0.045 | 0.062 |
| 2 | 199.70 | 49.96 | 0.0286 | 0.015 | 0.017 | 0.009 | 0.173 | 0.095 | 0.119 |
| 3 | 26.90 | 6.72 | 0.0329 | 0.017 | 0.050 | 0.028 | 0.243 | 0.134 | 0.179 |
| 4 | 60.90 | 15.20 | 0.0087 | 0.004 | 0.018 | 0.010 | 0.020 | 0.01 | 0.024 |
| 5 | 22.24 | 5.60 | 0.025 | 0.012 | 0.027 | 0.015 | 0.05 | 0.02 | 0.047 |
| 6 | 34.00 | 8.50 | 0.0085 | 0.004 | 0.040 | 0.022 | 0.075 | 0.04 | 0.067 |
| Mean ± *SE* | 60.68 ± 46.30 | 15.17 ± 11.59 | 0.017 ± 0.008 | 0.008 ± 0.004 | 0.030 ± 0.009 | 0.016 ± 0.005 | 0.107 ± 0.067 | 0.057 ± 0.037 | 0.083 ± 0.044 |

tograph[7] with dual flame-ionization detectors. The column dimensions were 1.5 m × 0.63 cm (5 ft × 0.25 in.) (glass). The packing was 20% Dowfax 9N9 with 2.5% NaOH on 60–80-mesh Diatomite C-AW. The column temperature was 120°, and the gas flow rates were: nitrogen (carrier), 30 ml/min; hydrogen, 30 ml/min; and overall, 500 ml/min. The attenuation was $1 \times 10^2$, and the injector sample was 0.5 μl. Under these conditions, the retention times of cyclopentylamine, cyclopentanone, cyclopentanol, and *n*-dodecane were 4.2, 6.2, 9.2, and 12.2 min, respectively (Fig. 1).

A standard curve was prepared by injecting 1-μl portions of amine, ketone, and alcohol into 5-ml samples of urine. Then 5 ml of 20% sulfosalicylic acid (w/v) was added, and the pH was adjusted to 12–13 with 1 ml of 10 *N* NaOH. Then 2-ml samples were taken and extracted with 2 × 3 ml of dichloromethane (containing 0.04 ml of *n*-dodecane/250 ml of dichloromethane). The samples were centrifuged[8], and 2-ml portions were transferred to 5-ml volumetric flasks containing approximately 2–4 mg of anhydrous sodium sulfate. Samples of 0.5 μl were injected onto the column. A plot of peak height ratios (peak height of the amine, ketone, or alcohol/peak height of *n*-dodecane) *versus* concentration was linear over the 10–100-μg/ml range for each compound. Urine samples taken before and after feeding were similarly analyzed.

For purposes of estimating the percent recovery, varying amounts of the amine, ketone, and alcohol were determined by this procedure (Table I).

**Visible Spectrophotometry Analysis and Standardization**—Quantities from 3 to 33 mg of sodium cyclopentylsulfamate were dissolved in 1-ml samples of urine in 10-ml volumetric flasks. Then 4 ml of dioxane and 0.5 ml of 5 *M* perchloric acid were added. The flasks were placed for 3.5 hr in an oil bath at 95°, after which 2-ml samples were removed and extracted with 2 × 10 ml of chloroform. The extracts were made up to 25 ml with chloroform and transferred to 50-ml volumetric flasks. Then 10 ml of 1% benzoquinone solution was added to each flask.

The solutions were then heated at 60° in a water bath for 1.25 hr, and the absorbances were read[9] at 490 nm. A plot of absorbance *versus* concentration was linear over the 3–33-mg/ml range of sodium cyclopentylsulfamate. Urine samples taken before and after feeding were similarly analyzed. Sometimes emulsions formed during the extraction procedure, but separation of the urine and chloroform layers was achieved by centrifugation at 3000 rpm for 30 min.

## RESULTS AND DISCUSSION

In choosing analytical techniques for the determination of sodium cyclopentylsulfamate and its metabolites, established methods for cyclamates and their metabolites should be adaptable with modifications. Accordingly, for the determination of sodium cyclopentylsulfamate in urine, the sulfamate was hydrolyzed to cyclopentylamine and reacted with *p*-benzoquinone and the absorbance of the colored product was determined. This method was mentioned in a survey of the analytical chemistry of cyclamates (20) and was used previously (21–23).

Johnson *et al.* (23) examined the method critically and found that the hydrolysis to the amine, which is carried out with hydrochloric acid in the presence of hydrogen peroxide, does not produce an equivalent amount of amine. They developed a method using hydrochloric acid but involving high temperatures and pressure. Previously, it was found that cyclamates can be hydrolyzed under less forcing conditions by employing an acidified aqueous dioxane medium, and this method has been used for hydrolyzing cyclopentylsulfamate. For the complete analysis of metabolites, the GLC method of Derse and Daun (24) was adapted; the amine, ketone, and alcohol have been determined from a single injection. Previous work with this method involved separate injections and different conditions for the determination of the amine and alcohol or ketone (25).

Results of analyses of the urine of rabbits and rats for unchanged sulfamate and various metabolites are given in Tables II and III, respectively. An average of about 56% of the sulfamate, either as "unchanged" sulfamate or as metabolites, was found for rabbits (Table II), which suggests that a little less than half of the sulfamate fed is excreted in the feces. From feeding studies with $^{14}$C-cyclamate sodium

[7] Pye-Unicam 104.
[8] Griffin Christ centrifuge.
[9] Perkin-Elmer 124 double-beam spectrophotometer.

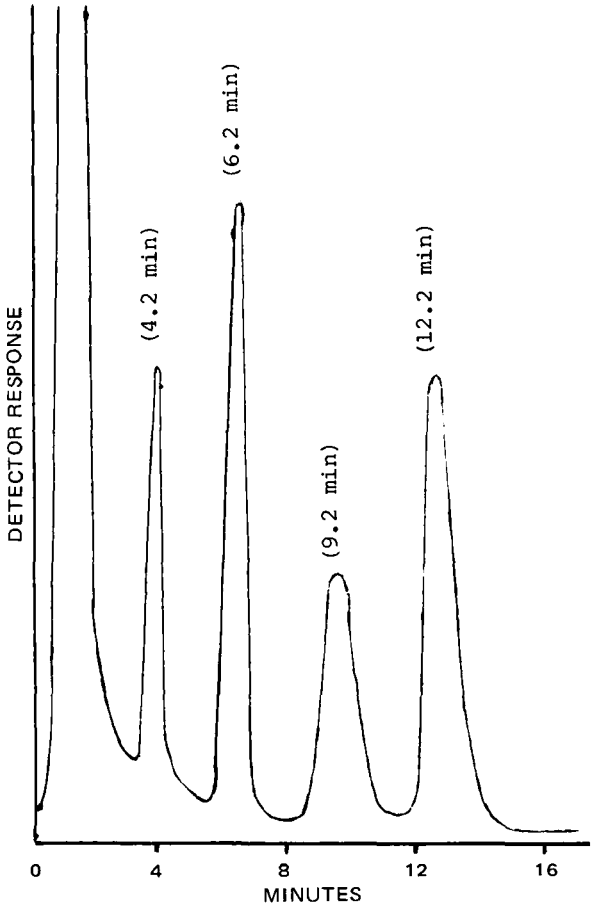


**Figure 1**—*Gas chromatogram of the urine metabolites of cyclopentylsulfamate: cyclopentylamine (4.2 min), cyclopentanone (6.2 min), and cyclopentanol (9.2 min). The internal standard was n-dodecane (12.2 min).*

in both control and pretreated (with unlabeled cyclamate) rabbits, Renwick and Williams (2) found that the radiolabel was excreted extensively in the urine and only about 6% was found in the feces. For rats, about 15% of the sulfamate was excreted in the urine (Table III); again, Renwick and Williams (2) found that for normal rats the distribution was about 40% in urine and 50% in feces. Derse and Daun (24), however, found that for rats receiving a level of sulfamate feed similar to ours, about 17% of the cyclamate was excreted in the urine and about 70% in the feces.

The conversion of sodium cyclopentylsulfamate to metabolites was calculated on a molar basis. Thus, a rabbit fed 1 g of sulfamate and excreting 0.3 mg of cyclopentylamine converted 0.066% of the sulfamate to cyclopentylamine:

$$(0.0003/85 \div 1/187)100 = 0.066\% \qquad \text{(Eq. 1)}$$

In the rabbit (Table II), the average conversion to metabolites was 0.103% for cyclopentylamine, 0.171% for cyclopentanone, and 0.054% for cyclopentanol. If these percentages are compared with those of previous studies on normal (*i.e.*, nonpretreated) rabbits that had received a single dose of cyclamate, they are found to be somewhat greater than the percentage conversion of cyclamate to cyclohexylamine, cyclohexanone, and cyclohexanol. Thus, the data of Ichibagase *et al.* (11) reveal that the average conversions to these latter three metabolites were 0.003, 0.002, and 0.003%, respectively, after feeding 200 mg/kg of cyclamate sodium. From another report (13) in which 200 mg/kg of cyclamate was also fed, average values of 0.0063, 0.0004, and 0.0004% can be calculated. Renwick and Williams (2) found a 0.04% conversion of cyclamate to cyclohexylamine in rabbits that had received 56 mg/kg. The degree of conversion may be dependent on the amount of cyclamate fed (even where only a single dose is given), since, for example, cyclamate-pretreated species convert a higher percent of fed cyclamate to metabolites than normal species (2, 12).

In the rat (Table III), the average conversions to metabolites were 0.057% for cyclopentylamine, 0.016% for cyclopentanone, and 0.008% for cyclopentanol. Another study (13) reported values of 0.0096, 0.001, and 0.0015% for the conversion of cyclamate to cyclohexylamine, cyclohexanone, and cyclohexanol, respectively, in normal rats receiving a single dose of cyclamate (500 mg/kg).

On the basis of the results presented here, it appears that cyclopentylsulfamate is metabolically cleaved to a somewhat greater extent than cyclamate. However, prolonged feeding experiments with cyclopentyl and other sweet sulfamates are planned to investigate the possibility of using other sulfamates as cyclamate alternatives.

## REFERENCES

(1) A. G. Renwick and R. T. Williams, *Biochem. J.*, **129,** 857(1972).

(2) *Ibid.*, **129,** 869(1972).

(3) M. Asahina, T. Yamaha, K. Watanabe, and G. Sarrazin, *Chem. Pharm. Bull.*, **19,** 628(1971), and references cited therein.

(4) *Ibid.*, **20,** 102(1972).

(5) F. J. C. Roe, L. S. Levy, and R. L. Carter, *Food Cosmet. Toxicol.*, **8,** 135(1970).

(6) G. T. Bryan and E. Erturk, *Science*, **167,** 996(1970).

(7) P. G. Brantom, I. F. Gaunt, and P. Grasso, *Food Cosmet. Toxicol.*, **11,** 735(1973).

(8) J. M. Price, C. G. Biava, B. L. Oser, E. E. Vogin, J. Steinfeld, and H. L. Ley, *Science*, **167,** 1131(1970).

(9) C. Parekh, E. K. Goldberg, and L. Golberg, *Toxical. Appl. Pharmacol.*, **17,** 282(1970).

(10) B. S. Drasar, A. G. Renwick, and R. T. Williams, *Biochem. J.*, **129,** 881(1972).

(11) H. Ichibagase, S. Kojima, K. Inoue, and A. Suenaga, *Chem. Pharm. Bull.*, **20,** 175(1972).

(12) *Ibid.*, **20,** 1093(1972).

(13) A. Suenaga, S. Kojima, and H. Ichibagase, *Chem. Pharm. Bull.*, **20,** 1357(1972).

(14) T. Niimura, T. Tokieda, and T. Yamaha, *J. Biochem. (Tokyo)*, **75,** 407(1974).

(15) L. F. Audrieth and M. Sveda, *J. Org. Chem.*, **9,** 89(1944).

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

(17) *Ibid.*, **149,** 227(1972).

(18) W. J. Spillane, *J. Pharm. Sci.*, **62,** 1394(1973).

(19) E. J. Eisenbraun, in "Organic Synthesis," coll. vol. V, H. E. Baumgarten, Ed., Wiley, New York, N.Y., 1973, p. 310.

(20) M. L. Richardson, *Talanta*, **14,** 385(1967).

(21) S. Kojima and H. Ichibagase, *Yakugaku Zasshi*, **83,** 1108(1963); through *Chem. Abstr.*, **60,** 15060(1964).

(22) M. Ichikawa, S. Kojima, and H. Ichibagase, *ibid.*, **84,** 563(1964); through *Chem. Abstr.*, **61,** 10045(1964).

(23) D. E. Johnson, H. E. Nunn, and S. Bruckenstein, *Anal. Chem.*, **40,** 368(1968).

(24) P. H. Derse and R. J. Daun, *J. Assoc. Offic. Anal. Chem.*, **49,** 1090(1966).

(25) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.*, **17,** 2620(1969).

## ACKNOWLEDGMENTS AND ADDRESSES

Received October 17, 1975, from the *Department of Chemistry, University College, Galway, Ireland.*

Accepted for publication February 19, 1976.

The authors thank the Department of Education (Ireland) for a grant to G. A. Benson.

[x] To whom inquiries should be directed.