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Gas Chromatography of D-Glucaric Acid and Its Lactones, and Application to Measuring Their Transformation

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A simple and reliable analytical method for D-glucaric acid, D-glucaro-1,4-lactone and -6,3-lactone was established; the sample solution was freeze-dried, trimethylsilylated and applied to a gas chromatograph.

The transformation of D-glucaro-1,4-lactone into D-glucaric acid and D-glucaro-6,3-lactone at physiological pH and temperature was traced by this method. Similarly, the transformations of L-gulono-γ-lactone into L-gulonic acid and of D-glucono-γ-lactone into D-gluconic acid and D-glucono-δ-lactone were traced.

It has been known that D-glucaro-1,4-lactone (I), one of the competitive inhibitors of β-glucuronidase, was transformed into D-glucaric acid (II) and D-glucaro-6,3-lactone (III) in aqueous solution.[2,3] We were interested in the transformation of I into II and III under physiological conditions, however there was no simple and reliable analytical method for them, although I and III had been determined by the hydroxamate method.[2,4,5]

In this paper, therefore, we investigated a gas chromatographic method to trace the transformation of I and other lactones.

Experimental

Materials and Reagents——Pyridine (GR; Kanto Chemical Co., Ltd.) was dried with NaOH pellets. N,O-Bis(trimethylsilyl)acetamide (GR; Tokyo Kasei Kogyo Co., Ltd.) and trimethylchlorosilane (EP; Tokyo Kasei Kogyo Co., Ltd.) were used directly. Aldonic acids, saccharic acids and their lactones were commercial samples or prepared in the laboratory and their purities were confirmed from the results of elemental analysis and the melting points.

Gas Chromatography——The sample was treated with bis(trimethylsilyl)acetamide (0.05 ml), 2 drops of pyridine and 4 drops of trimethylchlorosilane for 20 min at 60° and one μl of the reaction mixture was injected to the gas chromatograph. Gas chromatography was performed on a Shimadzu GC-4APF gas chromatograph equipped with hydrogen flame ionization detector. The glass tube (2.0 or 1.5 m×4 mm *i.d.*) was packed with 2% OV-17 or 2% GE XF-1105 on a support of Chromosorb W (60—80 mesh). Temperatures were as follows: column, 170°; injection port, 200°; detector oven, 200°.

Result and Discussion

Gas Chromatography of D-Glucaric Acid and Its Lactones

As reported in our previous paper,[6] these compounds were easily trimethylsilylated with bis(trimethylsilyl)acetamide, and the trimethylsilyl esters derived were stable in the presence of excess of reagent.

The resolution of trimethylsilyl derivatives of I, II, and III was examined on several columns. The satisfactory separation was achieved only on 2% OV-17 under isothermal condition (Fig. 1).

1) Location: *Hongo 7-3-1, Bunkyo-ku, Tokyo.*
2) Y. Hirasaka, K. Umemoto, M. Sukegawa and I. Matsunaga, Chem. *Pharm. Bull.* (Tokyo), **13**, 677 (1965).
3) C.A. Marsh, *Biochem. J.*, **99**, 22 (1966).
4) A. Kiyomoto, S. Harigaya, S. Ohshima and T. Morita, *Biochem. Pharmacol.*, **12**, 105 (1963).
5) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
6) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2535 (1970).

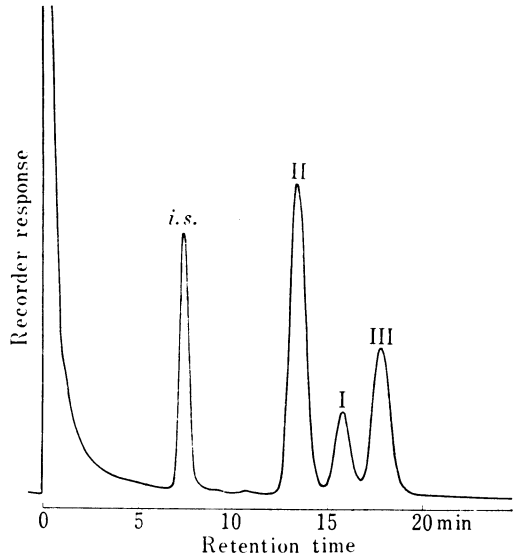

Fig. 1. Typical Gas Chromatogram of D-Glucaric Acid and Its Lactones as the Trimethylsilyl Derivatives

internal standard (*i.s.*): D-glucitol
conditions: 2% OV-17, 2.0m×4mm *i.d.*, 170°, N_2 44ml/min

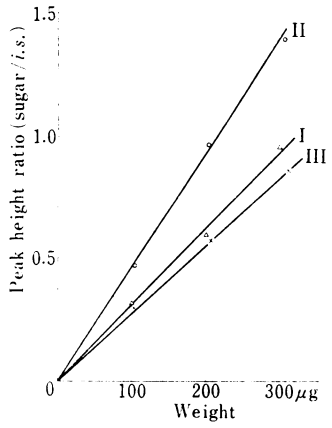

Fig. 2. Calibration Curves for D-Glucaric Acid, D-Glucaro-1,4-lactone and D-Glucaro-6,3-lactone using D-Glucitol (103 μg) as an Internal Standard (*i.s.*)

gas chromatographic conditions:
2% OV-17, 2.0 m×4 mm *i.d.*, 170° N_2 44 ml/min

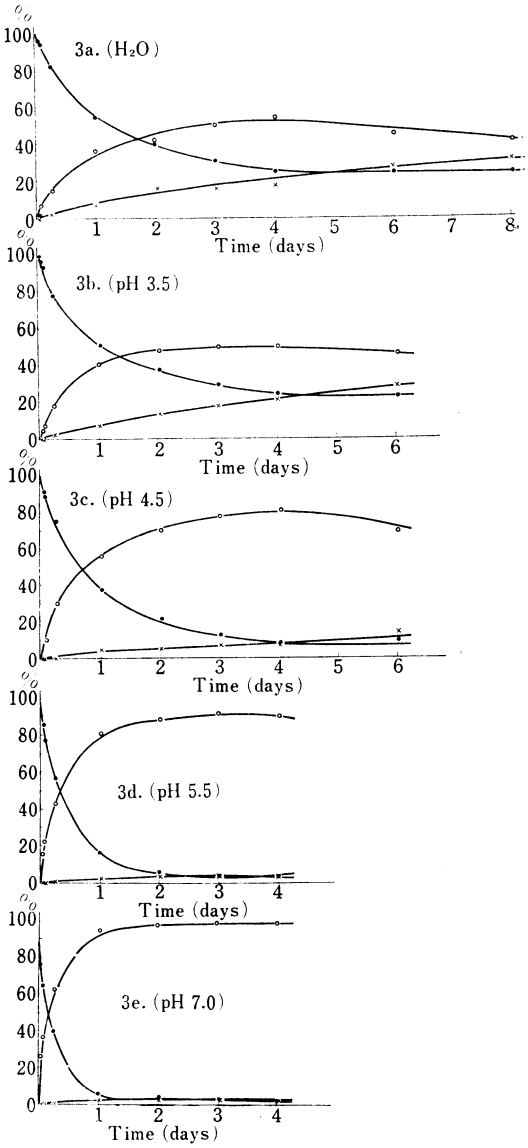

Fig. 3. Transformation of D-Glucaro-1,4-lactone (—●—) into D-Glucaric Acid (—○—) and D-Glucaro-6,3-lactone (—×—) at Various pH Values and 38°

Under the same condition, D-glucaro-1,4-6,3-dilactone and II (retention time: 12.92 and 13.44 min, respectively) were not separated perfectly, although the former is rapidly hydrolyzed in aqueous solution.

Stability during Freeze Drying

Since I, II, and III are transformed into each other throughout usual sample preparation, especially by evaporation, for gas chromatography, the reaction was intended to be stopped by freezing the solution.

One ml of the solution of I, II, or III in water or in a volatile buffer solution (0.2M pyridine +0.2M acetic acid) was frozen in dry ice–methanol and freeze-dried, and the stabilities of I, II, and III at pH 3.5—7.0 during freeze drying were analysed by gas chromatography. There appeared no other peaks than that of the original component in all cases tested.

Quantitative Analysis

The known quantity of D-glucitol was added to 1 ml of the sample solution before freezing and the analysis was achieved with peak height ratio method. The calibration curves obtained for I, II, and III are shown in Fig. 2.

Transformation of D-Glucaro-1,4-lactone into D-Glucaric Acid and D-Glucaro-6,3-lactone at Various pH Values

Eight mg of I and 3 mg of D-glucitol were dissolved in 25 ml of a buffer solution (0.2M pyridine + 0.2M acetic acid), and incubated at pH 3.5—7.0 and 38°. Each 1 ml of the solution was taken after an interval and the concentrations of I, II, and III were determined by the method mentioned above. As shown in Fig. 3, the transformation rate depended upon pH value and the amount of II formed by opening of lactone ring increased with rise of pH value.

Equilibrium in aqueous solution (Fig. 3a) took place after more than 8 days, on which the pH value was about 3.0.

Transformation of Aldonic Acids and Their Lactones

L-Gulonic acid, D-gluconic acid and their lactones were similarly determined by gas chromatography, using 2% XF-1105 in place of 2% OV-17.

The transformation of L-gulonate or D-gluconate at pH 5.5 and 38° was traced, using this method. The results were given in Fig. 4 and 5. The transformation of D-glucono-γ-lactone was more rapid than that of L-gulono-γ-lactone or I, and the stability of γ-lactone at pH 5.5 is indicated in following order: L-Gulono-γ-lactone>I>D-glucono-γ-lactone.

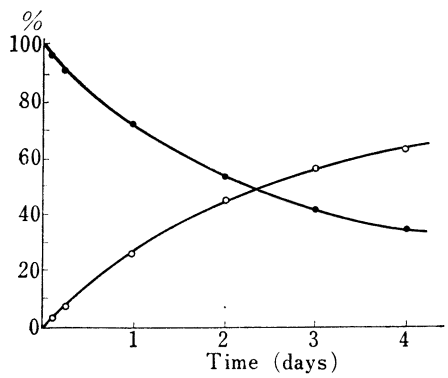

Fig. 4. Transformation of L-Gulono-γ-lactone (—●—) into L-Gulonic Acid (—○—) at pH 5.5 and 38°

Eight mg of L-gulono-γ-lactone and 3 mg of D-glucitol (*i.s.*) were dissolved in 25 ml of a buffer solution (0.2 M pyridine+0.2 M AcOH).
gas chromatographic conditions:
2% XF-1105, 1.5 m×4 mm *i.d.*, 170°, N_2 41 ml/min

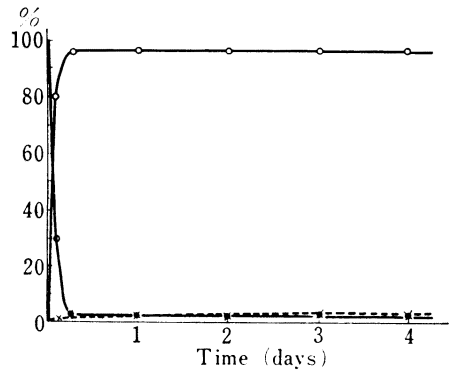

Fig. 5. Transformation of D-Glucono-γ-lactone (—●—) into D-Gluconic Acid (—○—) and D-Glucono-δ-lactone (---×---)at pH 5.5 and 38°

Eight mg of D-glucono-γ-lactone and 1.5 mg of D-xylitol (*i.s.*) were dissolved in 25 ml of a buffer solution (0.2 M pyridine+; 0.2 M AcOH).
gas chromatographic conditions:
2% XF-1105, 1.5 m×4 mm *i.d.*, 170°, N_2 41 ml/min