

CHROM. 7046

## Note

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### Separation by gas-liquid chromatography of mono- and symmetrically disubstituted ureas as their trifluoroacetates

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In the course of an investigation into the presence of monosubstituted aliphatic ureas in biological fluids a method of separation and identification of such compounds was an essential prerequisite. A colorimetric method has been described<sup>1</sup>, but since this is time consuming and laborious the possibility of utilising a gas-liquid chromatographic (GLC) technique seemed worthy of examination. Such methods have the virtues of being sensitive, reproducible and rapid.

Only one GLC method for ureas has, until now, appeared in the literature, namely that of Reiser<sup>2</sup>. In Reiser's method substituted ureas were chromatographed in the free form on a 2-ft. Carbowax 20M column. However, difficulties were encountered due to their thermal decomposition at the temperatures used, hence the very short column. The procedure was also insensitive and, in some instances, separation was inadequate.

This communication describes an alternative method which overcomes some of these difficulties and in which substituted ureas are chromatographed as their trifluoroacetyl derivatives.

## METHODS

### *Reagents*

The following ureas were obtained commercially: methylurea, ethylurea, phenylurea and N,N'-dimethylurea from Koch-Light (Colnbrook, Bucks., Great Britain) and propylurea and butylurea from Eastman-Kodak (Rochester, N.Y., U.S.A.).

Isopropyl-, isobutyl-, *sec.*-butyl-, *tert.*-butyl-, pentyl-, benzyl- and  $\beta$ -phenylethylureas were prepared in this laboratory from nitrourea according to the methods of Davis and Blanchard<sup>3</sup>.

Trifluoroacetic anhydride (TFAA) was obtained from British Drug Houses (Poole, Dorset, Great Britain).

### *Column conditions*

Chromatography has been performed on a 7 ft.  $\times$   $\frac{1}{4}$  in. O.D. ( $\frac{3}{16}$  in. I.D.) glass column packed with 10% polyethylene glycol adipate on acid-washed 100-120 mesh Diatomite C (J. J.'s Chromatography, Kings Lynn, Great Britain).

A Pye-Unicam Model 104 gas chromatograph (Pye-Unicam, Cambridge,

Great Britain) has been used throughout, incorporating a flame ionisation detector and a 1-mV Leeds & Northrup recorder (Leeds & Northrup, North Wales, Pa., U.S.A.) with a chart speed of 10 in./h.

The carrier gas was nitrogen at a flow-rate of 40 ml/min and all runs were performed isothermally. The attenuation was  $\times 500$ . All chromatograms shown were obtained by injection of the trifluoroacetates formed from 4  $\mu\text{g}$  of each urea used.

#### Preparation of derivatives

Mono- and symmetrically disubstituted ureas were converted to their trifluoroacetyl derivatives by addition of TFAA at room temperature. After allowing to stand for 2–3 min, excess TFAA was blown off under a stream of nitrogen before dissolving the residue in ethyl acetate for injection. Under these conditions, neither urea itself nor N,N-dimethylurea underwent trifluoroacetylation.

#### RESULTS AND DISCUSSION

A typical chromatogram obtained at 130° with nine aliphatic ureas is shown in Fig. 1 from which it is apparent that good separation has been obtained in all cases. A notable absentee from this chromatogram is, however, methylurea, which, under

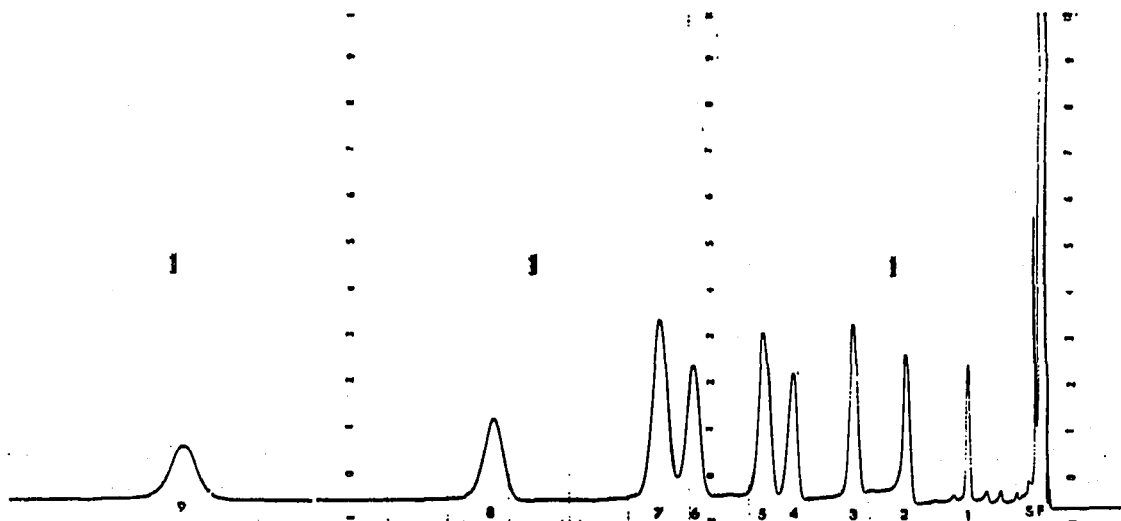


Fig. 1. Chromatogram of the trifluoroacetates of nine substituted ureas. Operating temperature, 130°; solvent, ethyl acetate. SF = Solvent front; 1 = N,N'-dimethylurea; 2 = *tert.*-butylurea; 3 = isopropylurea; 4 = ethylurea; 5 = *sec.*-butylurea; 6 = propylurea; 7 = isobutylurea; 8 = butylurea; 9 = pentylurea.

these conditions, runs between ethyl- and *sec.*-butylureas. These three compounds can be adequately separated and identified by chromatographing them at a slightly lower temperature, other conditions remaining the same. The separation achieved at 120° is shown in Fig. 2.

Chromatography of some ureas containing an aromatic ring has been examined

and a chromatogram of benzyl- and  $\beta$ -phenylethylureas at 180° is shown in Fig. 3. Unfortunately, phenylurea has proved less amenable to analysis by this technique since it gave rise to two peaks. It has not yet proved possible to explain this phenomenon.

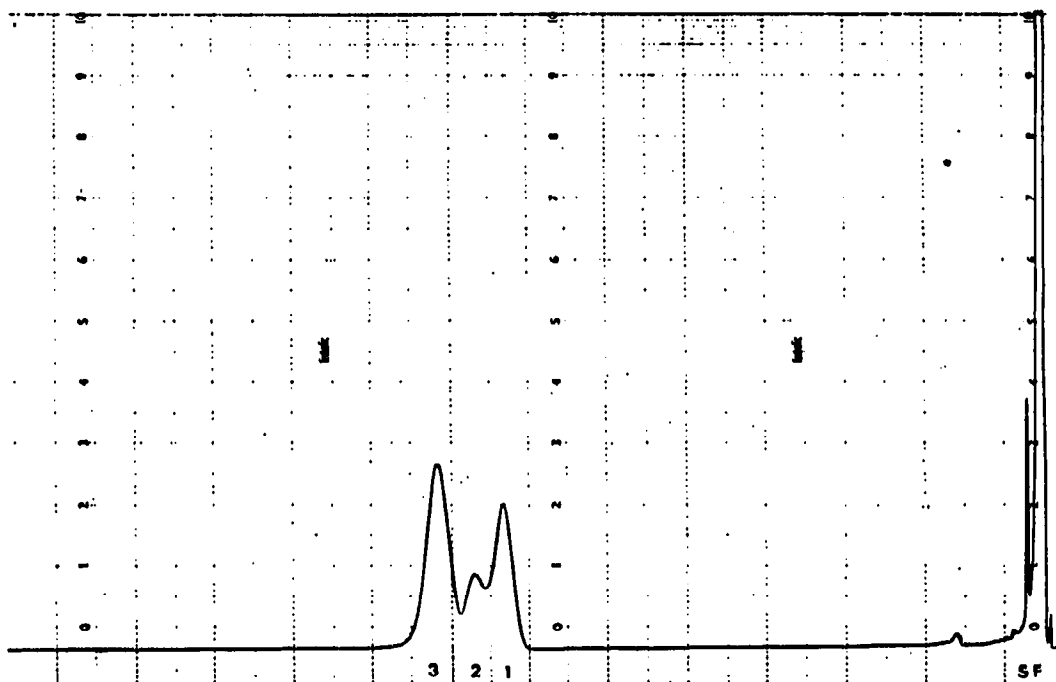


Fig. 2. Chromatogram of the trifluoroacetates of ethyl-, methyl- and *sec.*-butyl ureas. Operating temperature, 120°; solvent, ethyl acetate. SF = Solvent front; 1 = ethylurea; 2 = methylurea; 3 = *sec.*-butyl urea.

Retention times of all compounds examined under the appropriate conditions are shown in Table I.

The stability of the trifluoroacetyl derivatives of these compounds has been investigated. With the exception of that produced by *N,N'*-dimethylurea, which is liquid at room temperature, all derivatives are solids.

In this state they have a bench life at laboratory temperature of many weeks, if stored in an adequately sealed container. In ethyl acetate solution, however, they decay slowly over a period of a few days, presumably due to hydrolysis by water present as a contaminant in the ethyl acetate. This is suggested since a decrease in peak size of the trifluoroacetylated ureas is accompanied by an increase in the size of the peak due to trifluoroacetic acid.

Since unsubstituted and asymmetrically disubstituted ureas do not undergo trifluoroacetylation under the conditions described, it is reasonable to assume that the introduction of the trifluoroacetyl group occurs only on the nitrogens which bear both free hydrogen and a substituting group.

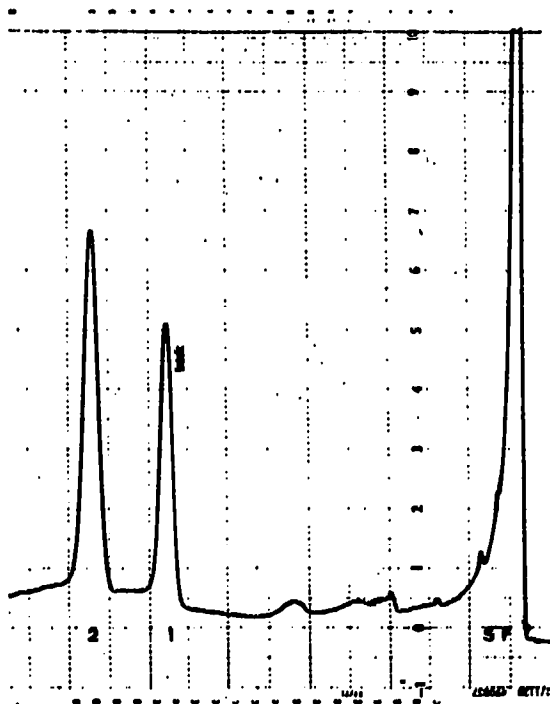


Fig. 3. Chromatogram of the trifluoroacetates of benzylurea and  $\beta$ -phenylethylurea. Operating temperature, 180°; solvent, ethyl acetate. SF = Solvent front; 1 = benzylurea; 2 =  $\beta$ -phenylethylurea.

A suggested reaction is shown below:

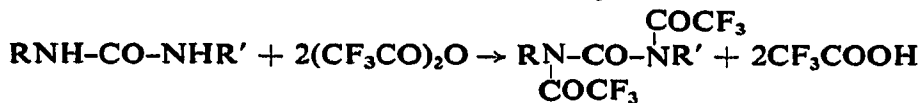
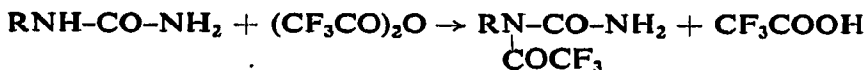


TABLE I

RETENTION TIMES OF SUBSTITUTED UREAS

Substituted group	Temperature (°C)	Retention time (min)
Methyl	120	44
Ethyl	120	41
sec.-Butyl	120	47
Ethyl	130	26
Propyl	130	36
Isopropyl	130	19
Butyl	130	56
Isobutyl	130	39
sec.-Butyl	130	29
tert.-Butyl	130	14
Pentyl	130	86
N,N'-Dimethyl	130	8
Benzyl	180	27
$\beta$ -Phenylethyl	180	33

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**REFERENCES**

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- 2 R. W. Reiser, *Anal. Chem.*, 36 (1964) 96.
- 3 T. L. Davis and K. C. Blanchard, *J. Amer. Chem. Soc.*, 51 (1929) 1790.