Colorless needles from petr. ether, mp 61°. *Anal.* Calcd. for $C_7H_5N_3Cl_2S_2$: C, 31.58; H, 1.88; N, 15.79. Found: C, 31.47; H, 1.73; N, 15.90.

7-Chlorothiazolo(5,4-*d*)pyrimidine (XVIII)——A suspension of 2 g of 5-amino-4-chloro-6-mercaptopyrimidine[9] in 20 ml of ethyl orthoformate was refluxed for 2 hr. After cooling, the deposited crystals were collected by filtration and concentration of the filtrate to almost dryness gave another crop of crystals which were combined to the above deposited crystals. Recrystallization from EtOH afforded colorless plates of XVIII, mp 156°. Yield, almost theoretical. *Anal.* Calcd. for $C_5H_2N_3ClS$: C, 34.99; H, 1.17. Found: C, 34.85; H, 1.35.

2,7-Dichlorothiazolo(5,4-*d*)pyrimidine (XX)——This compound was prepared from 1 g of 7-chloro-2-hydroxythiazolo(5,4-*d*)pyrimidine[10] and 5 ml of $POCl_3$ in the presence of 0.64 g of dimethylaniline by the same way as in the case of II. Recrystallization from benzene-hexane gave colorless needles, mp 100—101°. Yield, 1.1 g. *Anal.* Calcd. for $C_5NH_3Cl_2S$: C, 29.27; H, 0.49; N, 20.49. Found; C, 29.38; H, 0.36; N, 20.57.

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[9] S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 349 (1958).
[10] S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 675 (1958).

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Gas Chromatographic Separation of L-Idonate and D-Gluconate

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Catalytic hydrogenation of calcium D-xylo-5-hexulosonate (calcium 5-oxo-D-gluconate) by Raney nickel produces a mixture of two diastereoisomers calcium D-gluconate and calcium L-idonate.[2,3] The composition of the products has been determined so far by gravimetric,[4] polarimetric[5] or polarographic method.[6] We have found that the two diastereoisomers are completely separable by a gas chromatographic method when the sample is lactonized and trimethylsilylated. The method and result will be communicated here.

Experimental

A mixture of calcium L-idonate and D-gluconate was converted to acid by passage through Amberlite CG-120 (H^+) resin column and the sample eluted was lactonized after Morrison and Perry.[7] A conc. hydrochloric acid was added to the eluted solution adjusting its concentration to 2 N. The solution was then evaporated to dryness under a reduced pressure at 75—85°. The lactone sample was dissolved in 0.5 ml pyridine and trimethylsilylated by adding 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane followed by shaking for about 10 minutes at 75—85°. The sample was then evaporated to dryness and the

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[1] Location: *Bunkyo-ku, Tokyo.*
[2] G.E. Gray, U.S. Patent 2421611 (1947).
[3] T. Miki and Y. Sabashi, *J. Vitaminol.*, **15**, 361 (1958).
[4] S. Teramoto and I. Hori, *J. Fermentation Technol.*, **28**, 146 (1950).
[5] Y. Asahi and F. Kasahara, *Japan Analyst*, **14**, 614 (1965).
[6] Y. Asahi and F. Kasahara, *Japan Analyst*, **14**, 619 (1965).
[7] I. Morrison and M. Perry, *Can. J. Biochem.*, **44**, 1115 (1966).

trimethylsilyl derivatives of lactone were extracted with 2 ml *n*–hexane and freed from insoluble substances centrifugally. About one μl of the extract was subjected to gas chromatographic analysis.

Shimadzu gas chromatograph GC–1B, equipped with flame ionization detector, was used for the purpose. Gas Chrom P, coated with 2% SE–52, 3% QF–1 (FS–1265) or 2% CNSi (XF–1105) by a filtration method,[8] was packed in a glass tube of 1.8 m long and 4 mm internal diameter. The column temperature was set to 190° and nitrogen was passed through the column with a flow rate of 90 ml/min.

Result and Discussion

Preliminary experiment indicated that gas chromatographic separation is effective only for the sample lactonized; trimethylsilyl derivatives without lactonization appeared to be resolved to a less extent. A typical chromatogram obtained for the sample lactonized is shown in Fig. 1a. Three elution peaks of Fig. 1a obtained on 3% QF–1 column were identified, in the order of appearance, to *n*–hexane (solvent), D–gluconolactone and L–idonolactone by using an authentic sample respectively.

It is known that lactonization of D–gluconate yields a mixture of D–glucono–γ–lactone and D–glucono–δ–lactone.[7] These two lactone isomers were found to be resolved partially when 2% SE–52 or 2% CNSi coated column was employed. The chromatogram of this sort is illustrated in Fig. 1b with respect to CNSi column. It is noted that D–gluconolactone and L–idonolactone are resolved only poorly on 2% SE–52 column.

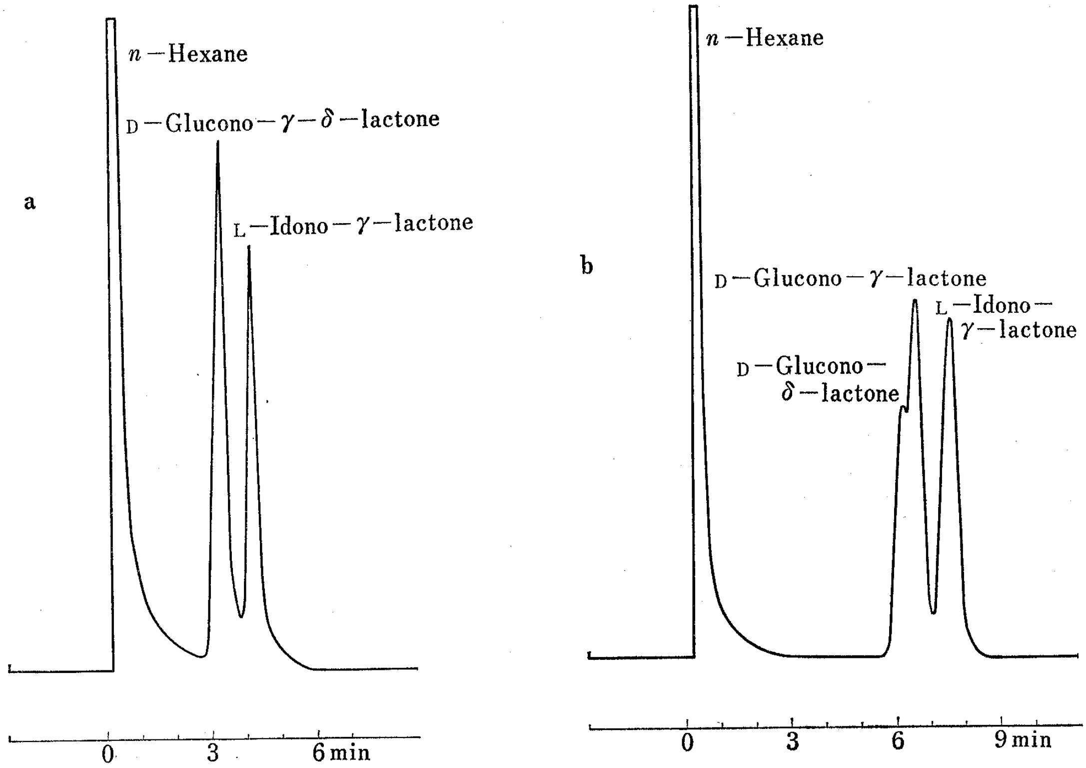

Fig. 1. Elution Chromatogram for a Sample of D–Gluconate and L–Idonate Lactonized and Trimethylsilylated

Column: a, 3% QF–1 on Gas Chrom P b, 2% CNSi on Gas Chrom P
Temperature: 190°
Carrier: Nitrogen
Flow Rate: 90 ml/min

Very recently, a paper[9] dealing with gas chromatographic separation of D–gluconate and L–idonate came to our notice. Accordingly, the separation was carried out on a SE–52

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[8] D. Glick, *Methods of Biochem. Analy.*, **11**, 80 (1963).

[9] E. Hardegger and N. Halder, *Helv. Chim. Acta*, **50**, 1275 (1967).

column although no chromatogram was shown in the paper. Our result indicated that CNSi or QF–1 is more favourable than SE–52.

The retention time for D–gluconolactone and L–idonolactone obtained on QF–1 column is shown in Table I together with those for γ and δ–lactones on SE–52 and CNSi columns respectively.

Table I. Retention Time for Lactones on Different Columns (min)

Sample / Column	D–Gluconolactone		L–Idonolactone
	(γ)	(δ)	(γ)
QF–1	3.0		3.9
SE–52	3.8	3.6	4.1
CNSi	6.6	6.1	7.6

Since we were interested in the selective hydrogenation of calcium 5–oxo–D–gluconate to give calcium L–idonate, further investigations were made on the quantitative determination of the mole fraction of L–idonate in a mixture of D–gluconate and L–idonate. Several samples of known composition were prepared from pure calcium salt of L–idonate and D–gluconate. After lactonizing and trimethylsilylating, each sample was subjected to gas chromatographic analysis. The area under a peak was assumed to give the amount of the component present.

It was found in this way that pure D–gluconate and L–idonate samples contain 4.5 and 3.0 mole % optical antipode respectively. If corrections are made for the impurities, it is concluded that the present method determines the composition of the two optical isomers within 2% accuracy. The method is now being applied to the study of the selective hydrogenation of calcium 5–oxo–D–gluconate.

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