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DERIVATIZATION OF DITHIOLS AND CERTAIN MONOTHIOLS WITH PHENYLARSINE OXIDE FOR GAS CHROMATOGRAPHY

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SUMMARY

Dithiols and certain monothiols reacted with phenylarsine oxide to give derivatives suitable for gas chromatography. Dihydrolipoic acid (6,8-dimercapto-*n*-octanoic acid) was thus determined after derivatization of the dithiol moiety to a dithiarsolane followed by extractive alkylation of the carboxyl group to the benzyl ester. 2,3-Dimercapto-1-propanol was converted into a dithiarsane, whereas bioxathiarsolane derivatives were obtained from dithiothreitol and dithioerythritol (the *threo* and *erythro* isomers of 1,4-dimercapto-2,3-butanediol) as each thiol group and its adjacent hydroxyl group in these compounds reacted with the arsenical. Mercaptoethanol and mercaptoacetic acid were also successfully derivatized for gas chromatography with phenylarsine oxide but α-toluenethiol and 1-dodecanethiol were not.

INTRODUCTION

Many thiol compounds are not suitable for direct gas chromatographic (GC) separation owing to their high polarity, thermal instability and sensitivity to oxidation. Such compounds may be analyzed after derivatization, and useful derivatives have been reported for monothiols[1–4]. Dithiols, however, pose problems due to steric hindrance of derivative formation, which we encountered in attempts to determine the biologically important compound dihydrolipoic acid (6,8-dimercapto-*n*-octanoic acid) by GC. We now report GC of dihydrolipoic acid (DHLA) after conversion of the dithiol moiety into the phenyldithiarsane (cyclic phenyldithioarsenite) with phenylarsine oxide, followed by extractive alkylation of the carboxyl group to the benzyl ester. The application of phenylarsine oxide to derivatization of other dithiols and monothiols was also studied.

EXPERIMENTAL

Reagents

DHLA was prepared by borohydride reduction[5] of lipoic acid obtained from E. Merck (Darmstadt, G.F.R.) which also supplied mercaptoacetic acid (thioglycolic

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acid). Dithiothreitol and dithioerythritol were products from Sigma (St. Louis, MO, U.S.A) whereas 2,3-dimercapto-1-propanol, 1-dodecanethiol, α-toluenethiol and mercaptoethanol were obtained from Fluka (Buchs, Switzerland). Phenylarsine oxide was purchased from Ega Chemie (Stannheim/Allbuch, G.F.R.) and benzyl bromide from E. Merck. The latter compound was purified by vacuum distillation before use. Tetrabutylammonium hydrogen sulfate (TBAHS) was obtained from Labkemi (Stockholm, Sweden) and purified by recrystallization from methyl isobutyl ketone.

Preparation of derivatives

Derivatization of DHLA was carried out as follows. A 0.2-ml aliquot of the DHLA containing sample was mixed with 0.2 ml of a 0.01 *M* solution of phenylarsine oxide in ethanol. After 10 min at room temperature, the ethanol was removed by evaporation *in vacuo* and 3.5 ml of water were added, followed by 0.2 ml of 0.18 *M* Na_2EDTA, 0.25 ml of 1 *M* TBAHS and 0.4 ml of 1 *M* $NaHCO_3$. The pH was adjusted to 9.0 with 2 *M* NaOH and the volume made up to 5.0 ml with water. To this mixture were added 2 ml of methylene chloride, containing 80 μmol of benzyl bromide and 10 nmol of cholesterol as an internal standard. Extractive alkylation was then performed at 37°C for 2 h with mechanical shaking (*cf.* ref. 6). The water phase was removed by aspiration and the organic phase placed under a stream of nitrogen to remove methylene chloride. The residue was dissolved in 2 ml of *n*-hexane and this solution was extracted with 3 ml of 0.01 *M* H_2SO_4 in order to remove interfering compounds. The organic phase was then dried in a stream of nitrogen and the residue dissolved in 25 μl of ethanol. Aliquots (2 μl) of this solution were used for GC.

Other derivatives were prepared by mixing a 0.005 *M* solution of the dithiol or monothiol in ethanol with an equal volume of a 0.01 *M* ethanolic solution of phenylarsine oxide.

Apparatus

A Hewlett-Packard 5750 G2 and a Hewlett-Packard 5700 gas chromatograph equipped with flame ionization detectors were used. The silanized glass columns (1.80 m × 3 mm I.D. and 1.20 × 2 mm I.D. respectively) were filled with 3% OV-17 on 100–120 mesh Chromosorb W HP. The flow-rate of nitrogen carrier gas was 35 ml/min. Samples were applied by on-column injection. Other operating conditions are indicated in figure legends.

Derivatives were identified on a Hewlett-Packard GC–mass spectrometry (MS) instrument 5981 in combination with the data processing system 5933 A. The glass column (0.90 m × 2 mm I.D.) contained 2% OV-101 on Chromosorb W HP, 100–120 mesh. Helium was used as carrier gas at a flow-rate of 30 ml/min. The ionization energy was 70 eV.

RESULTS AND DISCUSSION

In preliminary experiments we tried to derivatize DHLA by extractive alkylation with benzyl bromide using a technique previously[6] applied with success to certain monothiols also carrying a carboxyl group. Although the expected tribenzyl derivative of DHLA was obtained (as confirmed by MS), the yields were low and variable. Other methods for protecting the dithiol group of DHLA were consequently explored.

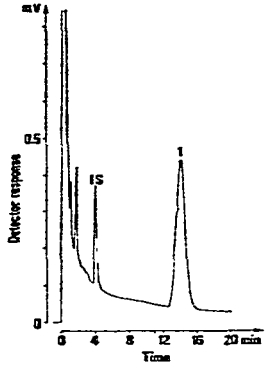

Fig. 1. Gas chromatogram of DHLA derivative. 1 = Reaction product of DHLA and phenylarsine oxide after benzylation. IS = Internal standard (cholesterol). Column: 1.80 m × 3 mm I.D. 3% OV-17. Temperatures: injector, 320°C; column oven, 300°C; detector, 340°C.

Dithiols are known to react with mono-substituted arsenicals to give very stable products[7], but their applicability to GC has apparently gone unnoticed. In fact, we found that after reaction with phenylarsine oxide, DHLA was amenable to extractive alkylation of the carboxyl group with benzyl bromide. The gas chromatogram in Fig. 1 shows the well-resolved peak of the derivatized DHLA. It should be noted that any phenylarsine oxide remaining in the sample leaves the column with the solvent front, as verified in separate experiments. The structure of the DHLA derivative was confirmed by MS (Fig. 2). The spectrum contains a significant molecular ion (*m/e* 448) and other fragments identified (*cf* refs. 6 and 8) are the base peak *m/e* 91 (benzylic cation or tropylium ion), 357 (M — 91), 296 (M — C_6H_5As) and 107 (AsS). The linear relationship between the amount of DHLA in the sample and the peak area of its derivative on GC is shown in Fig. 3.

Derivatization with phenylarsine oxide was also applied to other dithiols. The product obtained from the vicinal dithiol 2,3-dimercapto-1-propanol (BAL) gave

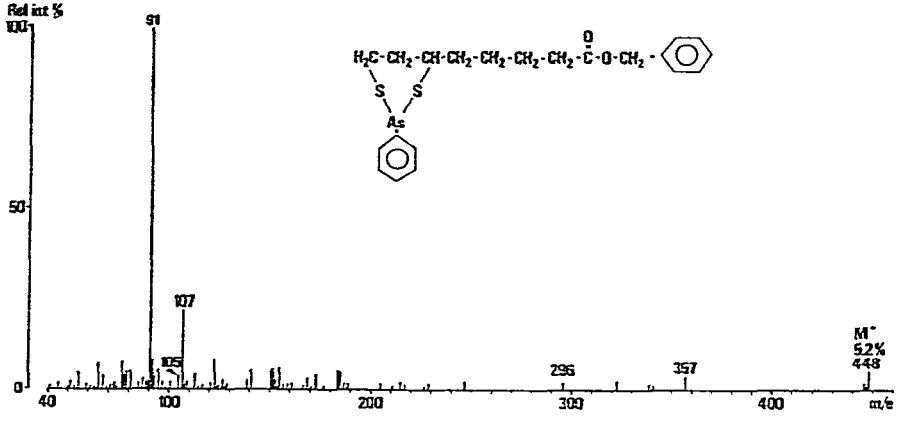

Fig. 2. Mass spectrum of DHLA derivative.

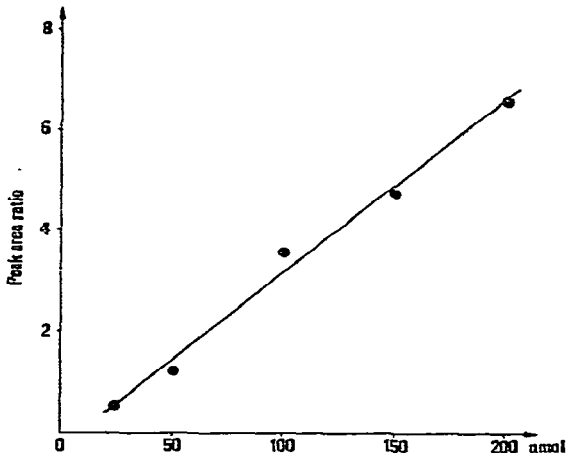

Fig. 3. Standard curve for GC of DHLA after derivatization.

a single GC peak, and its mass spectrum (Fig. 4) was compatible with the expected dithiarsane. A molecular ion at *m/e* 274 was thus observed, the base peak at *m/e* 107 was attributable to AsS, and other easily interpretable fragments were 216 (C_6H_5AsSS), 197 ($M-C_6H_5$) and 184 (C_6H_5AsS). However, the *threo* and *erythro* isomers of 1,4-dimercapto-2,3-butanediol[9] (dithiothreitol and dithioerythritol, respectively) gave unexpected results. Both compounds formed products, which gave single GC peaks with different retention times (Fig. 5) but with identical mass spectra. This indicated that each isomer of the dithiol formed a corresponding isomer of the derivative, which fragmentized by identical mechanisms in the mass spectrometer. The mass spectrum

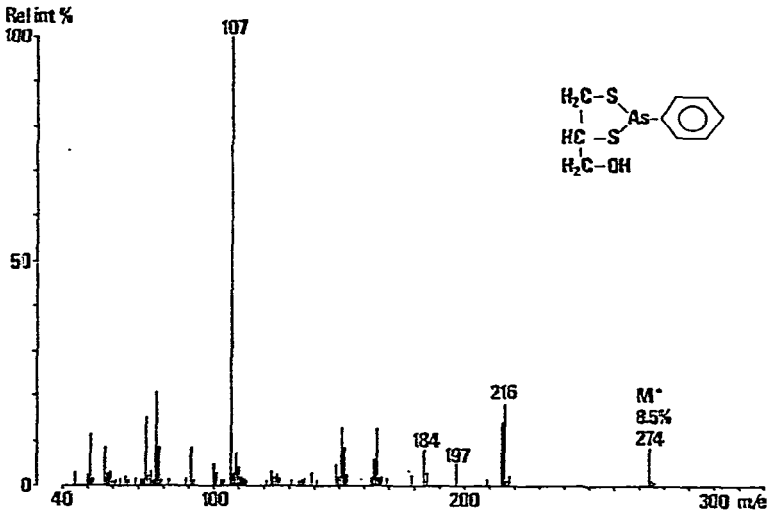

Fig. 4. Mass spectrum of BAL derivative.

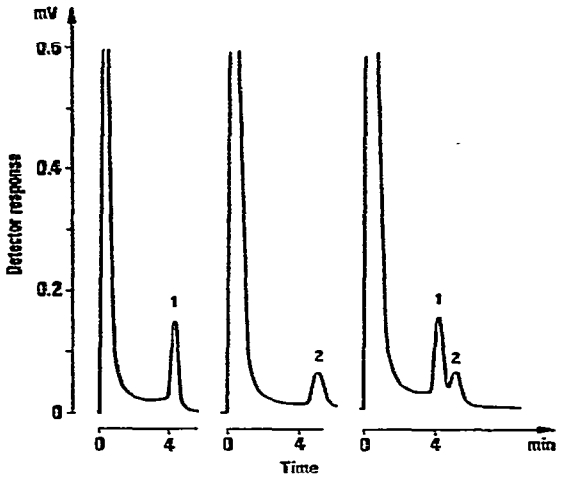

Fig. 5. Gas chromatograms of dithioerythritol (1) and dithiothreitol (2) derivatives. Column: 1.20 m × 2 mm I.D. 3% OV-17. Temperatures: injector, 300°C; column oven, 275°C; detector, 350°C.

(Fig. 6) showed a molecular ion at *m/e* 454, which was not compatible with that of the expected dithiarsepane (*m/e* 304) but indicated the formation of a phenyl-substituted bioxathiarsolane from one molecule of the dithiol and two molecules of phenylarsine oxide. The proposed structure of the derivatives is shown in Fig. 6. Other fragments supporting this structure (*cf.* ref. 8) were the base peak at *m/e* 107 (AsS), 377 ($M-C_6H_5$), 227 (M/2) and 91 (probably a mixture of the tropylium ion and AsO). It should be noted that dithiothreitol and dithioerythritol reacted at room temperature in ethanol solution with equimolar amounts of phenylarsine oxide as verified by spectrophotometric titration and determination of the sulfur and arsenic content of the isolated products. Under mild conditions the arsenical thus reacts only with the thiol groups to give dithiarsopane. A similar reaction between inorganic arsenite and dithiothreitol has earlier been reported[10]. The bioxathiarsolanes, obtained after GC, probably result

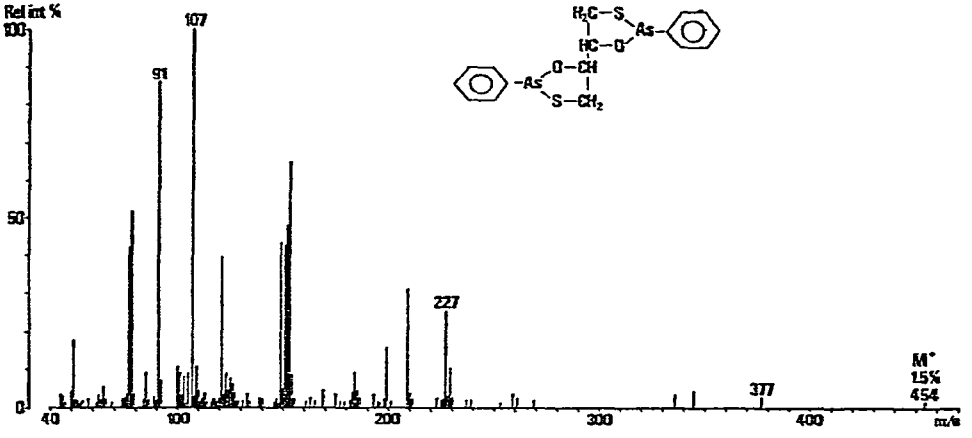

Fig. 6. Mass spectrum of dithioerythritol and dithiothreitol derivatives.

from a secondary reaction with excess phenylarsine oxide at the high temperature of the GC column.

Derivatization of monothiols with phenylarsine oxide was also attempted. The products from dodecanethiol and α-toluenethiol gave multiple peaks on GC, as explained by the known instability[7] of the dithioarsenites formed. With mercaptoethanol, however, a single, well-defined peak was observed on GC and its mass spectrum was identical with that earlier reported[8] for 2-phenyl-1,3,2-oxathiarsolane. This compound probably resulted from an on-column reaction of the dithioarsenite initially formed. In fact, $C_6H_5As(SCH_2CH_2OH)_2$ was found to be converted into the oxathiarsolane by prolonged heating[10]. Also 2-mercaptoacetic acid (thioglycolic acid) was found to give a single peak on GC after derivatization with phenylarsine oxide. The mass spectrum (Fig. 7) indicated the formation of an oxathiarsolane. Fragments identified were *m/e* 242 (M^+), 198 ($M-CO_2$), 152 (C_6H_5As), 107 (AsS) and the base peak 91 (mixture of tropylium ion and AsO).

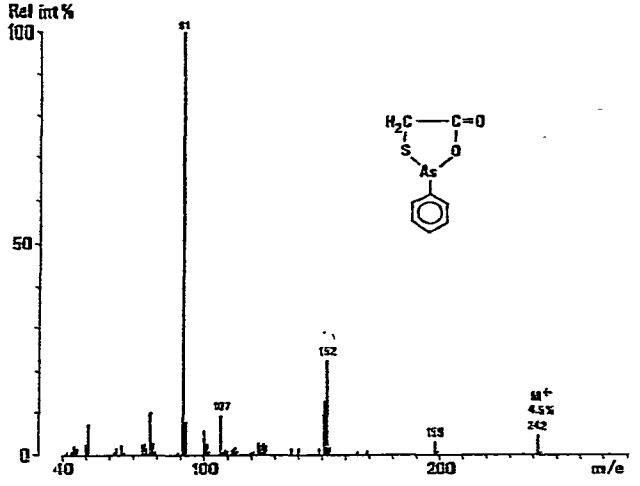

Fig. 7. Mass spectrum of mercaptoacetic acid derivative.

In conclusion, 1,2- and 1,3-dithiols reacted with phenylarsine oxide to give dithiarsanes and dithiarsolanes, respectively, which were suitable for GC separations. Derivatization with phenylarsine oxide of 1,4-dithiols also containing hydroxyl groups gave products amenable to GC, but secondary on-column reactions gave bioxathiarsolanes as the final products. Monothiols containing a hydroxyl or carboxyl group were also successfully derivatized for GC with phenylarsine oxide, but not simple monothiols.

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