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Note

Gas chromatographic determination of cyclic amines, ketones and alcohols, possible metabolites of sweet sulphamates

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Cyclamate was first discovered to be metabolized to cyclohexylamine in animals by Kojima and Ichibagase[1]. The cyclohexylamine in the urine was determined initially using thin-layer[1] and visible spectrophotometry[2]. These workers subsequently developed a gas chromatographic (GC) procedure for the determination of cyclohexylamine in the urine of rats which were fed cyclamate[3]. Two other metabolites namely cyclohexanol and cyclohexanone were subsequently identified in the urine of rats[4,5].

We were interested in studying the metabolic products (if any) of certain sweet-tasting cyclic and aliphatic sulphamates similar to cyclamate. This necessitated the development of an analytical procedure to identify and quantitatively determine amines, ketones and alcohols, possible metabolites which could arise due to metabolic breakdown of these sulphamates. The procedure of Kojima and Ichibagase[3] which is summarized in Fig. 1 was considered to be tedious and time consuming and we considered it desirable to have GC conditions and suitable column material which would allow the simultaneous separation of amine, ketone and alcohol from a single injection.

GC determination of amines has been a problem in the past due to strong adsorption of the compounds on the column resulting in badly tailed elution peaks. Modification of column materials by various groups of workers with sodium and potassium hydroxides has resulted in excellent peak shapes and resolution of aliphatic amines[6–8]. O'Donnell and Mann[9] have compared a Dowfax 9N9 column with 2.5% sodium hydroxide as partition liquid against Carbowax 400 and 20M columns for the separation of a series of aliphatic amines. They concluded that the Dowfax 9N9–NaOH column is the most effective for separating a wide range of basic organic compounds. Further Derse and Daun[10] have determined cyclamate by first converting it to cyclohexylamine, this amine was then estimated by GC using 20% Dowfax 9N9 and 2.5% NaOH on 60–80 mesh Gas-Chrom R.

This paper describes a GC separation on a Dowfax 9N9 with 2.5% NaOH on 60–80 mesh Diatomite C AW of aliphatic and cyclic amines from their corresponding ketones and alcohols and a procedure for the microdetermination of cyclic amines, ketones and alcohols in the urine of rats.

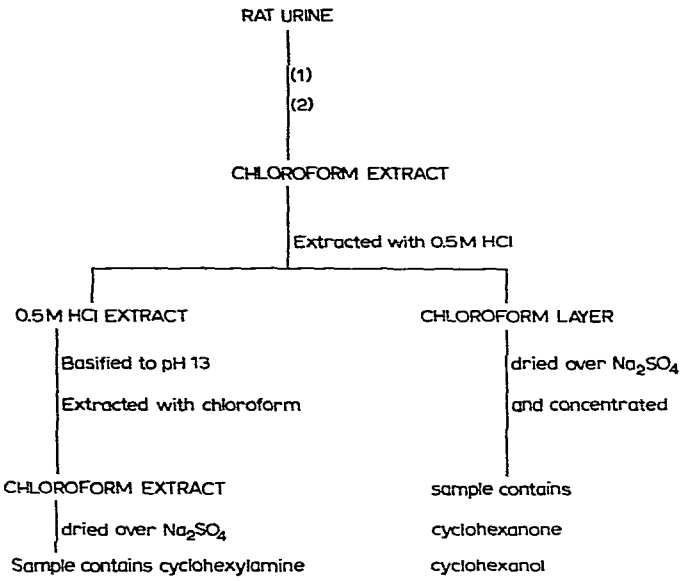

Fig. 1. Scheme for the separation of cyclohexylamine and cyclohexanone–cyclohexanol from urine according to Kojima and Ichibagase.

EXPERIMENTAL AND RESULTS

Chemicals and reagents

All amines, ketones and alcohols (supplied by Aldrich, Milwaukee, Wisc., U.S.A.) were distilled before use. *n*-Nonane, *n*-decane, *n*-dodecane and *n*-tetradecane (BDH, Poole, Great Britain) were used as obtained. Dichloromethane and sulphosalicylic acid (Analar; May & Baker, Dagenham, Great Britain) were used as obtained.

Rats and metabolism cages

Female Wistar albino rats were supplied by Trinity College (Dublin, Ireland) and were housed in individual metabolism cages supplied by Bowman Accessories (London, Great Britain). The urine from these animals was collected daily and refrigerated prior to being used.

Gas chromatograph and column

Chromatographic measurements were made on a Pye-Unicam 104 gas chromatograph with dual flame ionization detectors. The column was 5 ft. × $\frac{1}{4}$ in. glass. The column packing used in all measurements was 20% Dowfax 9N9 with 2.5% NaOH on 60–80 mesh Diatomite C AW.

Operating conditions for the separation of a cyclic or aliphatic amine from its corresponding ketone or alcohol

Table I summarizes the operating conditions and retention times for the separation of aliphatic and cyclic amines from their corresponding ketones or alcohols. The internal standard present in each separation was at a concentration of 0.02–

TABLE I

OPERATING CONDITIONS AND RETENTION TIMES FOR THE SEPARATION OF ALKYL AND CYCLIC AMINES, KETONES AND ALCOHOLS EXTRACTED FROM URINE USING A DOWFAX 9N9–2.5% NaOH ON 60–80 MESH DIATOMITE C AW COLUMN

Group	*Temp. (°C)*	*Nitrogen flow-rate (ml/min)*	*Retention time (min)*			*Internal Standard**
			Amine	*Ketone*	*Alcohol*	
Cyclopentyl	120	30	4.2	6.2	9.2	*n*-Dodecane (12.2)
Cyclohexyl	125	60	3.2	5.2	7.2	*n*-Tetradecane (16.4)
Cycloheptyl	130	75	6.3	8.4	13.0	*n*-Dodecane (4.6)
Cyclooctyl	138	75	8.4	9.5	17.0	*n*-Dodecane (3.2)
Cyclopentyl-methyl	120	60	4.8	—	12.4	*n*-Dodecane (7.8)
Isobutyl**	75	21	6.0	—	21.6	*n*-Nonane (13.0)
Isoamyl**	92	20	7.2	—	20.4	*n*-Decane (13.0)

* The number in parentheses is the retention time (min) for the internal standard.
** Determined in dichloromethane only.

0.04 ml per 250 ml dichloromethane. Separations were carried out isothermally and the carrier gas used was nitrogen.

Analytical procedure for the determination of cyclic amines, ketones and alcohols in rat urine

Calibration graphs were prepared for each of the separations outlined in Table I. A plot of peak height ratios (amine, ketone or alcohol/internal standard) *vs.* concentration, was linear over the range 0.006–0.2 mg/ml for each compound.

The calibration curves for the determination of cyclopentylamine, cyclopentanone and cyclopentanol are shown in Fig. 2. The analytical procedure employed for the setting up of the calibration graphs was as follows. Microliter quantities of cyclopentylamine, cyclopentanone and cyclopentanol were injected into 5-ml samples of rat urine. 5 ml of 20% sulphosalicylic acid (w/v) were added and the pH of the solution was adjusted to 12–13 with 1 ml of 10 *M* NaOH. Samples (2 ml) were then

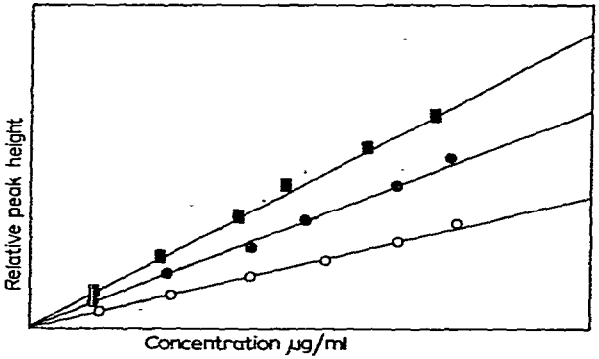

Fig. 2. Determination of cyclopentylamine (■), cyclopentanone (●) and cyclopentanol (○) by plotting relative peak heights (peak height of amine, ketone or alcohol divided by the peak height of *n*-dodecane) against concentration.

taken and extracted with 2 × 3 ml of dichloromethane (containing 0.04 ml *n*-dodecane per 250 ml dichloromethane). The samples were then centrifuged and 2-ml portions were transferred to 5-ml volumetric flasks containing approximately 2–4 mg of anhydrous sodium sulphate and 0.5 μl was injected on to the column. A similar procedure was employed to set up calibration curves for the other separations outlined in Table I.

For the purposes of estimating a percent recovery from urine cycloheptyl compounds were used and a calibration curve was prepared according to the procedure described above for the cyclopentyl compounds. The percent recoveries obtained for varying amounts of cycloheptylamine, cycloheptanone and cycloheptanol are given in Table II.

TABLE II

PER CENT RECOVERY OF CYCLOHEPTYLAMINE, CYCLOHEPTANONE AND CYCLOHEPTANOL FROM URINE

— = Not determined.

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	—
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	—
1166.4	94.6	1267.6	95.42	1263.2	94.9
1166.4	94.6	1267.6	104.91	1263.2	—
Mean ± S.E.	99.15 ± 4.75		98.82 ± 4.48		99.57 ± 7.64

DISCUSSION

Kojima and Ichibagase were the first workers to discover that the artificial sweetener cyclamate was metabolically cleaved in animals and humans. Following a series of further tests both for metabolites and toxicological effects the artificial sweetener was banned in 1969. We have been interested in other cyclic and aliphatic sulphamates, which are sweet tasting, from a structure–activity correlation point of view[11]. It occurred to us that if the structure of the sweet sulphamate was altered, on feeding it to animals its metabolic pattern might be different to that of cyclamate. The procedures outlined, and summarized in Table I, show that three metabolites can be determined directly by one injection on to the column in a very convenient time. Excellent calibration curves (see Fig. 2) and percentage recovery (see Table II) from urine were obtained. Fig. 3 shows the separations which were obtained for the cyclopentyl group.

n-Isoamyl and *n*-isobutyl sulphamates are sweet tasting compounds. Table I

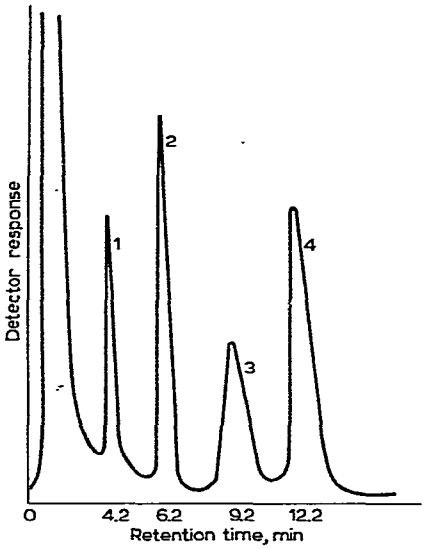

Fig. 3. Chromatographic separation of the urine metabolites of cyclopentyl sulphamate. Peaks: 1 = cyclopentylamine; 2 = cyclopentanone; 3 = cyclopentanol; 4 = *n*-dodecane (internal standard).

shows that we were able to separate the corresponding amines and ketones. However we failed to extract these compounds from rat urine quantitatively. The solvent was changed from dichloromethane to dimethyl ether but the extraction again failed. Work is continuing to obtain a satisfactory method for extracting aliphatic amines and alcohols from rat urine in order that the metabolism of *n*-isoamyl and *n*-isobutyl sulphamates can be studied.

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