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GAS CHROMATOGRAPHIC STUDIES OF PHENOBARBITAL AND DIPHENYLHYDANTOIN AFTER FLASH-HEATER ALKYLATION

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SUMMARY

The functionally excellent flash-heater methylation, ethylation and butylation techniques have been extended to include the higher alkyl homologs. Phenobarbital and diphenylhydantoin have been alkylated using the tetraalkylammonium hydroxides in which the alkyl group ranges from methyl to hexyl. The gas chromatographic properties of the resulting alkyl derivatives have been investigated.

INTRODUCTION

Several gas chromatographic methods for determining the anticonvulsant drugs diphenylhydantoin (DPH) and phenobarbital (PB) make use of their alkyl derivatives. During alkylation, these polar compounds are converted into suitable non-polar derivatives, thereby reducing irreversible adsorption to the solid support, contamination of the column and peak tailing, all of which contribute to an improved analysis.

Present alkylation techniques have been limited to methylation[1–10], ethylation[11] and, most recently, butylation[12,13]. As the use of other alkylating agents remains to be exploited, we decided to investigate the gas chromatographic properties of DPH and PB derivatives formed in the flash heater by several alkylating agents. Derivatives of the higher alkyl homologs may prove to be more practicable than those currently in use.

EXPERIMENTAL

Reagents

DPH and 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) both $\geq 99\%$ pure were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). PB (U.S.P. grade) and chloroform (nanograde) were obtained from Mallinckrodt (St. Louis, Mo., U.S.A.).

Solutions

Stock DPH–PB standard. Amounts of 100 mg each of DPH and PB were dissolved in 100 ml of absolute ethanol. This stock solution was stored at 4°.

Working DPH–PB standard. A 1.0-ml volume of the stock DPH–PB standard was diluted to 100 ml with absolute ethanol to give a solution containing 10 μg/ml each of DPH and PB. This working solution was prepared fresh just prior to use.

Stock MPPH standard. As MPPH is widely used as an internal standard for DPH analyses[5,6,10], it was also included in our studies. A 150-mg amount of MPPH was dissolved in 200 ml of chloroform. This stock solution was stored at 4°.

Working MPPH standard. A 0.5-ml volume of the stock MPPH standard was diluted to 250 ml with chloroform to give a solution containing 1.5 μg/ml of MPPH.

Preparation of alkylating agents

Tetraalkylammonium hydroxides (0.2 *M*) were used as alkylating agents and were prepared from their corresponding tetraalkylammonium iodides, obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). The appropriate tetraalkylammonium iodide (0.02 mole) was dissolved in 100 ml of absolute methanol, 3.5 g of finely divided silver oxide were added to the methanolic solution and the resulting slurry was mixed for at least 1 h at 20° so as to precipitate completely silver iodide. The mixture was then filtered and the filtrate, consisting of a 0.2 *M* methanolic solution of tetraalkylammonium hydroxide, was stored in well stoppered, amber-colored glass bottles. In this work, the following alkylating agents were prepared and studied: tetramethylammonium, tetraethylammonium, tetrapropylammonium, tetrabutylammonium, tetrapentylammonium, tetrahexylammonium and tetraheptylammonium hydroxides.

Apparatus

Gas chromatography was carried out using a Perkin-Elmer Model 3920 instrument equipped with a hydrogen flame ionization detector and a Perkin-Elmer Model 56 recorder with a range of 0–1 mV and a chart-speed of 5 mm/min. Glass columns 6 ft. long × ¼ in. O.D. packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh, were used. The columns were conditioned by heating them at 100° for 1 h and increasing the temperature at the rate of 1°/min until the temperature reached 275°, at which temperature it was maintained for 18 h. The detector end of the column was disconnected during the conditioning period.

The operating conditions were as follows: nitrogen carrier gas at a flow-rate of 30 ml/min; hydrogen produced by a Protran 150-A generator (Trienco, Raleigh, N.C., U.S.A.) at 21 p.s.i.; air at 50 p.s.i.; injector temperature, 300°; oven temperature, 250°; detector temperature, 300°; attenuation, ×16; range, ×10; recorder, 1 mV full-scale.

Procedure

A 1.0-ml volume of the working DPH–PB standard representing 10 μg each of DPH and PB was placed into a 15-ml glass conical centrifuge tube. To this were added 7.0 ml of the working MPPH standard. The final solution was then evaporated to dryness under a stream of nitrogen at 35–40°, and 25 μl of the 0.2 *M* tetraalkylammonium hydroxide (methyl, ethyl, propyl, butyl, pentyl, hexyl, or heptyl homolog) were added separately to the residues remaining after evaporation. Then 1–2 μl of the resulting solution of alkylated compounds were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Fig. 1 presents a series of gas chromatograms showing the results obtained when PB, DPH and MPPH are treated with each of the homologous series of alkylating agents. Each component in the mixture is separated with no detriment to peak symmetry, regardless of the alkylating agent used. Minor secondary peaks occur with tetrapropyl, terabutyl and tetrapentylammonium hydroxides. The use of trialkylanilinium hydroxides rather than tetraalkylammonium hydroxides might have eliminated these minor peaks. Our attempts to synthesize anilinium compounds with alkyl chains of greater length than the ethyl group were unsuccessful, the failure being attributed to steric hindrance[14].

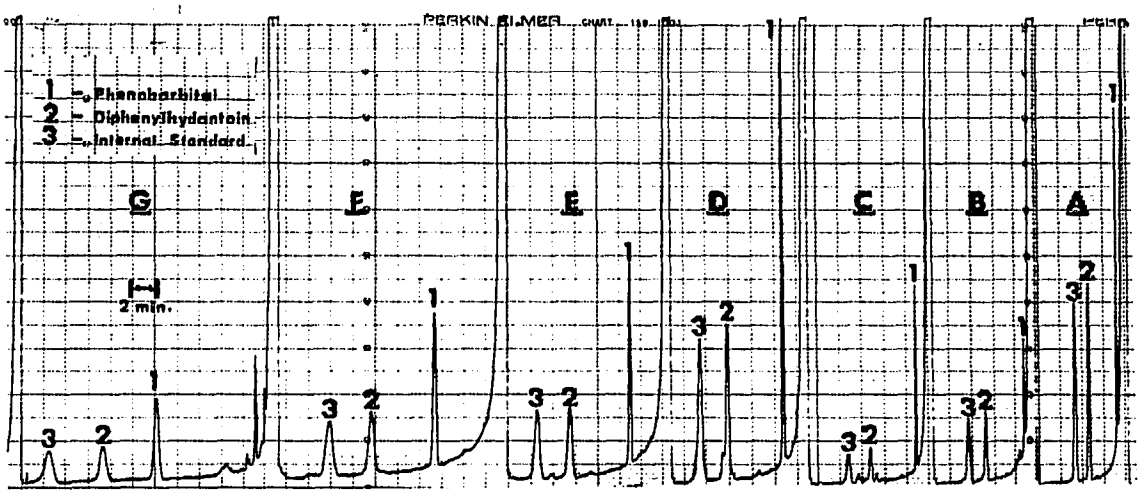

Fig. 1. Typical gas chromatograms of alkylated derivatives of PB (1), DPH (2) and MPPH as internal standard (3). Alkyl derivatives: methyl (A), ethyl (B), propyl (C), butyl (D), pentyl (E), hexyl (F) and heptyl (G).

With the lower homologs, PB emerges from the column too close to the solvent front to be of any analytical value. However, with increasing length of the carbon chain, it is well separated from the solvent front. This increase in retention time with increasing length of carbon chain is also evident with DPH and MPPH. Retention times (Table I) plotted on semi-logarithmic paper against the number of carbon atoms in the alkyl chain (Fig. 2) gave rise to straight lines, showing that the data follow the exponential growth law and that the increase in retention time for this series is proportional to the value already attained. The DPH and MPPH lines run parallel and close to one another, as would be expected, since the two compounds differ by only a methyl group.

Calculating the relative retention times (RRT) for both DPH and PB, using MPPH as the internal standard (Table I), we find that the RRTs for DPH are constant for the entire alkyl series studied, whereas those for PB change from C_5H_{11} through C_7H_{15}. This constancy of the RRTs for DPH indicates that both DPH and MPPH behave in a similar manner during alkylation, regardless of the alkylating agent used.

TABLE I

RETENTION TIMES AND RELATIVE RETENTION TIMES OF COMPONENTS RESOLVED IN FIG. 1

Alkyl group	*Retention time (min)*			*Relative retention time*	
	PB	*DPH*	*MPPH*	*PB*	*DPH*
CH_3	1.0	3.7	5.0	0.20	0.74
C_2H_5	1.0	4.5	6.0	0.17	0.75
C_3H_7	1.6	5.8	7.7	0.21	0.75
C_4H_9	2.4	7.4	9.7	0.25	0.76
C_5H_{11}	3.9	9.4	12.3	0.32	0.76
C_6H_{13}	6.6	12.2	16.0	0.41	0.76
C_7H_{15}	11.2	16.0	20.8	0.54	0.77

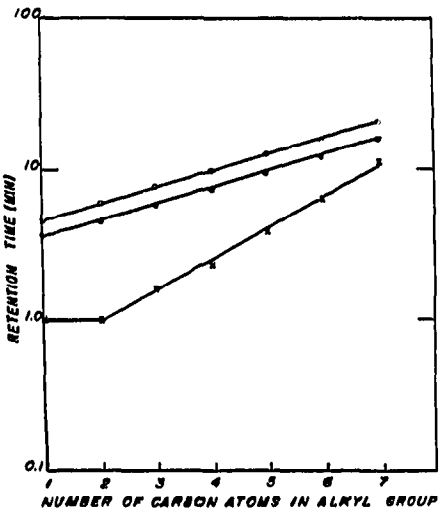

Fig. 2. Logarithmic plot of retention time as a function of the number of carbon atoms in the alkyl group for: ×, PB; ●, DPH; ○, MPPH.

This makes MPPH an excellent choice as an internal standard for DPH determinations.

These studies indicate several potential possibilities employing flash-heater alkylation with higher homologs. As the introduction of longer alkyl groups increases the retention time, it is possible to detect compounds that would otherwise have been lost in or near the solvent front. Also, the need for temperature programming can be eliminated if the retention time of a compound of interest has been sufficiently increased so as to be detectable at higher temperatures required for determining other components of a particular system. Further, in systems where the alkylation of two different compounds gives rise to the same derivative, such as occurs with the methylation of PB and mephobarbital, the introduction of a higher homolog can effect a satisfactory separation[11]. Finally, the use of higher homologs may eliminate the formation of extraneous peaks such as those found upon methylation of PB[1,8,10,15].

We feel that flash-heater alkylation with higher homologs will prove to offer distinct advantages in overcoming analytical limitations of alkylation using the lower homologs. We expect to see numerous applications being developed for this technique as it becomes more utilized in the future.

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