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Chromatographic examination of the *p*-nitrobenzoates and α-naphthylurethans of lower aliphatic alcohols

Gas chromatography (GC) has been commonly used for the analysis of the volatile components of alcoholic beverages as well as the derived distillates and fusel oils[1–4]. However, in many instances, the predominant lower aliphatic alcohols have been identified by retention time comparisons alone. In such complex mixtures the separation of individual alcohols on a preparative scale for conclusive identification by chemical and spectroscopic techniques is often difficult and only partially successful. However, complex mixtures of the alcohols can be subjected to further chromatographic examination after conversion to derivatives. Since the *p*-nitrobenzoates and α-naphthylurethans of the lower aliphatic alcohols are relatively stable and simple to prepare, these substances were selected for chromatographic investigation.

Paper chromatography has been used for the examination of various lower aliphatic alcohol derivatives[5–7] although chromatograms usually take relatively long times to obtain. Similarly one-dimensional thin-layer chromatography (TLC) has been investigated also, but only normal alcohol derivatives up to that of butanol were satisfactorily resolved[8]. More recently CHURÁČEK *et al.*[9] have successfully resolved lower aliphatic alcohols, as the coloured N,N-dimethyl-*p*-aminobenzeneazobenzoates, using both paper chromatography and TLC. GALETTO *et al.*[10] have used GC to resolve the lower alcohols as the 3,5-dinitrobenzoates.

Since, as a general rule, superior resolution and relatively rapid speed of analysis have been obtained with TLC and GC, these techniques were used in this investigation.

Experimental

Preparation of derivatives. Reference alcohols were commercial "Analytical Grade" products checked for purity by GC. Derivatives were prepared by the usual synthetic procedures and purified by recrystallisation. With crude mixtures, such as fusel oils, the reaction was performed after removal of water and the product analysed without further treatment.

Thin-layer chromatography. Silica gel (30% by weight; Macherey, Nagel and Co.; MN-Kieselgel G) was made into a slurry with water and spread on glass plates (5 × 20 cm) to a thickness of 0.3 mm using Shandon Unoplan apparatus. The plates were then air-dried at 20° for 16–20 h. The α-naphthylurethans were spotted onto the plates in acetone solution and developed to a distance of 12 cm from the origin in atmosphere saturated tanks at 20°. Developed plates were dried at 100° for 10 min and sprayed with 10% phosphomolybdic acid in ethanol. After 10 min at 100° the α-naphthylurethans appeared as dark blue spots on a yellow background. Two solvent systems were used and between fifteen and thirty replications of each compound made. Solvent 1 was petroleum ether (b.p. 80°–100°)–anhydrous diethyl ether–water (85:15:3), while solvent 2 was benzene–ethyl acetate (9:1). With solvent 1 after one development the plates were dried at room temperature and redeveloped before visualisation.

Gas chromatography. A Varian Model 1400 instrument fitted with 1/8 in. × 5 ft.

stainless-steel columns and a flame ionisation detector was used. Two liquid phases (OV-17 and SE-30) were absorbed (each 3% loading) on AW Chromosorb W (80–100 mesh) and used at temperatures of 130° and 125°, respectively, with N_2 as carrier gas at flow-rates of 25 and 15 ml/min, respectively. The detector and injector were maintained at 230° while the *p*-nitrobenzoates in a 5% (w/v) solution of carbon disulphide were injected (0.7 μl) into the gas chromatograph. Six replications of the R_t determinations for each compound were made.

Results and discussion

Although unsatisfactory for GC, the α-naphthylurethans of normal alcohols up to pentanol were resolved by TLC on silica gel (see Table I). The separation of the isomeric alcohols was limited as has been found in previous work with other derivatives. However, the derivatives used previously present greater difficulties in preparation than the α-naphthylurethans. Crude mixtures of alcohols, such as fusel oils, can be reacted to form the α-naphthylurethans and analysed directly without further purification.

TABLE I

CHROMATOGRAPHIC DATA

Parent alcohol	*TLC R_F values[a] of α-naphthylurethans*		*GC R_t values[b] of p-nitrobenzoates*	
	Solvent 1[c]	*Solvent 2[c]*	*OV-17 (130°)*	*SE-30 (125°)*
Methanol	0.61	0.77	0.45	0.42
Ethanol	0.85	0.91	0.62	0.61
n-Propanol	1.00	1.00	1.00	1.00
n-Butanol	1.11	1.07	1.69	1.67
n-Pentanol	1.15	1.11	2.84	2.63
n-Hexanol	1.21	1.14	—	—
i-Propanol	1.05	1.01	0.66	0.73
i-Butanol	1.09	—	1.26	1.33
i-Pentanol	1.17	—	2.25	2.30
sec.-Butanol	—	—	1.10	1.20
2 Methyl-*n*-butanol	1.17	—	2.14	2.18
Max. coeff. variation (%)[d]	1.6	1.6	1.4	1.9

[a] Relative to *n*-propyl-α-naphthylurethan = 1 (absolute R_F = 0.42 ± 0.08).
[b] Relative to *n*-propyl-*p*-nitrobenzoate = 1.
[c] See *Experimental*.
[d] $\frac{\text{Std. deviation}}{\text{Mean}} \times 100$.

The *p*-nitrobenzoates of the lower alcohols were found to be resolved by GC using packed columns with relatively low liquid phase loadings on the solid support. Reference retention times of the derivatives of a number of common alcohols are included in Table I. Conventional semilog plots of retention time against carbon number for those compounds forming a homologous series (*e.g.* normal and *iso* alcohol derivatives) were found to yield straight lines. Qualitative analyses of various fusel

oils were performed using this GC technique after reaction of the original crude mixture to form the *p*-nitrobenzoates.

It was found that, with comparatively large sample sizes, the use of low liquid phase loadings in this manner readily leads to overloading, *i.e.* production of elution curves exhibiting marked asymmetry. Increasing the size of injections onto the GC column leads to increased asymmetry of the resultant curves and a displacement of the curve maximum towards increased retention times. Thus if the retention time of a compound is measured from the maximum point on its elution curve it will exhibit an apparent increase. A quantitative measurement of the amount of overloading was taken as the difference between the apparent retention time as measured from an asymmetric elution curve and the normal retention time from a normal symmetric curve. To obtain comparative results this difference in retention times was divided by the normal retention time, giving an overloading factor,

$$i.e.\ \text{O/L factor} = \frac{\text{App. } R_t - \text{Norm. } R_t}{\text{Norm. } R_t}.$$

With the *p*-nitrobenzoates the quantity of the substance injected was plotted against the O/L factor exhibited by the resultant elution curve for a range of injection sizes. By extrapolation the maximum size of injection without causing excessive overloading was obtained (see Fig. 1). For a number of compounds this maximum was plotted against molecular weight. This indicated that the maximum decreased with increasing molecular weight in this series of compounds (see Fig. 2).

Therefore, as a general rule, overloading can be expected to be more evident with compounds having longer retention times, since these compounds fall into a

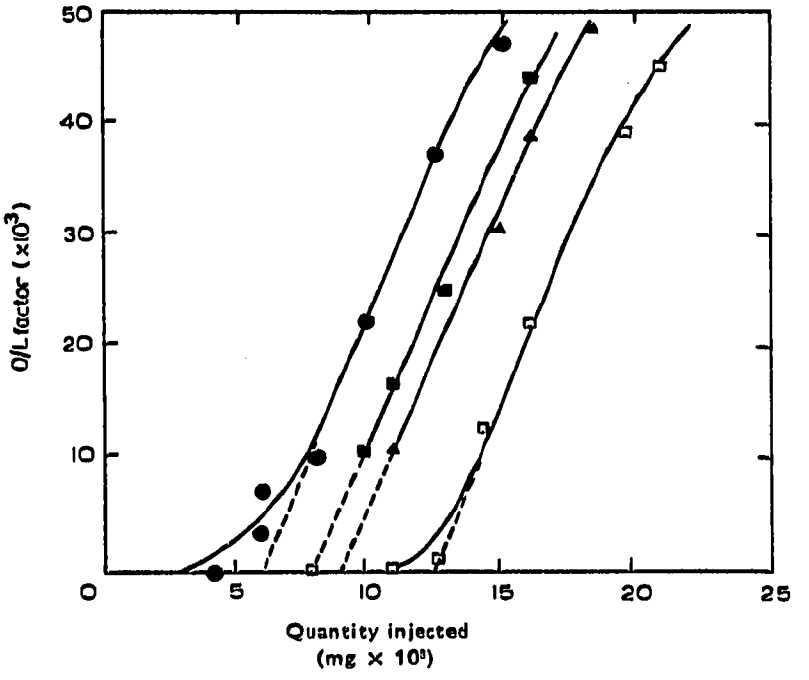

Fig. 1. Plot of the O/L factor against the quantity injected for some *p*-nitrobenzoates of alcohols. ●, *n*-pentanol; ■, *n*-propanol; ▲, ethanol; □, methanol. The broken line indicates extrapolation to obtain the maximum size injection which can be used without causing excessive overloading.

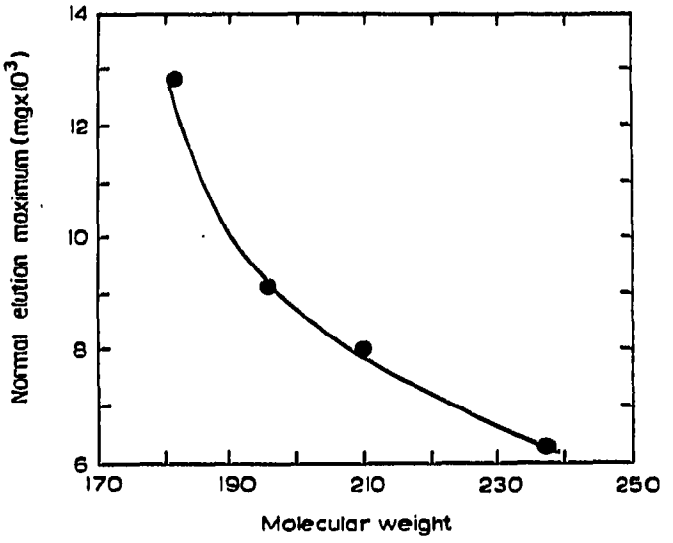

Fig. 2. Plot of the normal elution maximum against molecular weight for some *p*-nitrobenzoates (see Fig. 1).

high-molecular-weight range (see behaviour of homologous series described above). It is necessary to avoid overloading, since this will lead to retention variations dependent on injection size.

Conclusions

The results indicate that, with the lower aliphatic alcohols, TLC examination of the α-naphthylurethans and gas chromatography of the *p*-nitrobenzoates can be used to provide confirmatory evidence for identifications made by direct examination of the parent alcohols. The GC technique gives a comparatively high order of resolution and can give detailed information on qualitative composition.

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