[Chem. Pharm. Bull. 16(9)1851—1854(1968)]

UDC 612.393-085 : 547.541.08 : 543.544.25

Studies on Synthetic Sweetening Agents. XIII.[1] Metabolism of Sodium Cyclamate. (2).[2] Detection of Metabolites of Sodium Cyclamate in Rabbit and Rat by Gas-Liquid Chromatography

Shoji Kojima and Hisashi Ichibagase

Faculty of Pharmaceutical Sciences, Kumamoto University[3]

(Received May 13, 1968)

In the previous paper[4] on the metabolism of sodium cyclamate (CHS–Na) in human, dog, and rabbit using paper chromatography and thin-layer chromatography, the authors reported that cyclohexylamine, as a metabolite of CHS–Na, was found in the urine from human and dog, but was not detected in the urine from rabbit. The authors found recently that cyclohexylamine was excreted in the urine of rabbit and rat which had continued to receive CHS–Na orally, also, that the amount of urinary cyclohexylamine increased gradually in accordance with a prolongation of period administering CHS–Na.[5] In regard to the metabolites of CHS–Na, however, nothing has been known about any metabolites other than cyclohexylamine.

In this paper, the metabolism of CHS–Na in rabbit and rat, which had previously continued to receive CHS–Na, was investigated *in vivo* and *in vitro* by using a gas–liquid chromatography method. The results indicated that cyclohexylamine, cyclohexanol, and cyclohexanone were excreted in the urine from rabbit and rat receiving CHS–Na and also found in the metabolism of CHS–Na by rat liver.

Furthermore, in order to clarify the formation processes of cyclohexanol and cyclohexanone, an investigation on the metabolism of cyclohexylamine was carried out using rabbit and rat.

Experimental

Materials——CHS–Na was recrystallized from aqueous EtOH, and dried at 105° for 2 hr. Cyclohexylamine hydrochloride was recrystallized from EtOH, and dried at 100° for 1 hr, mp 204°. Cyclohexylamine, cyclohexanol, and cyclohexanone were purified by distillation of commercial products of reagent grade, bp 133—134°, bp 158°, and bp 155°, respectively.

Animals——Male rabbits (about 3 kg body wt.) were kept on the solid food[6] and water in individual cages. Wistar male rats (120—150 g body wt.) were maintained on the solid food[7] and water in individual metabolic cages.

Rabbits and rats, which had been administered orally 1 g and 100 mg of CHS–Na per day for 7 days respectively, were used for this investigation.

Administration of Drugs and Collections of Urines——In the rabbits which had been treated with CHS–Na as above mentioned, 1 g of CHS–Na or 200 mg of cyclohexylamine hydrochloride was administered orally by using Nelaton's catheter in the form of solution in 50 ml of water, and the urine was collected in the flask which contained toluene for preventing putrefaction.

1) Part XII: S. Kogima, and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 1619 (1968).
2) Previous paper, "Studies on Synthetic Sweetening Agents. VIII. Cyclohexylamine, A Metabolite of Sodium Cyclamate," *Chem. Pharm. Bull.* (Tokyo), **14**, 971 (1966), represents "Metabolism of Sodium Cyclamate (1)." in this series.
3) Location: *Oemoto-machi, Kumamoto.*
4) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **14**, 971 (1966).
5) Details will be published elsewhere in the near future.
6) "CR–2", Nippon Clea Co., Ltd. was used.
7) "CE–2", Nippon Clea Co., Ltd. was used.

In the rats which had been treated with CHS–Na as above mentioned, 100 mg of CHS–Na or 10 mg of cyclohexylamine hydrochloride dissolved in 5 ml of water was administered orally by using a stomach sonde, and the urine was collected in the flask which contained toluene.

Rat Liver Homogenate——The livers were removed from two rats, which were fasted about 24 hr prior to use in experiments, immediately after sacrifice and washed with ice–cold isotonic potassium chloride solution. The washed livers (20 g) were suspended in 30 ml of 0.1 M Tris buffer (pH 7.5) and homogenized in a teflon pestle glass homogenizer. The resulting homogenate was made to a total volume of 60 ml by adding 0.1 M Tris buffer, and 10 ml of 0.1 M nicotinamide was added.

Apparatus of Gas-Liquid Chromatography——A Shimadzu Model GC–1B dual column gas chromatograph equipped with a Model HFD–1 dual hydrogen flame ionization system was used. The carrier gas was nitrogen. The column was 3 meter × 4 mm i.d. stainless steel U–tube containing a packing of 20% PEG 20M and 2.5% NaOH on 60—80 mesh Shimalite.

Results and Discussion

Metabolism of CHS-Na in Rabbit *in Vivo*

The sample solutions (A and B) were prepared from two rabbit urines, which were collected for four days after oral administration of 1 g of CHS–Na once a day successively, using the procedure described in Fig. 1. Each sample solution was submitted to gas chromatography in order to examine the metabolites of CHS–Na.

The gas chromatogram of sample solution (A) showed one peak which was not identical with that of blank urine. The retention time (6.8 min) of the peak corresponded with that of authentic cyclohexylamine as shown in Fig. 2.

On the other hand, on the gas chromatogram of sample solution (B), two peaks were observed at the retention times of 5.8 and 7.2 min. These peaks had the same retention times with those of authentic cyclohexanone and cyclohexanol, respectively (see Fig. 3).

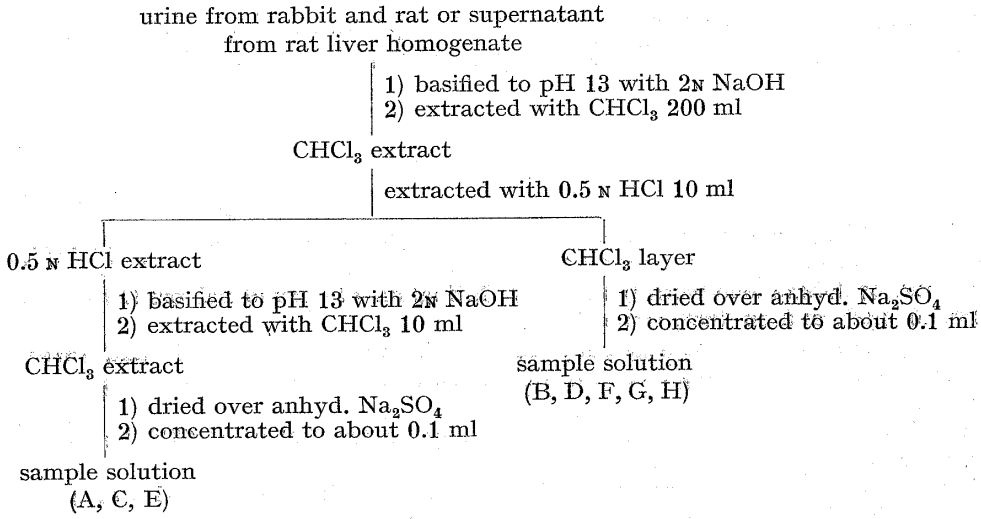

Fig. 1. Preparation Procedure of Sample Solution

A, B : from urine of rabbit receiving CHS–Na
C, D : from urine of rat receiving CHS–Na
E, F : from rat liver homogenate
G : from urine of rabbit receiving cyclohexylamine
H : from urine of rat receiving cyclohexylamine

Metabolism of CHS-Na in Rat *in Vivo*

The urines in three rats, which continued to receive orally 100 mg of CHS–Na per day to each animal, were collected for four days. The sample solutions (C and D) were prepared from the urine according to the method described in Fig. 1, and each sample solution was examined by gas chromatography.

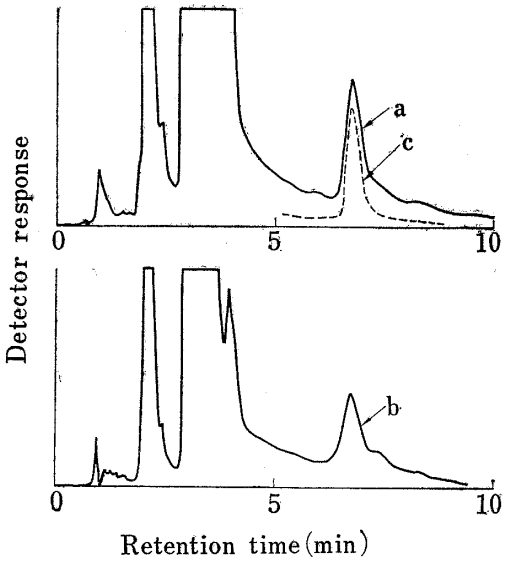

Fig. 2. Gas Chromatogram of Cyclohexylamine from Sample Solutions (A) and (C)

peak: a) cyclohexylamine from sample soln. (A)
b) cyclohexylamine from sample soln. (C)
c) authentic cyclohexylamine
condition: temp.—column 115°, injector 230°, detector 200°
gas flow rate—N_2 75 ml/min, H_2 55 ml/min, air 1000 ml/min
sens.—100, sample size—2 μl

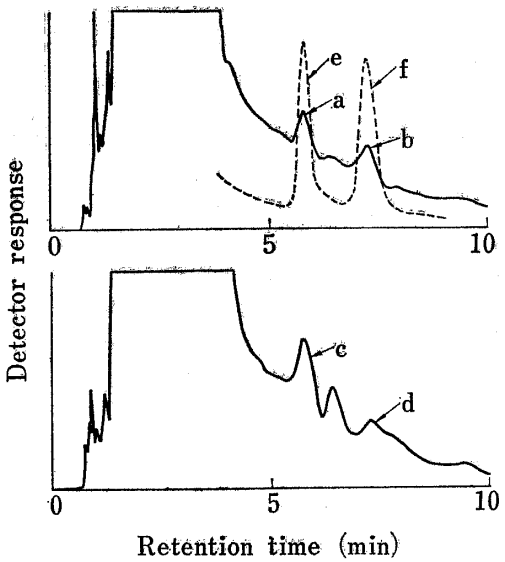

Fig. 3. Gas Chromatograms of Cyclohexanone and Cyclohexanol from Sample Solutions (B) and (D)

peak: **a**) cyclohexanone from sample soln. (B)
b) cyclohexanol from sample soln. (B)
c) cyclohexanone from sample soln. (D)
d) cyclohexanol from sample soln. (D)
e) authentic cyclohexanone
f) authentic cyclohexanol
condition: temp.—column 150°, injector 230°, detector 200°
gas flow rate—N_2 65 ml/min, H_2 55 ml/min, air 1000 ml/min
sens.—100, sample size—2 μl

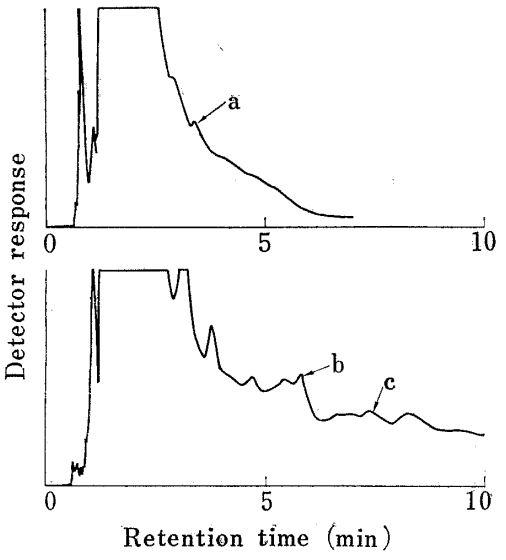

Fig. 4. Gas Chromatograms of Cyclohexylamine, Cyclohexanone, and Cyclohexanol from Sample Solutions (E) and (F)

peak: **a**) cyclohexylamine from sample soln. (E)
b) cyclohexanone from sample soln. (F)
c) cyclohexanol from sample soln. (F)
condition: the same as described in Fig. 3

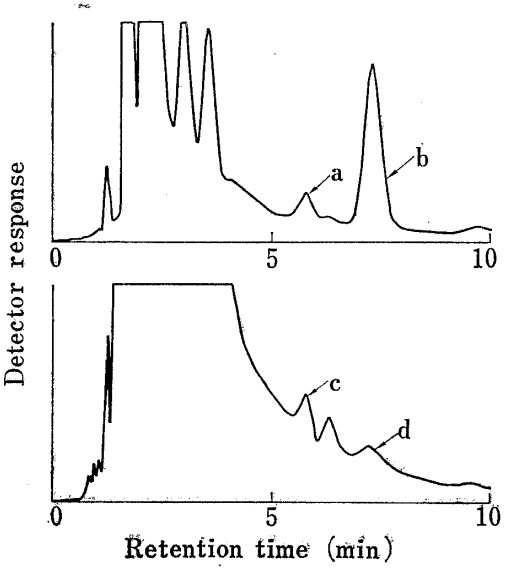

Fig. 5. Gas Chromatograms of Cyclohexanone and Cyclohexanol from Sample Solutions (G) and (H)

peak: **a**) cyclohexanone from sample soln. (G)
b) cyclohexanol from sample soln. (G)
c) cyclohexanone from sample soln. (H)
d) cyclohexanol from sample soln. (H)
condition: the same as described in Fig. 3

Therefore, three peaks corresponding to authentic cyclohexylamine, cyclohexanone, and cyclohexanol were detected from the sample solutions (C) and (D) as shown in Fig. 2 and 3.

Metabolism of CHS-Na in Rat Liver *in Vitro*

In order to study further on the CHS–Na metabolites detected in the urine from rabbit and rat, an experiment utilizing rat liver homogenate was carried out.

The rat liver homogenate containing 100 mg of CHS–Na was incubated on an incubator at a shake rate of 120 per minute and a temperature of 37° for 90 minutes. Then, the sample solutions (E and F) were prepared from the supernatant which was obtained by centrifuging the homogenate for 20 minutes at 9000 rpm (see Fig. 1), and were submitted to gas chromatography. As shown in Fig. 4, the gas chromatograms of the above sample solutions indicated the presence of cyclohexylamine, cyclohexanone, and cyclohexanol as metabolic products of CHS–Na, though these metabolites were a very small amount.

Metabolism of Cyclohexylamine in Rabbit and Rat *in Vivo*

From the above results, it was found that in addition to cyclohexylamine, cyclohexanone and cyclohexanol were detected as metabolites of CHS–Na in rabbit and rat. In order to study the formation processes of cyclohexanone and cyclohexanol, further investigation was carried out using cyclohexylamine.

On the gas chromatograms of sample solutions (G and H) (see Fig. 1), which were prepared from urine of rabbit and rat receiving cyclohexylamine as described in experimental, two peaks corresponding to cyclohexanone and cyclohexanol respectively were detected distinctly as shown in Fig. 5.

Accordingly, it was postulated that CHS–Na was metabolized primarily by the formation of cyclohexylamine, and the metabolite was oxidized further to afford the formation of cyclohexanone and cyclohexanol.

Further studies on these metabolites of CHS–Na are in progress.

[Chem. Pharm. Bull. 16(9)1854—1857(1968)]

UDC 615.217.011.5 : 547.565.2.07

Synthesis of Epinephrine Monosulfates

KAZUHIRO IMAI and ZENZO TAMURA

Faculty of Pharmaceutical Sciences, University of Tokyo[1)]

(Received May 25, 1968)

In the course of our studies on catecholamine metabolism, the need of the authentic catecholamine conjugates became evident. As to epinephrine, Richter's suggestion[2)] was given that the urinary epinephrine conjugate was a sulfate since its isolated conjugate from urine was positive for sulfate ester tests, but the lack of the authentic samples have obstructed the following progress. Now we wish to report the synthesis of two epinephrine monosulfates, namely epinephrine 3′–sulfate and epinephrine 4′–sulfate. The synthetic route is shown in Chart I.

1) Location: *Hongo–7–chome, Bunkyo–ku, Tokyo.*
2) D. Richter, *J. Physiol.* (London), **98**, 361 (1940).