Determination of 1-Alkene-1,3-sultones in Alkylethoxy Sulfates

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ABSTRACT

1-Alkene-1,3-sultones, which have been found as skin sensitizers in certain batches of alkylethoxy sulfates, are measured by an isotope dilution method, using a ^{13}C-labeled sultone. The sultone is recovered by thin layer chromatography and gas chromatography, and its dilution with unlabeled sultone is measured by mass spectrometry. Chlorosultones, also implicated as sensitizers, are determined at the same time by a dehydrohalogenation treatment that converts them to unsaturated sultones.

INTRODUCTION

A widespread outbreak of dermatitis in Scandinavia was attributed to an alkylethoxy sulfate (AES) that had been used as the surfactant in a washing product (1). More recent work (2) has shown that the AES itself was not the sensitizer, but rather than sensitization resulted from the presence in the AES of 1-dodecene-1,3-sultone (DDS) and 1-tetradecene-1,3-sultone, probably accompanied by 2-chlorododecane-1,3-sultone and 2-chlorotetradecane-1,3-sultone (3). Because all of these sultones are strong sensitizers (4), it was necessary to devise a way of detecting and determining them in AES and in alkyl sulfate, preferably at levels below 1 ppm.

This paper describes such an analytical method. The analysis depends on isotope dilution. A known quantity of 3-^{13}C-DDS is added as an internal standard to a sample of the AES being examined. The mixture is extracted with petroleum ether, and the extract is dehydrohalogenated to convert any chlorosultone that might be present to unsaturated sultone. The sultones are concentrated by two-stage thin layer chromatography (TLC), giving an overall concentration of ca. 10^5. The concentrate is finally examined by combined gas chromatography-mass spectrometry (GC-MS), and selected ions are monitored. Quantitation is achieved by comparing the response of the mass spectrometer at two masses, one characteristic of the internal standard and the other characteristic of the DDS to be assayed. The method is also applicable to alkyl sulfates.

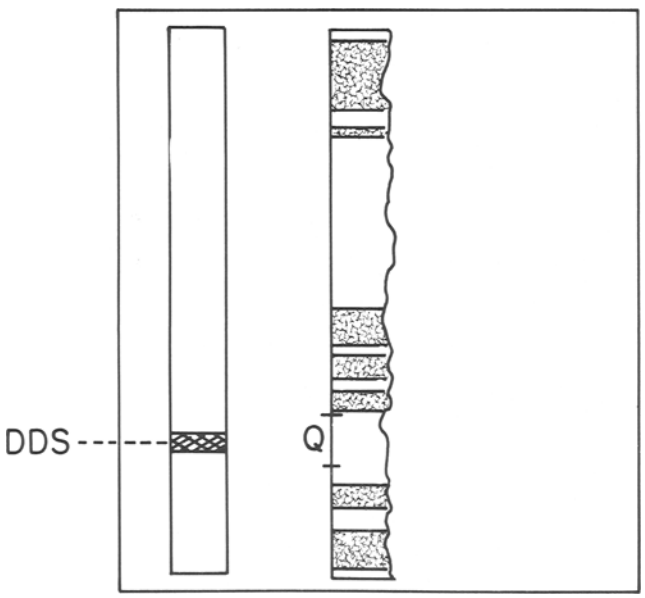

FIG. 1. Typical first stage chromatogram of extract from alkylethoxy sulfate (right lane), along with chromatogram of authentic 1-dodecene-1,3-sultone (DDS) (left lane). The silica gel G plate was developed in petroleum ether:ether (1:1).

EXPERIMENTAL PROCEDURES

Reagents

All solvents, acids and salts were purchased from Fisher Scientific Co. (Fairlawn, NJ) as certified grade, except the pentane, which was chromatographic grade, and petroleum ether, which was ACS grade. γ-Collidine from MCB Manufacturing Chemists (Norwood, OH) was redistilled before use. Ethanol was denatured, formula 3-A.

The 3-^{13}C-DDS was synthesized by the method of Connor et al. (3) from a mixture of 3-^{13}C-1-bromo-2-dodecene and 3-^{13}C-3-bromo-1-dodecene, which had been prepared in 90% isotopic purity by conventional methods at Stanford Research Institute.

Addition of ^{13}C-DDS

To a 50 ml sample of AES paste (usually containing 25-30% AES) was added 500 μg of ^{13}C-DDS in the form of 500 μl of a solution containing 1 mg of ^{13}C-DDS per ml of acetone.

Extraction

The 50 ml portion of AES was divided between two 2-liter separatory funnels and dissolved by adding 750 ml of 40% aqueous alcohol to each. After 25 g NaCl had been added and dissolved, each portion of the sample was extracted with three 500-ml and two 250-ml portions of pentane. The combined pentane extracts stood for 1 hr while water droplets settled and then were decanted and evaporated under reduced pressure at 40-45 C. The residue typically weighed ca. 800 mg.

Dehydrochlorination

For conversion of any chlorosultone in the extract to unsaturated sultone, the extract was heated for 2 hr at 96-98 C with 0.1 ml collidine under nitrogen. The mixture was taken up in 25 ml hexane and 25 ml ether and was washed 5 times with 15 ml 0.01 N HCl and 3 times with water. After being dried over sodium sulfate and filtered, the organic phase was evaporated under reduced pressure.

Thin Layer Chromatography

For the first stage of TLC, ca. 25 mg of the dehydrochlorinated extract in 0.4 ml acetone was applied as a uniform series of spots near the edge of a 20 x 20 cm prescored plate coated with a 250 μ layer of Silica Gel G (Analtech, Wilmington, DE), which had been prewashed in hexane. Eighteen plates were required for the analysis of each sample of AES. Plates were developed in petroleum ether:ether (1:1). After a plate was developed and dried, a 5 cm strip was broken from one edge, sprayed with 25% sulfuric acid, and charred on a hot plate at 260 C. This strip

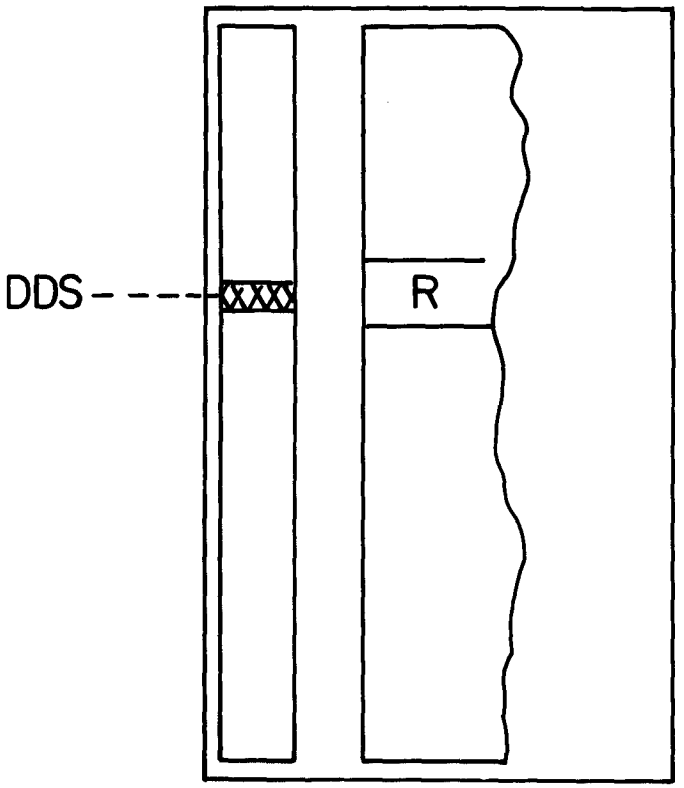

FIG. 2. Typical second stage chromatogram of isolate (right lane), along with 1-dodecene-1,3-sultone (DDS) (left lane). The silica gel G plate was developed in benzene:acetone (9:1).

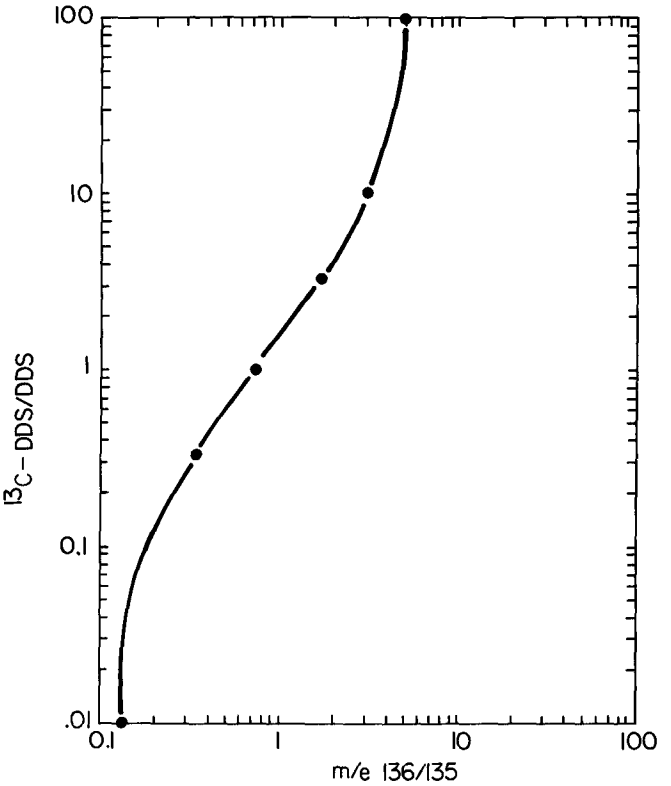

FIG. 3. Calibration curve showing ratio of added ^{13}C-DDS to native DDS in a sample as a function of the ratio of peak *m/e* = 136 to peak *m/e* = 135. DDS = 1-dodecene-1,3-sultone.

served as a guide for removal of the area that was known, from preliminary experiments, to contain the DDS. Figure 1 shows a typical chromatogram, along with the chromatogram of authentic DDS. The area of interest (Q) was scraped from each plate, and the composited material was covered with acetone, soaked for 5 min, and filtered. The insoluble material was washed twice with acetone, and the combined acetone solutions were evaporated to dryness under reduced pressure.

For the second stage of TLC, the concentrate from the first stage was spotted onto a 15 x 20 cm plate as described above. Simultaneously a 5 x 20 cm plate was spotted with 2 μl of a 0.1% solution of DDS in acetone. Both plates were developed together in benzene:acetone (9:1). The small plate was sprayed and charred as a guide in scraping the area of interest (R) from the large plate (Fig. 2). Material was recovered from the adsorbent as described above. The residue usually amounted to 0.1-0.5 mg, representing a concentration of 500,000-100,000 from the AES.

Gas Chromatography–Mass Spectrometry

The concentrate from TLC was taken up in bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) and injected into a 2mm x 3m glass column packed with 1% SE-30 on Gas Chrom Q (100-120 mesh), which was programmed from 150 C to 300 C at 5 C/min with a He flow rate of 30 ml/min. The injection port was at 300 C. Effluent from the column went into an LKB-9000 mass spectrometer which was equipped with a computer-generated variable accelerating voltage for monitoring of selected ions (5). The following masses, known to be characteristic of DDS (3), were monitored throughout the GC run: *m/e* 119, 121, 135, and 139. Masses characteristic of ^{13}C-DDS were simultaneously monitored: *m/e* 120, 122, 136, and 140. At the retention time of DDS, the ratio of the peak area for *m/e* 136 to the peak area for *m/e* 135 was determined. The corresponding ratio of ^{13}C-DDS to unlabeled DDS was read from the calibration curve (Fig. 3) which had been prepared using known mixtures of the two compounds. The concentration of DDS in the original sample was then calculated by dividing by this ratio the known concentration of ^{13}C-DDS that had been added to the sample.

The mass pair, *m/e* 135/136, was used for quantitation because these are the most abundant peaks in the region of the mass spectrum (above *m/e* 100) that is most characteristic of DDS. The remaining mass pairs, *m/e* 119/120, 121/122, and 139/140, could also be used for quantitation with appropriate calibration curves. The consistency of data resulting from these additional mass pairs was used primarily to confirm the absence of interfering compounds at the same retention time.

RESULTS AND DISCUSSION

The calibration graph used for these assays (Fig. 3) shows a marked departure from linearity. The curvature at the upper end is caused by the presence of ca. 10% of unlabeled compound in the synthetic labeled standard, and the reverse curvature at the lower end is caused by the presence of naturally abundant ^{13}C in the unlabeled DDS. Consequently, for maximum sensitivity with this method, the ^{13}C:^{12}C ratio in the sample should be between 10:1 and 1:10. Appropriate ratios are attained by adjusting the quantity of ^{13}C-DDS added to the sample; preliminary experiments indicate the level that should be used.

A few samples contained extraneous substances that had nearly the same separation characteristics as DDS, and that interfered with the reading of the ratios from the mass spectra. Such samples were handled by taking narrower cuts in the TLC separation, although to do this may sacrifice some sensitivity.

The sensitivity of the method is limited by GC rather than by MS. The maximum usable size of injection into the

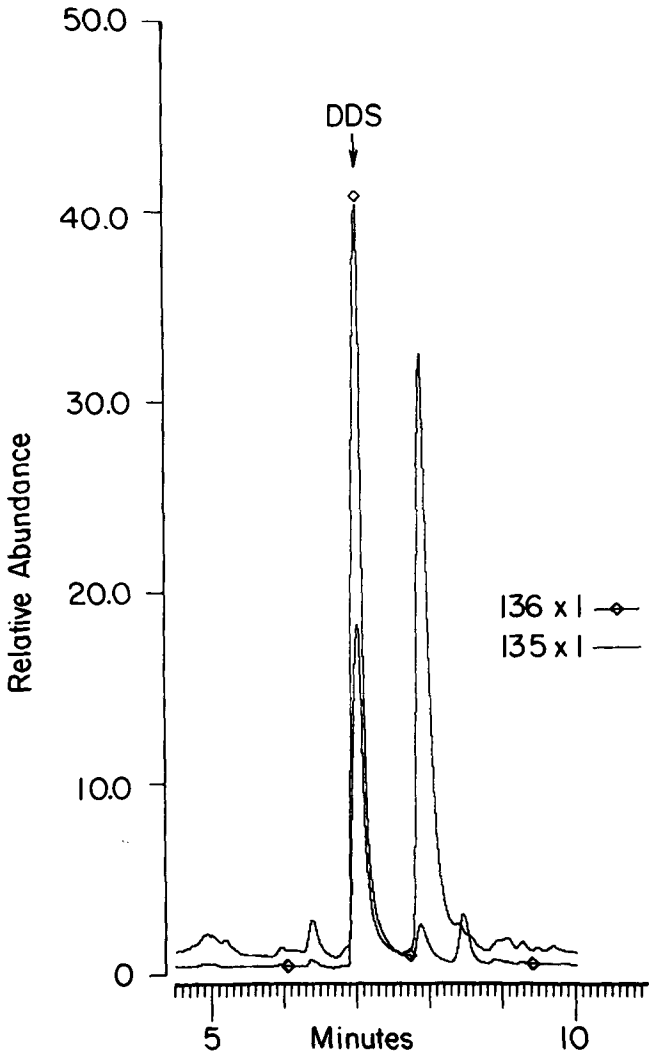

FIG. 4. Chromatogram of an alkylethoxy sulfate thin layer chromatography fraction as detected by measuring *m/e* 135 and *m/e* 136 fragments. DDS = 1-dodecene-1,3-sultone.

gas chromatograph is ca. 1 mg. For a usable peak to be seen, this injection must contain 200-500 ng of DDS, because appreciable amounts of DDS appear to be irreversibly adsorbed in the GC column. Therefore, if a maximum $^{13}C:^{12}C$ ratio of 10:1 is to be maintained, the minimum quantity of unlabeled DDS that can be measured with reasonable precision is of the order of 20-50 ng. Although DDS does not form a trimethylsilyl derivative, BSTFA + 1% TMCS was used as a solvent to keep the GC column conditioned and thus to minimize the adsorption problem.

An important feature of this method is that it measures halogenated sultones as well as unsaturated sultones. It is appropriate that both kinds of material be measured together, since they have similar biological effects. Dehydrohalogenation experiments on samples that had been deliberately enriched with 2-chlorododecane-1,3-sultone showed that the conversion of this chlorosultone to DDS is essentially quantitative under the conditions used.

Although the method is specific for DDS and 2-chlorododecane-1,3-sultone, the homologous sultones in a sample of AES can be estimated by assuming that the ratio of C_{12} sultones to homologous sultones is equal to the ratio of dodecylethoxy sulfate to homologous alkylethoxy sulfates in the material being analyzed, which ratio can be determined by familiar methods.

A number of samples of AES have been analyzed by this method. Results are shown in Table I. Figure 4 shows the chromatograms recorded for fragments *m/e* 135 and 136 from sample E. The measured ratio of the *m/e* 136 peak to the *m/e* 135 peak at the retention time of DDS (7.0 min) is 2.3, which gives a ratio (from the calibration curve) of ^{13}C-DDS:DDS = 5.4.

Samples A-C were from the batch of AES—referred to elsewhere (3) as LES 13-20355—that had been implicated in the original outbreak of dermatitis. The three samples had been stored under different conditions, and the different analytical results evidently reflect changes that had occurred in them during storage. Sample B had been stored for some time as the petroleum ether extract of the AES material, but the DDS content was calculated on the basis of the AES paste itself. The levels of sultone found in these samples are consistent with the levels that had been estimated from their biological activities on guinea pigs in 1974. Biological testing on guinea pigs during the period 1972-73, before the sensitizer had been identified, indicated that LES 13-2035 was losing activity with passing time. Although a precise measure of earlier activity cannot be made, the activity of LES 13-2035 is estimated to have been 3-10 times higher in 1972 than when it was analyzed

TABLE I

Determination of 1-Dodecene-1,3-Sultone (DDS) +(2-Chlorododecane-1,3-Sultone) in Alkylethoxy Sulfates

Sample	Concentration of added ^{13}C-DDS (ppm)	Measured ratio of ^{13}C-DDS to DDS	Concentration of DDS in sample (ppm)
A	10	3.0	3.3
B	10	2.1	4.8
C	10	6.4	1.6
D	10	20	0.5
E	1	5.4	0.2
F	1	1.1	0.9
G	0.2	3.2	0.06
H	0.2	> 20	< 0.01
I	0.2	10	0.02
J	0.2	> 20	< 0.01
K[a]	0.2	12	0.02
L[a]	0.2	> 20	< 0.01
M	0.1	> 10	< 0.01
N	0.1	0.17	0.6
O	0.1	9	< 0.01
P	0.1	> 10	< 0.01
Q	0.1	> 10	0.01
R	1	1.2	0.8

[a]Alkyl sulfate.

here; it may have been much higher even than that when the outbreak of dermatitis occurred in 1966.

Samples D through Q were evaluated in an attempt to define the processing conditions that might have given rise to the sensitizer. Most of them showed a DDS content markedly less than the DDS content of the material that had caused contact sensitization. Samples K and L were alkyl sulfates.

To establish the validity of the method, Sample R was prepared by adding 1 ppm of authentic DDS to a portion of Sample P. Analysis of this enriched sample showed 0.8 ppm DDS, representing an 80% recovery of the added DDS. The other values found in Table I are uncorrected.

The fatty alcohols that were used for making these various samples of surfactant came from three different sources: natural fat, oxo process, and Ziegler process. It appears that none of these sources contributed trace materials that interfere with the analysis.

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