because the peak of 8-hydroxyquinoline overlapped with the strong UV-absorbing background peaks present in cucumber extract, whereas, as shown in Fig. 3, only the peak of 8-hydroxyquinoline appeared on the chromatogram obtained by the fluorimetric detector. The reliability of the present method was assessed by comparison with the results obtained by the conventional colorimetric method with *p*-nitrobenzene diazonium fluoroborate.[4] The results (Table II) obtained between both methods show a good agreement. The proposed method is suitable for the routine analysis of copper-oxinate residue in plant tissues, and is applicable to other pesticides and drugs that yield fluorescent complexes with metal ion.

Table II. Copper-oxinate Residues (ppm) in Fortified Samples after Spraying

Sample	Number of spraying	Amount of spraying	Days after the last spray	Number of determinations	Colorimetry[b]	HPLC
Tomato	0	—	—	2	0.05	0.01
	5	200 l[a]/10 a	3	2	0.26	0.23
Cucumber	0	—	—	2	0.05	0.01
	7	150 l[a]/10 a	3	2	0.14	0.16

a) 4% aqueous solution.
b) *p*-nitrobenzene diazonium fluoroborate colorimetric method.[4]

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Gas-Liquid Chromatographic Determination of Inositol in Multivitamin Preparation containing Large Amounts of Sugars

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A simple gas–liquid chromatographic method was developed for the determination of inositol in multivitamin preparation containing large amounts of sugars. Sample multivitamin preparation was trimethylsilylated and separated on a 2% OV-101 column. The quantitations were achieved by the peak height ratio method using methyl arachidate as an internal standard. Inositol in 13 synthetic mixtures containing large amount of various sugars (200 to 1000-fold amount) was determined successfully by the present method.

Keywords——inositol; sugars; multivitamin preparation; trimethylsilylation; gas–liquid chromatography

Niwa, *et al.*[2] established a spectrophotometric method for the determination of inositol in synthetic mixtures containing large amounts of sucrose using the color-reaction with hydroxamate following acetylation or the periodate oxidation. The reaction used, however, is not specific to inositol and complicated procedures are required for elimination of sucrose which interferes with the determination of inositol. As a result, their method is time-con-

1) Location: *1-403, Yoshino-cho, Omiya-shi, Saitama, 330, Japan.*
2) S. Niwa, A. Kotaki, and K. Yagi, *Bitamin*, 30, 23 (1964).

suming and may lose a portion of inositol. Moreover, conventional methods (*e.g.* spectrophotometry,[3] gravimetry[4] and titrimetry[5]) have the same deficits for the determination of inositol containing large amounts of sugars. Microbiological assay and enzymic analysis are specific to inositol, but require tedious procedures and are easily affected by inherent factors.

On the other hand, gas–liquid chromatographic (GLC) methods[6] for the determination of inositol in natural products as trimethylsilyl (TMS) derivatives have been reported since 1965 and it is necessary to remove interfering substances by ion exchange resins prior to gas-liquid chromatography. However, no paper has dealt with GLC determination of inositol in the presence of large amounts of sugars. This report describes a simple and rapid method which involves trimethylsilylation according to a modification of the method of Sweeley, *et al.*[7] followed by GLC separation on 2% OV-101 column for the determination of inositol in multivitamin preparation containing large amounts of sugars (MPCS).

Experimental

Reagents and Materials——Methyl arachidate was purchased from Sigma Chemical Co. Inositol, hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), pyridine and ethanol (99.5 v/v%) were obtained from Wako Pure Chemical Ind., Ltd. Sugars were obtained from commercial source. Multivitamin preparation (MPCS) contains 50 mg of inositol, 5.5 mg of thiamine mononitrate, 5.75 mg of sodium riboflavin phosphate, 5.0 mg of pyridoxine hydrochloride, 20 mg of nicotinamide, 1000 mg of taurine, 50 mg of caffeine, 16000 mg of sucrose and 5000 mg of sorbitol in 100 ml of aqueous solution.

Gas Chromatography——A Shimazu Model GC-6AM gas chromatograph equipped with a flame ionization detector was used. A glass column (2 m × 3 mm i.d.) was packed with 2% OV-101 coated on Shimalite W (80—100 mesh). The column temperature was programmed from 180° to 300° at a rate of 5° per min. The carrier gas (nitrogen) was used at a flow rate of 17 ml per min.

Determination of Inositol in MPCS——To 0.2 ml of MPCS containing 0.1 mg of inositol was added 5 ml of internal standard solution (0.05 mg of methyl arachidate/ml of ethanol) and the mixed solution was evaporated to complete dryness by a rotary vacuum evaporator. The residue was dissolved in 2.5 ml of anhydrous pyridine, 1 ml of HMDS and 0.5 ml of TMCS and trimethylsilylated at 60° for 40 min on a water bath. After cooled down to room temperature, 3 μl of the reaction mixture was injected into a gas chromatograph. The quantitations were achieved by the peak height ratio method.

Results and Discussion

Trimethylsilylation of Inositol in MPCS

Sweeley, *et al.*[7] presented a method for the trimethylsilylation of carbohydrates, that is, 10 mg of carbohydrates were trimethylsilylated by 0.2 ml of HMDS and 0.1 ml of TMCS in 1 ml of pyridine at room temperature. It was found from the preliminary experiment, however, their method gave incomplete trimethylsilylation of inositol in MPCS. Therefore, we modified the method of Sweeley, *et al.*[7] using an aliquot of MPCS containing 0.1 mg of inositol. Based on the fact that the highest peak of inositol was obtained by gas-liquid chromatography under the conditions of heating at 60° for 30 to 50 min during silylating period, trimethylsilylation was performed at 60° for 40 min. Moreover, it was found that 5 times as much amounts of TMS reagent as Sweeley, *et al.*[7] gave better reproducibility. The conditions of trimethylsilylation was chosen through these experiments.

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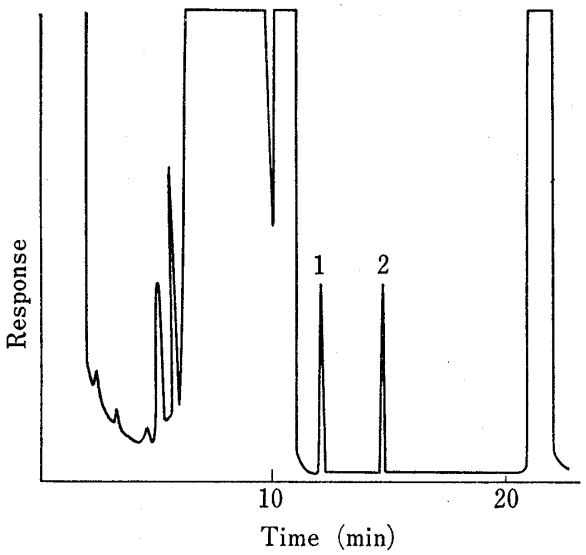

Fig. 1. Gas Chromatogram of Multivitamin Preparation

1. TMS-inositol, 2. methyl arachidate.

Gas-Liquid Chromatography of MPCS

Among the several stationary phases (SE-30, OV-17 and OV-101) examined for the separation of inositol from other sugars in MPCS, the OV-101 column was found to be the best choice. For the rapid analysis of inositol, a linear temperature-programming method was employed. A chromatogram of MPCS on a 2% OV-101 column with linear temperature-programming technique is shown in Fig. 1. The retention times of inositol and methyl arachidate (internal standard) were 12.5 and 14.3 min, respectively.

Quantitative Analysis

The calibration curve for inositol in the concentration range of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml using methyl arachidate as an internal standard was given by the regression equation;

$$Y = 1.822X - 0.121 \quad (r=0.999)$$

Table I. Recovery of Inositol from Multivitamin Preparation

Inositol added (mg)	Inositol found (mg)	Recovery (%)
50	49.6	99.2
50	51.7	103.5
50	50.1	100.2
50	51.0	102.0
50	50.5	101.0
Mean	50.6	101.2
Coeff. of var.		1.63

Table II. Recoveries of Inositol from Synthetic Mixtures

No.	Sugars	Amount of sugar (fold)	Recovery (%)
1	Arabinose	1000	102.1
2	Xylose	1000	99.8
3	Ribose	1000	102.1
4	Rhamnose	1000	99.3
5	Glucose	1000	101.0
6	Mannose	1000	100.2
7	Galactose	1000	100.1
8	Fructose	1000	98.1
9	Maltose	1000	107.5
	Maltose	500	98.8
10	Sucrose	1000	101.5
11	Lactose	1000	102.5
12	Mannitol	1000	99.4
13	Sorbitol	1000	112.8
	Sorbitol	200	100.7

where X is concentration of inositol (mg/ml) and Y is ratio of peak height (inositol/methyl arachidate). The limit of determination was 0.1 mg/ml. The variation and average recovery of 5 samples are shown in Table I, indicating that the present method is enough accurate for quantitative analysis of inositol in MPCS.

Influences of Sugars

In order to examine the interference with large amounts of various sugars, 13 kinds of synthetic mixture which contained inositol and 1000-fold amount of each sugars (see Table II) were prepared and treated according to the above procedure. As a result, the sugars except maltose and sorbitol had no influences on the recovery of inositol, whereas maltose and sorbitol made the recovery of inositol a little higher, as shown in Table II. It was found that 500-fold amount of maltose or 200-fold amount of sorbitol did not interfere any more.

From these results, it was proved that the present method is applicable to the determination of inositol in samples containing large amounts of sugars.

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Reaction of N,N-Dialkylaminomethyl Arenesulfonates with Diazoalkanes

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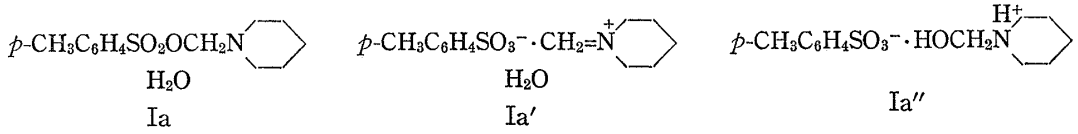
The present paper describes our finding that diazoalkanes react with N,N-dialkylaminomethyl arenesulfonates affecting the insertion of alkylidenes into the methylene carbon-oxygen bond.

Keywords——diazoalkane; methyleniminium salt; N,N-dialkylaminomethyl arenesulfonate; insertion reaction; aziridinium intermediate; anion exchange; piperidineethanol

We describe here a new reaction of diazoalkanes with N,N-dialkylaminomethyl arenesulfonates affecting the insertion of alkylidenes into the methylene carbon-oxygen bond.

The reactions of N,N-dialkyl-N-chloromethylamines with methyl *p*-toluenesulfonate and of methylenebisamines with benzenesulfonic benzoic anhydride have been reported by Böhme *et al.*[2)] to give N,N-dialkyl-N-methyleniminium arenesulfonates without description of their melting points. In an attempt to synthesize 1-methylenepiperidinium *p*-toluenesulfonate according to the former method, recrystallization of the product from benzene, however, gave crystals of mp 85—87°, which were analyzed as a compound corresponding to its monohydrate. For this hydrate the following three structures, Ia, Ia′ and Ia″, can be



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