Gas chromatography of methyl derivatives of some barbiturates

With the increasing therapeutic use of barbiturate mixtures, it would be valuable to have an analytical method in forensic chemistry for identifying individual acids in these mixtures. Paper chromatography has been used for the purpose[1] but hitherto it has not been possible to separate the various malonylureas by gas chromatography because they decompose at high temperatures. JANÁK[2] made use of this property by separating the pyrolytic products of the acids by gas chromatography. Characteristic elution patterns were obtained with single barbiturates but the method does not lend itself to identification of individual acids in a mixture since several of the breakdown products were common to several acids.

Experiments carried out with 1,3-dimethyl derivatives of the barbituric acids showed that these compounds were thermostable and in this paper a method is described for separating these derivatives by gas chromatography.

Experimental

Initial attempts to separate the barbituric acids by gas chromatography proved unsuccessful, but after methylation with diazomethane satisfactory elution patterns were obtained. The 1,3-dimethyl derivatives were prepared as follows:

An excess of diazomethane in ethereal solution was poured on to the solid barbituric acid or its sodium salt in a test tube and allowed to stand overnight at room temperature in the fume cupboard. The ethereal solution was washed twice with an equal volume of saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulphate and filtered. The ether was evaporated under reduced pressure leaving the methylated barbiturates either as viscous liquids or solids of low melting point.

Gas chromatography

Both apiezon L and Polyethylene Glycol Adipate[3] were used as stationary phases. The column support was Celite 545 prepared as described by ORR AND CALLEN[4]. The proportions, mesh sizes of the column pack and the operating conditions are as described in Table I. Columns of 130 cm by 4 mm were packed by vibration. The carrier gas was Argon and a Strontium 90 ionisation detector[5] was used. Samples were transferred to the columns with a micro pipette, the solid specimens being melted on the boiling water bath.

TABLE I

Stationary phase	*Mesh size of Celite 545*	*% stationary phase to support medium*	*Column working temperature °C*	*Argon*	
				Inlet pressure cm Hg	*Flow rate ml/min*
Apiezon L	60/80	15	197	96	86
Polyethylene glycol adipate	30/60	10	180	40	46

Retention volumes were calculated relative to Quinalbarbitone and the results are shown in Table II. Typical elution patterns are shown in Figs. 1 and 2.

TABLE II

Barbiturate		*Relative retention volumes*	
Pharmaceutical name	*Chemical name*	*Apiezon*	*Polyethylene glycol adipate*
Barbitone	5,5-Diethylbarbituric acid	0.37	0.51
Allobarbitone	5,5-Diallylbarbituric acid	0.48	0.72
Butobarbitone	5-Ethyl-5-*n*-butyl-barbituric acid	0.63	0.72
Amylobarbitone	5-Ethyl-5-isoamyl-barbituric acid	0.72	0.72
Pentobarbitone	5-Ethyl-5-(1-methylbutyl)-barbituric acid	0.87	0.85
Quinalbarbitone	5-Allyl-5-(1-methylbutyl)-2-barbituric acid	1.0	1.0
Hexobarbitone	5-(1-Cyclohexen-1-yl)-3,5-dimethyl-barbituric acid	1.98	3.87
Cyclobarbitone	5-Ethyl-5-(1-cyclohexen-1-yl)-barbituric acid	2.3	3.31
Phenyl-methyl-barbituric acid	5-Methyl-5-phenyl-barbituric acid	1.98	6.35
Phenobarbitone	5-Ethyl-5-phenyl-barbituric acid	2.3	5.38
Thiopentone	5-Ethyl-5-(1-methylbutyl)-2-thio-barbituric acid	3.76	1.41
		0.87	0.85
		1.98	3.87
		2.3	5.38
			3.31

Discussion

In order to identify the full range of barbiturates selected, two types of stationary phase were necessary for gas chromatography. The first, Apiezon L, separated completely those barbiturates with different alkyl substituents in the 5,5 positions according to chain length, those with shorter side chains being eluted earlier. Branching of the chain had the effect of shortening it so that Amylobarbitone was eluted before Pentobarbitone. On polyethylene glycol adipate the resolution of these alkyl derivatives was poor and Allobarbitone, Amylobarbitone and Butobarbitone emerged as a single peak.

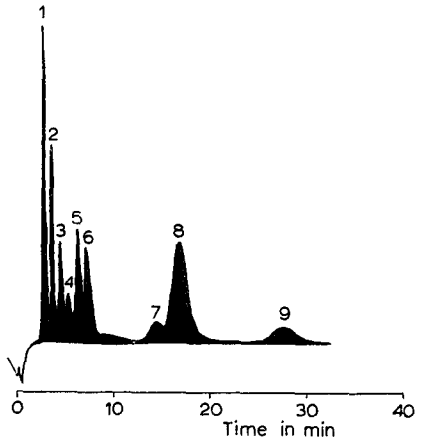

Fig. 1. Elution pattern on Apiezon L. 1 = Barbitone; 2 = Allobarbitone; 3 = Butobarbitone; 4 = Amylobarbitone; 5 = Pentobarbitone; 6 = Quinalbarbitone; 7 = Hexabarbitone; 8 = Phenobarbitone; 9 = Thiopentone.

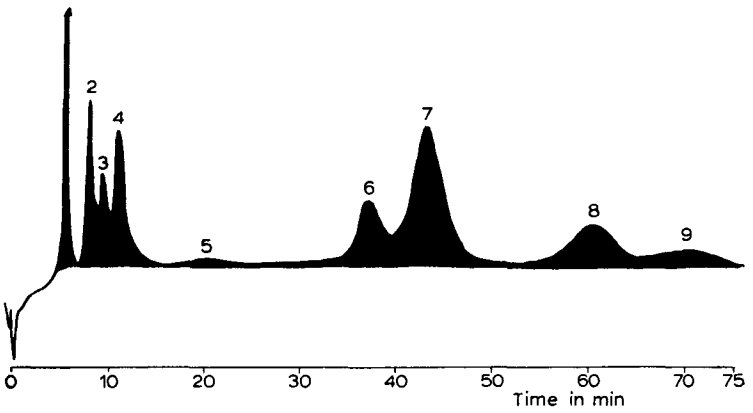

Fig. 2. Elution pattern on polyethylene glycol adipate. 1 = Barbitone; 2 = Allobarbitone, Butobarbitone, Amylobarbitone; 3 = Pentobarbitone; 4 = Quinalbarbitone; 5 = Unidentified impurity; 6 = Cyclobarbitone; 7 = Hexobarbitone; 8 = Phenobarbitone; 9 = Phenyl-methyl-barbituric acid.

On both types of column Thiopentone gave more than one peak, only one of which was characteristic. On Apiezon L it gave four peaks three of which were coincident with those of Pentobarbitone, Hexobarbitone and Phenobarbitone. On glycol adipate there was also a fifth peak in the Cyclobarbitone position. The occurrence of a peak in the Pentobarbitone position is readily explained since it is the oxygen analogue of Thiopentone but the reason for the occurrence of the other peaks is not clear, possibly they are due to pyrolytic decomposition of the more labile sulphur analogue.

Separation of phenyl and cyclohexenyl derivatives was only achieved by using polyethylene glycol adipate. Separation on this type of column depends on polarity with the result that the cyclical barbiturates emerged in the following order: Cyclobarbitone, Hexabarbitone, Phenobarbitone and Rutanol. On Apiezon L Cyclobarbitone and Phenobarbitone emerged as a single peak after the combined Hexobarbitone and phenyl-methyl-barbituric acid peak.

Methylation takes place by substitution in the 1 and 3 positions of the barbituric acid nucleus. For this reason it is impossible to separate barbituric acid and N-methyl-barbituric acid derivatives with identical groups in the 5,5 positions, *e.g.* Phenobarbitone and Prominal. Ethylation instead of methylation may allow such separations but this has not yet been explored.

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Notes

Paper chromatography of 2,4-dinitrophenylhydrazones. Extension of the Huelin method

During the course of certain studies on the carbonyl components of cigar tobacco smoke, it became necessary to separate these compounds as their 2,4-dinitrophenylhydrazones (DNPH's). For this purpose the paper chromatographic method of HUELIN[1] was selected and used with only slight modifications.

Admittedly, there have been newer methods for 2,4-dinitrophenylhydrazones published since the work of HUELIN and, as MACEK states[2], this method may no longer be in great use. Initially, several of these newer techniques were tried, but they failed to give satisfactory results in our hands. For example, one method[3] failed to give sufficient movement or discrete spots, while another[4] was not reproducible due to lack of information from the authors. The precoated-paper systems[5–7] seemed too troublesome to control. In contrast, the method of HUELIN was found to be very simple, highly reproducible and provided good separation and spot formation.

To establish R_F values under our own conditions, some 25 known DNPH's were investigated. Since HUELIN'S paper dealt with only a limited number of DNPH's, it seemed advisable to submit our data as an extension of the original work. These are presented in Table I. Each R_F value listed is the average of several runs on different days. The reproducibility was generally good, varying only a few hundredths, except in the cases where a range is given. The only compound presenting any difficulty was propanal-DNPH, which exhibited extremely poor reproducibility.

The variation between these data and those of HUELIN may be attributed to differences in operating conditions. The temperature was probably the most significant factor in this; HUELIN failed to mention any operating temperature.

In the present work, chromatograms were run in a cylindrical glass jar 22 cm in diameter and 46 cm high. The solvent container at the bottom was a crystallizing dish 15×7.5 cm in size. Whatman No. 1 paper was used in sheets 42×30 cm. Samples were placed along a line 2 cm from the bottom and 3 cm apart. The solvent system