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Short Communication

Capillary gas chromatography method for the analysis of the *trans* isomers of ceralure, a medfly attractant

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Abstract

A capillary GC method has been developed to analyze laboratory or commercial samples of the medfly attractant ceralure [ethyl 4- (and 5-)iodo-*trans*-2-methylcyclohexane-1-carboxylate]. The method utilizes a specially prepared fused-silica column with a bonded phenyl-methyl polysiloxane liquid phase. Baseline separation was achieved for three of the four *trans*-ceralure isomers. Difficulties encountered with other columns investigated are also discussed.

1. Introduction

Ceralure [ethyl 4- (and 5-)iodo-*trans*-2-methylcyclohexane-1-carboxylate] is a potent and persistent synthetic attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) [1–3], commonly referred to as the medfly. The medfly, a worldwide pest, feeds on 253 varieties of fruit, nuts and vegetables [4] and if established in California, the total yearly recurrent costs for crop damage are estimated to be as much as US$ 756 089 000 [5]. Early warning systems to detect medfly infestations involve traps baited with the synthetic attractant trimedlure [TML, 1,1-dimethylethyl 4- (and 5-)chloro-*trans*-2-methylcyclohexane-1-carboxylate] [6]. Although TML has proven extremely useful in the medfly surveillance program, research to discover attractants with unusual properties that may lead to novel detection and/or control techniques, still continues. The highly persistent nature of ceralure (an iodo analogue of TML) combined with its inherent activity has stimulated considerable interest in this attractant and, as a result, large-scale field trials were recently initiated to exploit these properties.

Methods used to synthesize TML [6] and ceralure [1,2] result in mixtures containing a preponderance (90–95%) of the four *trans* isomers A, B_1, B_2 and C (see Fig. 1) [7]. The *trans* refers to the configurational relationship of the methyl and ester moieties on the cyclohexane ring and descriptors A, B_1, B_2 and C refer to relative order of gas chromatographic (GC) retention times arbitrarily assigned for TML [8]. The remainder (5–10%) of the synthetic mixture is usually comprised of the four *cis* isomers [*i.e.*, for TML 1,1-dimethylethyl *cis*- and *trans*-4- (and

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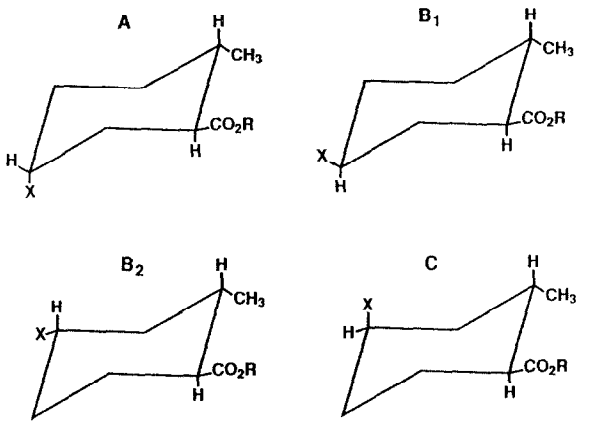

Fig. 1. Structures of the *trans* isomers of ceralure and trimedlure. Ceralure: X = I, R = ethyl; trimedlure: X = Cl, R = 1,1-dimethylethyl.

5-)chloro-*cis*-2-methylcyclohexane-1-carboxylate] [6]. Medfly attractancy to TML or ceralure is correlated with orientation (*i.e.*, position, configuration) of the halogen, methyl and ester moieties. For example, while the C isomer [7,9] is the most attractive isomer in TML (specifically the 1*S*,2*S*,4*R*-enantiomer [10,11]), the B_1 isomer is the most attractive in ceralure [2,12,13].

Since the relative distribution of the four *trans* isomers in ceralure is influenced by variations in synthetic procedures [2], an accurate method to detect and quantify these isomers is indispensable to the optimization of the synthesis procedure and the success of pest management programs using this attractant. High-performance liquid chromatography (HPLC) was used on a semi-preparative scale to separate the four *trans* isomers [14]; isomers B_1 and B_2 were separated directly, while isomers A and C were separated as their *tert.*-butyl esters and then subsequently converted to their corresponding ethyl esters. A GC method using a packed-column was also reported [14] for *trans*-ceralure isomers but retention times were too close to effect an accurate analysis. We wish to report here an efficient capillary GC method to determine the *trans* isomer content of ceralure mixtures.

2. Experimental

2.1. Materials and sample preparation

The ceralure standard, >50% in B_1 and B_2 isomers, was synthesized from predominantly (90–95%) *trans*-6-methyl-3-cyclohexenecarboxylic acid (Albany International, Columbus, OH, USA) according to published procedures [2]. Distillation afforded a pale-yellow liquid, b.p. 81°C (0.15 mmHg; 1 mmHg = 133.322 Pa), n_D(25°C) 1.5130. The standard was stored in an amber-colored vial over copper wire.

Ceralure B_1 (ethyl *cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate) and ceralure B_2 (ethyl *trans*-4-iodo-*trans*-2-methylcyclohexane-1-carboxylate) were obtained by semi-preparative HPLC [14]. Ceralure A (ethyl *trans*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate) and ceralure C (ethyl *cis*-4-iodo-*trans*-2-methylcyclohexane-1-carboxylate) were obtained by converting their corresponding *tert.*-butyl ester isomers [14] to free acids, then to acid chlorides and finally to the desired ethyl esters. A technical grade amber-colored sample of ceralure [n_D(25°C) 1.5165] was obtained from AgriSense/Biosys (Fresno, CA, USA).

Samples for analyses were prepared by dissolving 1 μl of a standard or test material in 100 μl of acetone (Fisher A19-4); 0.25 μl of pure isomer standard or 1 μl of ceralure standard or test material was injected into the column.

2.2. Capillary gas chromatography

Samples were analyzed on a Shimadzu GC-9A chromatograph (Columbia, MD, USA), equipped with a flame ionization detector and Shimadzu C-R4A data processor. The column was a specially prepared SPB-608 column (Supelco, Bellefonte, PA, USA); 60 m × 0.25 mm I.D. × 0.25 μm film. The stationary phase is a proprietary blend of bonded methyl and phenyl polysiloxanes. GC conditions were: column temperature 180°C (isothermal), injector/detector temperature 220°C and carrier gas (helium) head pressure 2.0 kg/cm^2 (19.4 cm/s linear velocity at

180°C). Samples were injected in the split mode (1:100).

2.3. GC–MS analyses

Electron impact (EI) spectra were recorded on a Hewlett-Packard 5971A mass spectrometer equipped with the identical specially prepared SPB-608 column. GC conditions for the mass spectrometer were identical to those described for the GC analysis of ceralure.

3. Results and discussion

Ceralure's reported [14] instability during capillary GC analysis was not unexpected since many iodo compounds are susceptible to various photolytic, hydrolytic, thermolytic or oxidative decomposition processes. Although TML has been successfully analyzed by capillary GC on a Carbowax 20M column [15], attempts to analyze ceralure on a 60-m Supelcowax-10 (Supelco) column were unsuccessful. Ceralure isomers eluting from this column, although well resolved, showed signs of decomposing (A and C mostly). Similar results were observed with a 60-m dicyanopropyl polysiloxane SPB-2340 (Supelco) column.

Decomposition by these polar columns did not appear to be thermally induced (*i.e.*, excessive column/injector temperatures) but rather seemed to be caused by build up of column contaminants. This hypothesis was supported by the facts that decomposition increased with increased column use and was eliminated (or substantially reduced) by washing poorly performing columns with polar solvents.

Although non-polar methyl polysiloxane columns failed to resolve the ceralure isomers, medium-polarity columns were substantially more effective. For example, near-baseline separation and good peak symmetry were achieved by either a 60-m SPB-35 (Supelco) or a 15-m DB-17 (Durabond) column. Optimum results, however, were obtained with a specially prepared 60-m phenyl-methyl polysiloxane SPB-608 column operated at isothermal conditions. Fig. 2

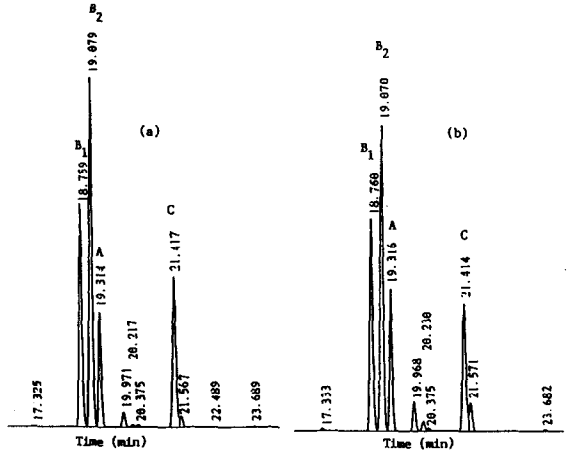

Fig. 2. GC traces of ceralure standard (a) and commercial ceralure (b). B_1, B_2, A and C identify specific isomers.

shows typical GC traces on the SPB-608 column for a ceralure standard and commercial sample. Retention times (min) for the isomers, in order of elution, were B_1 (18.76), B_2 (19.08), A (19.31) and C (21.42). GC profiles in Fig. 2 show acceptable peak symmetry and except for ceralure C (small peak overlap at 21.57 min), isomers were baseline resolved. Percentages of the B_1, B_2, A and C isomers (based on total *trans* isomer content) for the ceralure standard were 27.10, 41.53, 12.44 and 18.92%, respectively and for the commercial sample were 26.83, 38.57, 16.39 and 18.21%, respectively. Interestingly, baseline separation of the four *trans* and four *cis* isomers of TML was also achieved on the SPB-608 column (isothermal conditions, 155°C); the order of elution for *trans* isomers was identical to that observed for ceralure.

GC–EI-MS data were obtained for the four *trans* isomers in the commercial sample. As expected, the B_1, B_2, A and C isomers gave nearly identical fragmentation patterns. Intensities of molecular ions (M^+ 296) were extremely low making it difficult to identify these ions over background noise. Mass-to-charge ratios (m/z) and corresponding relative intensities of fragmentation ions for the B_1 isomer follow: 251 (5.5%), 169 (41.0%), 128 (5.5%), 123 (11.1%), 95 (100%), 81 (7.1%), 67 (9.5%), 55 (11.9%).

GC–MS spectra for minor components eluting at 19.97 and 21.57 min (Fig. 2) were nearly identical to those obtained for the *trans* isomers, suggesting that these compounds are *cis* isomers.

In summary, a capillary GC method has been developed to analyze the principal isomers of ceralure. The method is reproducible and requires less than 30 min for completion. Over 100 ceralure samples have been analyzed on the SPB-608 column with no apparent signs of column deterioration or performance problems. Research is in progress to fully define the elution characteristics of the four *cis* isomers of ceralure on this column.

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Names of products in this paper are included for the benefit of the reader and do not imply endorsement or preferential treatment by the US Department of Agriculture.

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