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Note

Determination of 2,4-dinitrophenyl ether derivatives of the phenolic metabolites of carbofuran by high-performance liquid chromatography

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Many methods have been published for the analysis of the systemic carbamate insecticide-nematicide carbofuran (CF), 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, from various substrates. Fewer methods describe the analysis of the metabolites, in particular the free phenols, 2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol (I), 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol (II) and 2,3-dihydro-2,2-dimethyl-7-benzofuranol (III). Several methods[1–3] which describe the analysis of these phenolic metabolites have used derivitisation with 1-fluoro-2,4-dinitrobenzene (FDNB) to give 2,4-dinitrophenyl ether (DNPE) derivatives followed by analysis by gas chromatography (GC). This derivatization reagent has also been used for analysis of CF and its carbamate metabolites, 3-hydroxy-CF and 3-keto-CF, by hydrolysis of the carbamate group at the derivatisation step[2].

This note reports that the DNPE derivatives of the phenolic metabolites of carbofuran may be analysed using reversed-phase high-performance liquid chromatography (HPLC). In practice, detection limits are effectively similar to those obtainable using GC with thermionic detection. The information should find use in commodity testing situations where positive component identification is required.

EXPERIMENTAL

Reagents

Solvents were analytical grade (BDH or Ajax) except for diethyl ether which was U.S.P. (J. T. Baker). Water was glass distilled and then passed through a Millipore Milli-Q water purifier. FDNB was analytical reagent grade (Merck). Phosphoric acid was analytical grade (Ajax).

Chemical standards

Crystalline DNPE derivatives of the 7-phenols, I, II and III, were prepared from the parent carbamates using the method of Tannock and Wessels[2], and the structure verified by mass spectrometry. Standard solutions of these derivatives were made in water–methanol (20:80).

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Apparatus

The liquid chromatograph was a two-pump gradient system with a fixed loop injector and variable-wavelength detector as previously described[4]. Absolute HPLC detection limits for the DNPE derivatives were determined using a Shimadzu SPD-2A variable-wavelength detector. The analytical column was a 15 × 4.6 mm I.D. column slurry-packed with Zorbax C_8 (DuPont, Wilmington, DE, U.S.A.). The analytical column was preceded by a 2-μm in-line filter (Rheodyne, Berkeley, CA, U.S.A.) and a MPLC RP-8 guard column (Brownlee Labs., Santa Clara, CA, U.S.A.).

The gas chromatograph was a Varian Model 3700 with heated bead alkali flame ionisation detector. The 1-m columns were either glass (2.7 mm I.D.) or nickel (2 mm I.D.) packed with 2.5% SE 30 on 80–100 mesh Gas-Chrom Q. The gas flow-rates were: nitrogen carrier gas 30 ml/min, air 200 ml/min and hydrogen 4.5 ml/min. The temperatures were: column 200°C or 210°C, injector 240°C and detector 240°C. The injection volume was 2 μl.

HPLC conditions

The analyses were performed at room temperature (20–22°C). For isocratic elution water–methanol (32:68) at 1 ml/min was used. For gradient elution a flow-rate of 1.2 ml/min with water–methanol (36:64) for 12 min followed by water–methanol (28:72) was used. Due to gradient mixer and in-line volume, the step gradient solvent change occurred at the column inlet during 1–2 min after activation. The detector wavelength was set at 280 nm or 300 nm, sensitivity 0.04 a.u.f.s. A 20-μl sample loop was used.

RESULTS AND DISCUSSION

We had found that the simplified procedure described by Tannock and Wessels[2] did not give sufficiently clean extracts for analysis by GC of the carbofuran metabolites in our grape and grass samples. The possibility of using HPLC for the analysis of the DNPE derivatives of I, II and III became apparent during the investigation of reversed-phase clean-up procedures for these derivatised extracts. The three derivatives were found to be strongly absorbing over a wide range of UV wavelengths and to be generally separated from contaminants using reversed-phase HPLC. The three DNPE derivatives had absorption peaks near 285 nm and were again strongly absorbing below 240 nm, with II-DNPE having additional absorption peaks at 329 nm and 253 nm. In all cases, absorption was strong below 310 nm (Table I). When a variable-wavelength detector was used, 300 nm was often preferred for analysis since the absorption of interfering co-extractives was relatively reduced at this wavelength compared with 280 nm and below. This is illustrated in Fig. 1 with the analysis of a soil extract at both 280 nm and 300 nm.

In our extracts prepared according to Tannock and Wessels[2], co-extractive interference, especially for the 3-oxygenated derivatives, sometimes occurred at low levels when the isocratic separation method was used. In these cases a step-gradient elution allowed better resolution of these derivatives and hence gave more reliable results. This is illustrated in Fig. 2, although as Fig. 2d shows, interference may still occur in some instances. Comparison of Fig. 2c and e also shows that recovery of

TABLE I

RELATIVE ABSORPTION OF DNPE DERIVATIVES AT SEVERAL WAVELENGTHS UNDER ISOCRATIC HPLC CONDITIONS WITH WATER–METHANOL (24:76)

Peak height at 300 nm defined as 1.00. Detection limits were similar for each compound; 5 ng gave between 0.8% and 1% full scale deflection at 0.04 a.u.f.s. at this wavelength.

Wavelength (nm)	*I-DNPE*	*II-DNPE*	*III-DNPE*
220	1.72	2.63	1.70
240	1.00	1.72	1.06
254	0.96	2.03	0.98
280	1.14	1.13	1.15
300	1.00	1.00	1.00
315	0.74	1.02	0.76
330	0.52	1.14	0.48
340	0.32	0.99	0.33

the free carbamates is not as efficient with chloroform as with 0.25 *M* hydrochloric acid.

With gradient analysis the detection limits of I-DNPE and II-DNPE increased relative to III-DNPE, and the compounds required 11, 15 and 9 ng for 1% deflection of 0.04 a.u.f.s. at 280 nm. The detector response was linear up to at least 2500 ng

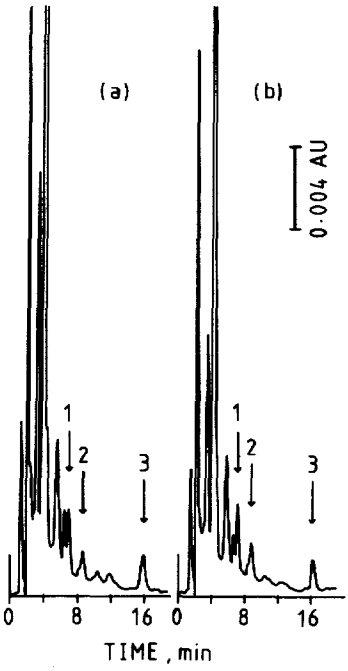

Fig. 1. Isocratic HPLC chromatograms of a FDNB derivatised chloroform extract (according to ref. 2) from soil spiked at 2 μg/g with each of CF, 3-keto-CF and 3-hydroxy-CF. (a) 280 nm; (b) 300 nm. 1, 2 and 3 show retention times of I-DNPE, II-DNPE and III-DNPE, respectively. Chromatographic conditions in text.

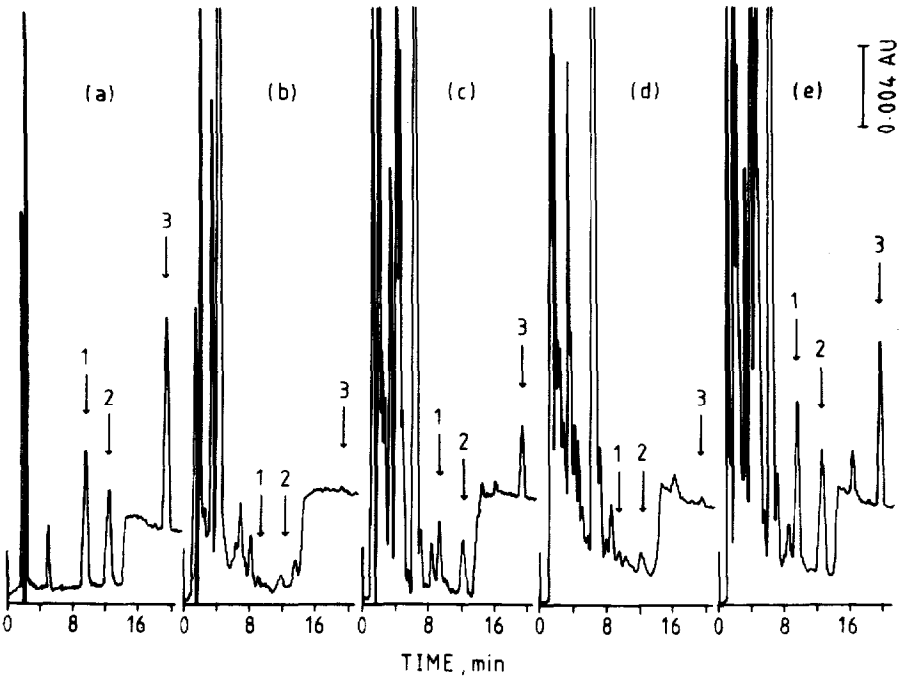

Fig. 2. Gradient HPLC chromatograms of FDNB derivatised standards and grape extracts. (a) Standard solution in water–methanol (20:80) of I-DNPE (8.8 μg/ml), II-DNPE (8.85 μg/ml) and III-DNPE (11.15 μg/ml); (b) derivatised chloroform extract from untreated grapes; (c) derivatised chloroform extract from grapes spiked at 5 μg/g with CF, 3-keto-CF and 3-hydroxy-CF; (d) derivatised dilute HCl extract from untreated grapes; (e) derivatised 0.25 *M* HCl extract from grapes spiked after chloroform extraction, level as in (c). Extraction procedures according to ref. 2. 1, 2 and 3 as for Fig. 1. Chromatographic conditions in text with detector wavelength 280 nm.

which gives ample range to cover the residue levels of the phenolic metabolites or carbamates commonly encountered in plant samples.

The absolute detection limits of the DNPE derivatives (quantities required to give at least five times the noise level) were determined to be between 2–4 ng (HPLC at 280 nm) and 0.2–1 ng (GC). While these limits for standards were lower by GC than by HPLC, the effective concentration limits for samples are similar, since in HPLC, injection volumes are commonly larger. The fact that these derivatives can be used for GC and HPLC at similar concentrations should be useful for positive characterisation of carbofuran residues.

When DNPE derivatives have been used for analysing carbofuran metabolites by GC[1,2,3,5], clean-up requirements have generally varied from one substrate to another. Column chromatography[1], simple coagulation[2] and HPLC[3] have been used to prepare an extract suitable for analysis by GC after derivatisation. These existing methods comment on the precautions required for quantitative and reproducible extraction and derivatisation of I, II and III. In our experience, the coagulation clean-up could not be recommended as a general procedure since significant co-extractive interferences can occur with some substrates. We have noted, however, that these interferences are more easily avoided by using HPLC analysis which would

make this analytical technique a favourable option to GC.

In instances where positive identification of a residual metabolite is required, the availability of two completely different analytical techniques for analysis at similar sensitivity levels is valuable. A single extraction and clean-up procedure may be used, and after derivatization, the extracts simply require dividing and dissolving in different solvents to prepare them for analysis by the different methods. The added option of multiple-wavelength determination by HPLC (see Table I) would strengthen this characterisation potential. A recently published method[6] for determination of I, II and III in plants using gas chromatography–mass spectrometry, employs a simplified and general clean-up procedure which should be applicable to sample treatment prior to FDNB derivitisation and HPLC analysis.

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