An 801 Perkin-Elmer Gas Chromatograph was used. This is a dual-column temperature-programmed instrument with glass inlet and glass columns and a flame ionisation detector. The column packing was 20% Apiezon K on Kieselguhr ("Embacel") and the flow rate of the carrier gas, nitrogen, was 38.5 c.c./min. The inlet and detector were maintained at 250°.

Under these conditions an isothermal chromatogram of an acetone solution of bisphenol A at 250° (Fig. 1a) shows a retention time for bisphenol of 10.8 min.

One possible reason for the failure of previous attempts at direct gas chromatography of bisphenol A is the use of a metal injector and/or metal columns. Bisphenol A when heated in the presence and absence of nichrome wire for 30 min at 250° is partially degraded (Fig. 1b and c, respectively). The formation of phenol and isopropylphenol under these conditions is accelerated by metal. Another possible reason for previous lack of success is acid or base catalysis of the degradation of bisphenol A[4]. Thus, incorporation of phosphoric acid into the liquid phase, which has been done when analysing for bisphenol A[3], could also accelerate the degradation. Fig. 1d shows that when bisphenol A is heated with a trace of phosphoric acid in vacuum at 250° for 5 min it is completely destroyed.

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1 L.-H. LEE, *J. Polymer Sci.*, A2 (1964) 2859.
2 L.-H. LEE, *J. Appl. Polymer Sci.*, 9 (1965) 1981.
3 H. H. GILL, *Anal. Chem.*, 36 (1964) 1201.
4 V. H. SCHNELL AND H. KRIMM, *Angew. Chem.*, 75 (1963) 662.

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Separation of mercapturic acids and related compounds by gas chromatography and a method for the determination of hippuric acid

Mercapturic acids (N-acetyl S-substituted cysteines) are of interest because they are formed *in vivo* as metabolites of certain foreign organic compounds. Many halogeno-alkanes are precursors, in rabbits and rats, of alkyl mercapturic acids which are excreted in the urine[1,2]. In addition to mercapturic acids some halogeno-alkanes form further sulphur-containing metabolites. BARNSLEY[3], for example, found that bromopropane formed N-acetyl-S-2-hydroxypropylcysteine and JAMES AND JEFFERY[4] thought a similar compound from bromobutane was a hydroxy derivative of butylmercapturic acid. As the isolation of these compounds and their identification by paper chromatography was difficult the possibility of separating mercapturic acids and their derivatives by vapour phase chromatography has been investigated. The behaviour of hippuric acid, a normal urinary constituent, has also been examined on the gas chromatogram and a method worked out for its determination.

Materials

Butyl-, pentyl-, hexyl-, heptyl- and octylmercapturic acids were synthesised as described by BRAY *et al*[2].

Propylmercapturic acid, and the dicyclohexylammonium salts of 2-hydroxypropylmercapturic acid ,3-hydroxypropylmercapturic acid and 2-hydroxy-1-methylethylmercapturic acid were gifts from Dr E. BARNSLEY. 2-Hydroxybutyl-L-cysteine was prepared by the interaction of butylene oxide and cysteine and acetylated to give 2-hydroxybutylmercapturic acid; the mercapturic acid was also prepared from butylene oxide and N-acetylcysteine. The hydroxymercapturic acid was isolated as the dicyclohexylammonium salt, m.p. 146–148° (Found: C, 60.6; H, 9.3; N, 6.5; S, 7.8. $C_{21}H_{40}N_2O_4S$ requires: C, 60.6; H, 9.6; N, 6.7; S, 7.7 %) and was not separated into diastereoisomers. N-Tosylbutylcysteine, prepared by the action of *p*-toluenesulphonylchloride on butylcysteine, had m.p. 78° (Found: S, 19.2; $C_{14}H_{21}NO_4S_2$ requires S, 19.4 %). N-Tosyl-2-hydroxybutyl-L-cysteine was prepared as described by JAMES AND JEFFERY[4]. The sulphoxides of ethyl- and propylmercapturic acids were a gift from Dr BARNSLEY; other sulphoxides were prepared by oxidation of the mercapturic acid in ethereal solution with hydrogen peroxide. The sulphoxide of butylmercapturic acid had m.p. 123–124° (Found: S, 13.8; $C_9H_{17}NO_4S$ requires S, 13.6 %). Hexylmercapturic acid sulphoxide had m.p. 106–108° (Found: S, 12.4. $C_{11}H_{21}NO_4S$ requires S, 12.2 %). Octylmercapturic acid sulphoxide had m.p. 115–118° (Found: S, 11.24. $C_{13}H_{25}NO_4S$ requires S, 11.0 %).

The free acids were liberated from the dicyclohexylammonium salts by passing a solution of the salt down a column of Zeo-karb 225. The solutions obtained were evaporated to dryness and the residues methylated by the addition of diazomethane to

TABLE I

RETENTION TIMES OF THE METHYL ESTERS OF SOME MERCAPTURIC ACIDS AND RELATED COMPOUNDS DETERMINED IN THE FLAME IONISATION CHROMATOGRAPH

Methyl ester of	*Retention time in min at*		
	190°	*205°*	*220°*
N-Acetylcysteine	1.4		
Propylmercapturic acid	2.8	1.28	
Butylmercapturic acid	3.44	1.62	
Pentylmercapturic acid	4.24		
Hexylmercapturic acid	5.44		
Heptylmercapturic acid	6.88		
Octylmercapturic acid	8.8		
2-Hydroxypropylmercapturic acid	5.6	2.72	
3-Hydroxypropylmercapturic acid	8.8	3.6	
2-Hydroxy-l-methylethylmercapturic acid		2.56	
2-Hydroxybutylmercapturic acid	7.21	3.9	
N-Tosylbutyl-L-cysteine		5.20	
N-Tosyl-2-hydroxybutyl-L-cysteine		7.48	
Ethylmercapturic acid sulphoxide			3.2
Propylmercapturic acid sulphoxide			4.0
Butylmercapturic acid sulphoxide			4.96
Hexylmercapturic acid sulphoxide			8.0
Octylmercapturic acid sulphoxide			12.6
Hippuric acid	3.52	1.70	

their ethereal or ethanolic–ethereal solutions. Other free acids were methylated in the same way.

Method

A Pye series "104" dual flame ionisation chromatograph (W.G. Pye & Co. Ltd., Cambridge) was used for the chromatography; the two 5 ft. long columns of internal diameter 4 mm were packed with 5 % QF_1 on acid-washed silanised Chromosorb W (Wilkens Instrument & Research (U.K.) Ltd., Manchester). The argon flow was 45 ml/min.

Results

The retention times of the compounds examined are given in Table I and in Fig. 1 the log of the retention time has been plotted against the number of carbon atoms in the alkyl chain for the homologous series of alkylmercapturic acids and alkylmercapturic acid sulphoxides.

Determination of hippuric acid. This method was designed to determine the amount of hippuric acid excreted in urine. The column operated at 170° and hexylmercapturic acid was used as an internal standard. The retention times for the methyl esters of hippuric acid and hexylmercapturic acid were 4.8 and 8.6 min, respectively.

Calibration curve. Weighed amounts of hippuric acid and hexylmercapturic acid were dissolved in ether and esterified with diazomethane. The solutions of the esters were then made up to a known volume. From these standards solutions were prepared containing equal amounts of the two esters. Amounts of these solutions corresponding to from 10 to 100 μg of acid were applied to the column. Good agreement between the areas of the peaks was obtained over the range 10–50 μg and at 100 μg the error was less than 10 %.

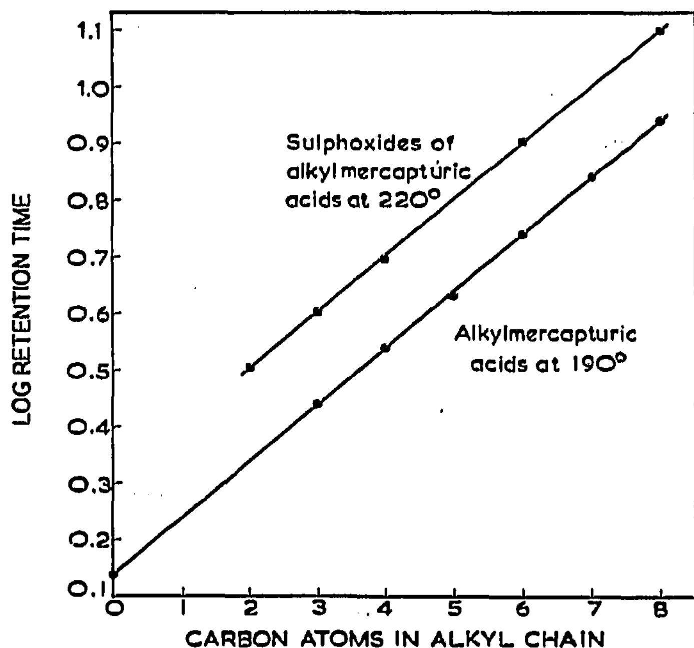

Fig. 1. Plot relating the log of the retention time on a column of 5 % QF_1 on silanised Chromosorb W of the methyl esters of alkylmercapturic acids and their sulphoxides to the number of carbon atoms in the alkyl chain.

Preparations of urine samples. Individual rabbit urine was collected for 24 h and the volume made up to 500 ml. Duplicate samples (20 ml) were adjusted to pH 2 and continuously extracted with ether for 12 h. The extracts were dried over anhydrous $CaSO_4$ and then esterified by the addition of excess ethereal diazomethane. The filtered solutions were evaporated and the residual ester made up to 20 ml in ether. Samples of these solutions were mixed with a known amount of methyl hexylmercapturate and the mixture was applied to the column. The amounts of methyl hippurate were calculated from the areas of the peaks. The recovery of hippuric acid added to normal urine was 100 ± 5 %.

Conclusions

The mercapturic acids and their derivatives are easily separated with good resolution under the given conditions. The plot of the log of retention times against the number of carbon atoms in the alkyl chain shows a satisfactory linearity in the homologous series of the alkylmercapturic acids and their sulphoxides. It was shown previously[2] that a similar linearity was obtained for the R_M values for the alkylmercapturic acids by paper chromatography. The technique of vapour phase chromatography offers advantages in the separation and identification of acids excreted as metabolites in urine. Hippuric acid can easily be distinguished from the mercapturic acid and hydroxymercapturic acids present in the urines of animals dosed with some bromoalkanes and can readily be determined by the method.

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1 T. H. GRENBY AND L. YOUNG, *Biochem. J.*, 75 (1960) 28.
2 H. G. BRAY, J. C. CAYGILL, S. P. JAMES AND P. B. WOOD, *Biochem. J.*, 90 (1964) 127.
3 E. A. BARNSLEY, *Biochem. J.*, 93 (1964) 15P.
4 S. P. JAMES AND D. J. JEFFERY, *Biochem. J.*, 93 (1964) 16P.

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A simple device for the automatic introduction of samples in gas–liquid chromatography

PODMORE[1] described an electromagnetic device for the automatic introduction of samples to a gas-chromatographic column. A mechanical device is commercially available (Barber-Colman Co.; see THOMAS[2]). The former technique presents no problem with the isolation of the column from the atmosphere, but requires that samples