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Note

Analysis of sulfonic acids by gas chromatography—mass spectrometry of trimethylsilyl derivatives

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Several sulfonic acids are known to exist in biological material. Most are formed in the metabolism of the sulfur-containing amino acids and little is known about their metabolic significance. Taurine, the sulfonic acid wich has been most extensively studied, is present in large amounts in mammalian skeletal and heart muscle, and is thought to play a role in the regulation of membrane potentials [1]. Even less is known about the biological actions of the others [1, 2].

Taurine gives a positive ninhydrin reaction, and can be determined by conventional ion-exchange amino acid analysis. The identification and determination of the other sulfonic acids was more complicated, involving laborious chromatographic, electrophoretic or ion-exchange techniques.

Methods have also been developed to determine sulfonic acids by gas chromatography (GC). Thus, Caldwell and Tappel [3] and Rosei et al. [4] described analytical methods for taurine and isethionic acid, respectively, using silylated derivatives and GC instruments equipped with hydrogen flame ionization detectors. Recently, Remtulla et al. [5] have published a GC method for isethionic acid, determined as the methyl ester.

We have repeated the above methods, and found that the methyl ester derivatives of sulfonic acids can easily be detected by flame ionization GC. However, we found that the flame ionization detectors were insensitive to the trimethylsilyl (TMS) derivatives.

In the present paper it is shown that silylated sulfonic acids can be detected by the use of combined GC—mass spectrometry (MS), where the total ion current of the ionization chamber is used as detector. Furthermore, it is

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shown that an ordinary OV-17 gas chromatographic column gives good separation among the different acids tested.

MATERIALS AND METHODS

Chemicals

Sulfuric acid, sulfosalicylic acid, sulfanilic acid, *p*-toluenesulfonic acid, and N,N-dimethylformamide were purchased from Merck, Darmstadt, G.F.R. Isethionic acid (sodium salt) and cysteic acid were obtained from Sigma, St. Louis, Mo., U.S.A. Sulfamic acid and taurine were the products of Fluka, Buchs, Switzerland. *o*-Sulfobenzoic acid was purchased from Schuchardt, Görlitz, G.F.R. BSTFA (bis (trimethylsilyl) trifluoroacetamide) and the hydrocarbon standards were obtained from Supelco, Bellafonte, Pa., U.S.A.

Instrumentation

The combined GC—MS instrument used was a Varian CH 7, manufactured by Varian-MAT, Bremen, G.F.R. It consisted of a Varian 1400 gas chromatograph with a coiled-glass column (6 ft × 1/8 in I.D.), a molecular separator of the glass frit type, and a single-focusing mass spectrometer operated with an ionization energy of 70 eV. The total ion current of the mass spectrometric ionization chamber served as detector for the gas chromatograph. Helium was the carrier gas (15 ml/min).

In addition, two Varian chromatographs model 2100 (Varian Aerograph, Walnut Creek, Calif., U.S.A.), each equipped with 2 U-columns of glass (6 ft × 1/4 in. I.D.) and one Varian 1400 chromatograph fitted with a coiled-glass column (6 ft × 1/4 in. I.D.), were used. These chromatographs had hydrogen flame ionization detectors. Nitrogen was the carrier gas (30 ml/min). The standard injection temperature was 230° and the detectors were usually kept at 250°.

The GC column material used was 10% OV-17 on Gas-Chrom Q (80—100 mesh), obtained from Applied Science Labs., State College, Pa., U.S.A.

Preparation of derivatives

In a small test tube with a PTFE screw cap were placed about 5 mg of sulfonic acid, 300 μl of dimethylformamide as solvent, and 300 μl of BSTFA. The tube was tightly capped, and placed in a sand bath at 110° for 60 min. The mixture was then ready for analysis. Heating at a temperature above 120° or at 110° for more than 2 h led to decomposition; the most unstable compounds were taurine and cysteic acid.

RESULTS

TMS derivatives were made of nine different sulfonic acids. None of these derivatives gave any response on the hydrogen flame ionization detectors. All chromatograms contained a peak with a methylene unit value of 14.5, representing a compound which was formed when dimethylformamide was heated for more than 20 min at a temperature of about 110°. The structure

TABLE I

GC–MS DATA FOR TRIMETHYLSILYL DERIVATIVES OF SULFONIC ACIDS

M.U. value = methylene unit value. The analytical details are given in the text.

Compound	TMS deriv.	Mol.wt.	M.U. value	10 most abundant *m/e* fragments									
Sulfuric acid	diTMS	242	12.8	147,	73,	227,	102,	148,	45,	59,	75,	66,	149
Isethionic acid	diTMS	270	15.1	255,	73,	147,	75,	74,	45,	66,	59,	256,	43
Taurine	diTMS	269	16.0	254,	102,	147,	73,	115,	116,	45,	59,	100,	74
	triTMS	341	17.9	326,	73,	147,	174,	327,	59,	45,	133,	100,	130
Cysteic acid	triTMS	385	18.6	73,	147,	75,	268,	45,	44,	77,	100,	59,	229
	tetraTMS	457	20.6	147,	73,	340,	241,	45,	74,	75,	268,	303,	59
Sulfamic acid	diTMS	241	14.3	147,	226,	73,	146,	148,	66,	45,	75,	59,	149
	triTMS	313	14.9	298	147,	73,	133,	148,	299,	130,	45,	59,	149
p-Toluenesulfonic acid	monoTMS	244	18.8	229,	149,	147,	91,	165,	75,	73,	65,	230,	231
o-Sulfobenzoic acid	diTMS	346	21.4	331,	147,	73,	148,	45,	75,	135,	76,	149,	119
Sulfosalicylic acid	triTMS	434	23.3	419,	73,	420,	421,	251,	331,	45,	74,	147,	149
Sulfanilic acid	triTMS	389	23.6	374,	73,	229,	375,	221,	45,	75,	149,	376,	230

of this artifact has not been determined. It has, however, no relation to any of the sulfonic acids.

When analyzed in the combined GC—MS instrument, the sulfonic acids gave rise to sharp and symmetrical peaks. Two derivatives were found for taurine, sulfamic acid and cysteic acid, representing either one or two TMS groups attached to the amino group of these compounds. The conditions used favored the formation of an amino—diTMS group over the monoTMS form. For each of the other sulfonic acids only one derivative could be detected.

In Table I the methylene unit values and the 10 most abundant *m/e* fragments of the sulfonic acids are given. A characteristic feature of the mass spectra is a dominating M—15 fragment; this is the base peak for many of the derivatives.

The mass spectra of the two taurine derivatives and of isethionic acid di-TMS given in Fig. 1 illustrate the typical pattern of fragmentation for this class of compounds. The sulfonic group is present only in a small number of the fragments; most stem from the other part of the sulfonic acid molecules.

The response of the total ion current detector to the sulfonic acid derivatives is of the same magnitude as to other organic acids. The detector response to taurine (sum of both derivatives) was found to be 1.3 relative to that of nonanedioic acid.

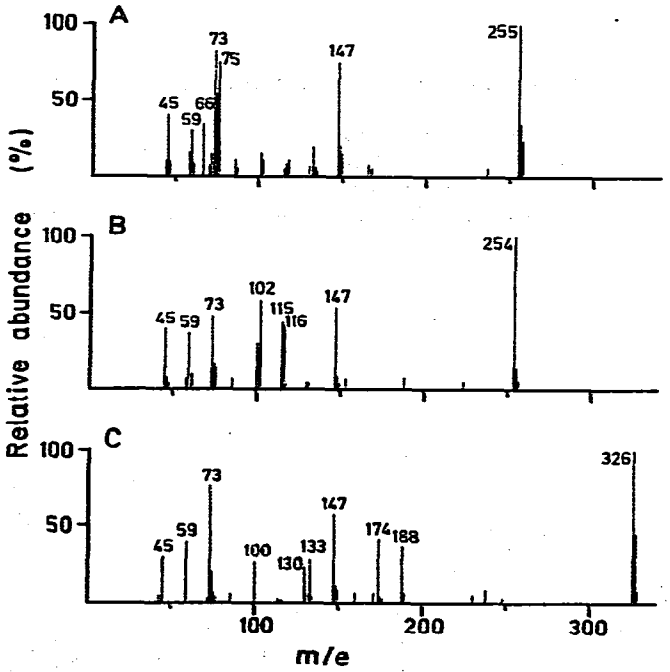

Fig. 1. Electron impact mass spectrum of (A) isethionic acid—diTMS, (B) taurine—diTMS, and (C) taurine—triTMS. The molecular ions are not visible. M—15 is base peak in all three spectra.

DISCUSSION

Sulfonic acids will easily form TMS derivatives, which are well separated by a standard gas chromatographic column. Trimethylsilylation has the advantage over methylation that a larger number of compounds becomes volatile by the former technique than by the latter. Of the nine acids analyzed in the present paper, only four could have been visualized as methyl esters (toluenesulfonic, sulfobenzoic, sulfosalicylic and isethionic acids). The sensitivity is good for both types of derivatives.

Methyl esters of the sulfonic acid are detected by the ordinary hydrogen flame ionization detector [5]. This detector is, however, insensitive to the TMS derivatives. The reason for this is unknown. Apart from containing a sulfonic group, the structures of the acids tested differed substantially from each other. It is, therefore, reasonable to conclude that the lack of response is related to some property of the sulfonic group, preventing the formation of charged radicals in the hydrogen flame.

It is difficult to understand how TMS derivatives of taurine and isethionic acid could have been detected by flame ionization in previously described methods [3, 4]. In both cases dimethylformamide was used as a solvent during derivatization. One possibility is that the artifact, which is formed when dimethylformamide is heated, has been misinterpreted and taken for the silylated derivative of the sulfonic acid in question.

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