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Note

Gas chromatographic detection of small amounts of formic acid as dimethylformamide

B. P. MOORE and W. V. BROWN
Division of Entomology, CSIRO, P.O. Box 1700, Canberra City 2601 (Australia)
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Formic acid is an important metabolite of certain groups of insects, notably formicine ants[1] and carabid beetles[2], where it is employed in a defensive role and is produced in relatively large amounts that are readily detected by standard laboratory tests. However, the identification of traces of the acid in smaller organisms and in other substrates has been hampered by the lack of sensitivity of the methods available. In gas chromatography (GC), the major difficulty has been the almost zero response of formic acid in the generally used flame ionisation detector and although other detectors based upon argon ionisation or thermal conductivity show better responses, their sensitivity is not of a high order. The marked variability in retention times shown by free formic acid[2] has also provided a further complication.

Esters of formic acid are less subject to these difficulties but with the exception of the methyl ester, which is too volatile for convenient GC, their preparation is not suited to a microgram scale. Likewise, the anilide method of Umeh[3] appears to be limited to analyses at the macro-level.

We now report a quick and simple method for the detection of small quantities of formic acid, through conversion to dimethylformamide (DMF) by successive treatments with diazomethane and dimethylamine. The DMF so formed can readily be detected by GC on Carbowax 20M–KOH or EGSS-X–Versamid columns, with the sensitive flame ionisation detector.

Satisfactory results were obtained with standards containing as little as 10 μg of formic acid. Higher acids did not interfere as their methyl esters apparently did not react with dimethylamine under the experimental conditions employed. Of such acids commonly found in carabid defensive secretions, tiglic and caproic acids showed the

TABLE I

RETENTION TIMES OF METHYL ESTERS AND DMF AT 90°

Compound	*Retention time (min)*	
	Carbowax 20M–KOH	*EGSS-X–Versamid*
Methyl caproate	4.30	2.11
Methyl tiglate	4.46	2.23
Dimethylformamide	9.08	7.04

longest retention times, as methyl esters, but these esters were eluted from both columns well before the emergence of the DMF, as indicated in Table I.

The new technique has been used successfully to detect formic acid in the defensive glands of several further carabid species, including small forms represented by single specimens that would not have been amenable to examination in earlier work. The method is therefore likely to prove a useful addition to general GC technology.

EXPERIMENTAL

GC was conducted on a Varian Model 2100 instrument with flame ionisation detectors and a Hewlett-Packard 3370A digital integrator. A nitrogen flow-rate of 25 ml/min was used throughout and columns were all-glass, 2 m × 3 mm I.D. Packings were 6% Carbowax 20M–2% KOH on Gas-Chrom Z and 6% EGSS-X–3% Versamid 900 on Gas-Chrom Q and columns were maintained at 90°.

Ethereal diazomethane was prepared freshly as required from Diazald®, by the distillation method.

Samples were prepared from aliquots of solutions of 1% of the test acids in diethyl ether as follows: A, formic acid (10 μg); B, formic acid (10 μg) + methacrylic acid (20 μg) + tiglic acid (4 μg); C, formic acid (10 μg) + methacrylic acid (10 μg) + tiglic acid (2 μg) + acetic acid (10 μg) + caproic acid (10 μg).

Each sample was treated dropwise with ethereal diazomethane until a faint yellow colour persisted, followed by 2 μl of dimethylamine–water (1:2). After brief shaking, the mixtures were analysed by GC without further delay. Substantial peaks corresponding with DMF were obtained from each sample, together with others corresponding with the methyl esters of higher acids, when such were present. When the reaction mixtures, or blank runs without the acids, were allowed to stand for some minutes before analysis two further peaks (at 5.0 and 8.3 min on Carbowax 20M–KOH) appeared and increased gradually with time. These were evidently due to unidentified artefacts resulting from a slow interaction between excess diazomethane and dimethylamine and, although they did not seriously interfere with the analyses, they complicated the chromatograms and were best avoided by prompt treatment.

REFERENCES

1 M. Pavan, *Proc. Int. Congr. Biochem., 4th*, 12 (1958) 15.
2 B. P. Moore and B. E. Wallbank, *Proc. Roy. Entomol. Soc., London, Ser. B*, 37 (1968) 62.
3 E. O. Umeh, *J. Chromatogr.*, 51 (1970) 139.