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Note

Determination of isethionic acid by gas chromatography with flame photometric detection

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Isethionic acid (2-hydroxyethanesulphonic acid, ISA) was first found in squid axoplasm [1] and its presence in mammalian tissues has been reported [2–7]. Although ISA is the hydroxy analogue of taurine, its biological role is not fully understood.

The determination of ISA in biological materials has been carried out by gas chromatography (GC) based on the preparation of methyl ether/methyl ester [3], trimethylsilyl [4–6,8] or 2-chloroethylsulphonyl chloride [7,9] derivatives. However, these derivatives are generally unstable and are apt to undergo thermal decomposition during the GC analysis. Further, these previously reported methods usually require complicated preliminary clean-up of the sample.

Recently, we developed a convenient method for the conversion of the sulphonic acid into its dibutylsulphonamide derivative, and by using this method, we successfully achieved GC analysis of sulphonic acids of biochemical interest, such as taurine [10,11] and cysteic acid [12]. The method involved ion-pair extraction of the compounds into organic phase, followed by chlorination and amidation. This paper reports the logical extension of the work to the analysis of ISA.

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EXPERIMENTAL

Chemicals

ISA as the sodium salt and 3-hydroxypropanesulphonic acid (HPS) as an internal standard (I.S.) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and each was dissolved in water to make a stock solution at a concentration of 1 m*M*. Tetrahexylammonium hydroxide (THA-OH; Nacalai Tesque, Kyoto, Japan) was prepared from its iodide with silver oxide as described earlier [10] and used as a 10% methanolic solution. Thionyl chloride (Nacalai Tesque) was used after distillation. Dibutylamine (DBA; Nacalai Tesque) was used at a concentration of 2 *M* in acetonitrile. All other chemicals were of analytical grade.

Derivatization procedure

The chemical reactions involved in the derivatization procedure are shown in Fig. 1. An aliquot of the sample solution containing 0.5–10 nmol of ISA was pipetted into a 10-ml Pyrex glass tube with a PTFE-lined screw cap. After addition of 0.25 ml of 20 μM HPS (I.S.), the total reaction volume was made up to 1 ml with distilled water if necessary. Immediately after the addition of 0.05 ml of 10% THA-OH and 2 ml of methylene chloride, the tube was shaken with a shaker set at 300 rpm (up and down) for 3 min at room temperature. After centrifugation at 2000 *g* for 1 min, the organic layer was transferred to another tube and the solvent was evaporated to dryness under a stream of dry air. To the residue was added 0.2 ml of thionyl chloride, and the tube was tightly capped and heated at 80°C for 15 min. The excess of thionyl chloride was removed at 50°C under a stream of dry air. To the residue was added 0.2 ml of 2 *M* DBA, and the mixture was allowed to stand for 5 min at room temperature after tightly capping. The reaction mixture was acidified with 1 ml of 20% orthophosphoric acid and then extracted twice with 3 ml of *n*-pentane. After the solvent was evaporated to dryness at 50°C, the residue was dissolved in 0.1 ml of ethyl acetate, and 0.5 μl of this solution was injected into the gas chromatograph.

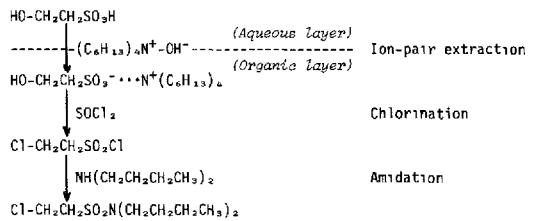

Fig. 1. Derivatization process of isethionic acid.

Preparation of samples

Ten male mice were used for experimentation. Immediately after dissection each organ was removed and frozen, and these frozen tissues were stored at −20°C until each assay. Each tissue was chopped up with a Model LK-21 ultra-dispersor (Yamato Kagaku, Tokyo, Japan) before being used, and then 0.1–0.2 g of each tissue was homogenized with 50 volumes of 0.1 m*M* HPS (I.S.) using an ultra-dispersor. The homogenate was diluted with ten volumes of water, and a 0.5-ml portion of this solution was used for the analysis.

Gas chromatography

GC analysis was carried out with a Shimadzu 14A gas chromatograph equipped with a flame photometric detector (S-filter). A fused-silica capillary column (15 m×0.53 mm I.D., 1.0 μm film thickness) with cross-linked DB-17 (J & W, Folson, CA, U.S.A.) was used. The operating conditions were as follows: column temperature, programmed at 10°C/min from 130 to 230°C; injection and detector temperature, 240°C; nitrogen flow-rate, 15 ml/min. The peak heights of ISA and HPS (I.S.) were measured, and the peak-height ratios against the I.S. were calculated for the construction of calibration curve.

Gas chromatography–mass spectrometry (GC–MS)

A Hewlett-Packard 5890A gas chromatograph was operated in connection with a VG Analytical 70-SE mass spectrometer and a VG 11-250J mass data system. The GC column was of the same type as used for GC analysis, with an ionizing voltage of 40 eV, an ion-source temperature of 240°C and a helium flow-rate of 8 ml/min.

RESULTS AND DISCUSSION

In the first step of the derivatization, ISA is extracted from the aqueous layer into the methylene chloride layer by ion-pair extraction [13] using tetraalkylammonium as the counter-ion. Of several alkylammonium ions tested, THA (hydroxy form) proved to be the most satisfactory. It was found that the hydroxy function as well as the sulphonic acid function of ISA were chlorinated with thionyl chloride under the conditions described in Experimental (80°C, 15 min), and 2-chloroethylsulphonyl chloride was formed. The reaction of the sulphonyl chloride with DBA proceeded rapidly at room temperature and gave the final derivative, 2-chloroethylsulphonic acid dibutylamide. The total derivatization process could be performed in 40 min.

The structure of the derivative was confirmed by GC–MS. The mass spectrum of the ISA derivative is shown in Fig. 2A. Although a molecular ion peak (*m/z* 255) was not observed, the prominent fragment ion peaks, *m/z* 177 ($M^+ - Cl$ and $-C_3H_7$), *m/z* 134 [$M^+ - Cl$ and $2(-C_3H_7)$] and *m/z* 120

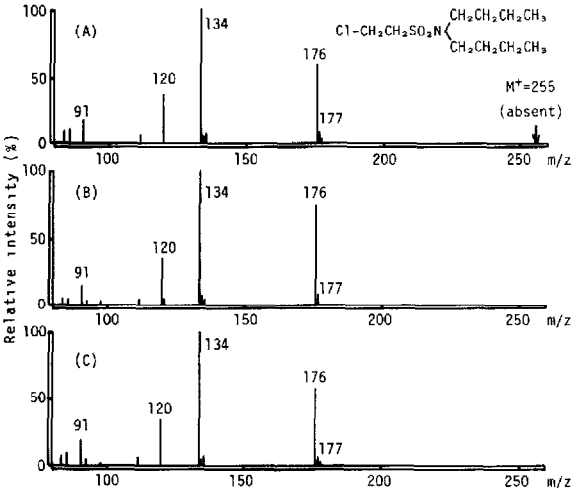

Fig. 2. GC-MS spectra obtained from the 2-chloroethyldibutylsulphonamide derivative of authentic ISA and from the peak identified with ISA in mouse tissues. (A) Authentic ISA; (B) mouse heart; (C) mouse liver.

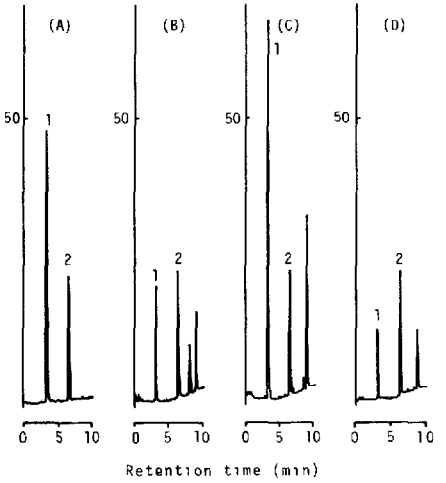

Fig. 3. Gas chromatograms obtained from standard solution and mouse tissues. (A) Standard (containing 5 nmol of ISA); (B) brain; (C) heart; (D) liver. GC conditions as in Experimental; attenuation, 10×32. Peaks 1=ISA; 2=HPS (I.S.).

($M^+ - Cl$, $-C_3H_7$ and $-C_4H_9$) were observed, and these peaks were useful for structure elucidation.

The derivative was found to be very stable under normal laboratory conditions, and no thermal decomposition was observed during GC analysis.

As shown in Fig. 3A, the ISA derivative was eluted as a single peak and

TABLE I

ISETHIONIC ACID CONTENT IN MOUSE TISSUES

Tissue	Isethionic acid content (mean ± S.D., $n=3$) (μmol/g wet weight)
Brain	1.90 ± 0.05
Heart	5.35 ± 0.07
Lung	2.98 ± 0.10
Liver	1.39 ± 0.06
Spleen	4.64 ± 0.30
Pancreas	0.63 ± 0.05
Kidney	1.16 ± 0.01
Stomach	1.53 ± 0.07
Intestine	1 93 ± 0.07
Testis	0.50 ± 0.03
Muscle	1.76 ± 0.11

provided an excellent response in the flame photometric detector. The minimum detectable amount of ISA to give a signal three times as high as the noise under our instrumental conditions was ca. 1 pmol.

In order to test the linearity of the calibration curve, various amounts of ISA ranging from 0.5 to 10.0 nmol were derivatized, and aliquots representing 2.5–50 pmol of ISA were injected. A linear relationship was obtained from both logarithmic plots, and the regression line was $\log y = 1.415 \log x - 0.673$ ($r = 0.990$, $n = 15$), where y is the peak-height ratio and x is the amount of ISA.

The method developed was successfully applied to biological materials without prior clean-up of the sample. Fig. 3B–D shows the chromatograms obtained from mouse tissues. ISA in the tissues could be detected without any influence from coexistent substances. The ISA peak obtained from each tissue sample was confirmed by GC–MS (Fig. 2). It was difficult to perform this analysis with a flame ionization detector because of interfering peaks derived from non-sulpho components. The recoveries of ISA added to mouse tissues (brain, heart, liver and kidney) were 94–100%, and the relative standard deviations were 1.9–7.4% ($n=3$). The ISA contents of mouse tissues are shown in Table I.

In conclusion, these experiments have conclusively demonstrated that ISA can be accurately and precisely determined by GC with flame photometric detection as 2-chloroethyldibutylsulphonamide derivative. The method is selective and sensitive, and complex biological materials can be analysed without prior clean-up of the sample. We believe that this method provides a useful tool in biochemical research where ISA assay is required.

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