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Note

Rapid method for esterification of trace levels of carboxylic acids for analysis by gas chromatography–electron-capture detection

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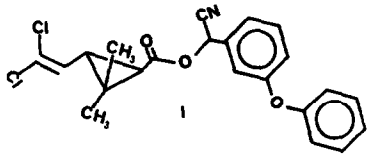
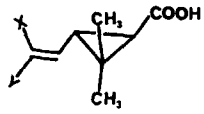
Pyrethroid insecticides are widely used because of their effectiveness, short lifetime in the field and relatively low mammalian toxicity. The principal reason for the short lifetime and low toxicity is the ease with which these esters are hydrolyzed on exposure to sunlight or in contact with esterase enzymes[1]. Cypermethrin (**I**), for example, yields permethrin acid (**II**) and the cyanohydrin of 3-phenoxybenzaldehyde on hydrolysis. The latter product is subsequently oxidized to phenoxybenzoic acid

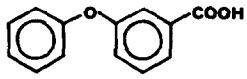


I



II: X = Y = Cl

III: X = Cl, Y = CF_3



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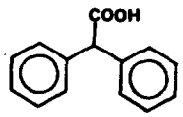

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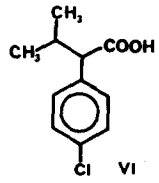

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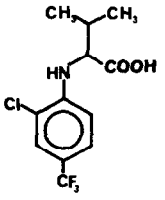

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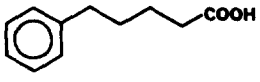
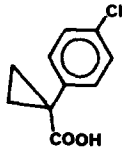


VIII



IX

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(IV). Because of the halogen atoms present in acid **II**, methylation yields a volatile ester which is readily analyzed by gas chromatography (GC) with electron-capture detection (ECD). In order to detect acid **IV** by ECD, however, it is necessary to employ a derivatization agent which itself contains the detectable function. There are a number of these reagents available, such as pentafluorobenzyl bromide[2–6], halogen-containing silylating agents[7] or halogenated alcohols[8,9], but the conditions of derivatization typically require long reaction times or elevated temperatures. In some instances, the need to remove excess reagent in order to minimize background interference can also be problematic. For example, esterification of carboxylic acids with trichloroethanol (100°C for 10 min) left an excess of high-boiling trichloroethanol that required separation on a silica column[9].

The carbodiimide-coupled esterification of carboxylic acids is a well known reaction which has been done under a variety of conditions[10]. Although hexafluoroisopropanol and dicyclohexylcarbodiimide have been used to esterify amino acids on a synthetic scale for peptide synthesis[11], there appears to be no reference to the use of these two reagents for the purpose of trace analysis. Poole and Schuette[8] do refer, however, to esterification with a number of halogenated alcohols via mixed anhydrides, including hexafluoroisopropanol. For example, hydroxyphenylacetic acids were esterified with hexafluoroisopropanol and pentafluoropropionic anhydride at 65°C for 90 min[12].

Inspite of its limited use, hexafluoroisopropanol should be an excellent derivatization reagent for carboxylic acids: its volatility (b.p. 59°C) and water solubility would facilitate removal, it is commercially available at reasonable cost and in high purity, and the six fluorine atoms would make its derivatives ECD sensitive. While attempting to circumvent the limitations of present derivatization methods, it was observed that the carbodiimide coupled esterification of 3-phenoxybenzoic acid (**IV**) occurred very rapidly at room temperature when the alcohol was hexafluoroisopropanol. This report concerns carbodiimide-coupled esterification with hexafluoroisopropanol as a simple and rapid means of preparing volatile derivatives of low concentrations of carboxylic acids for analysis by GC–ECD.

EXPERIMENTAL

Instrumentation

GC was carried out on a Varian 3700 instrument equipped with a ^{63}Ni electron-capture detector an a flame-ionization detector, a SGE on-column injector, a 30 m × 0.32 mm DB-1701 column (unless otherwise stated) with a 0.25 μm film thickness, and a HP3300A integrator. Helium carrier gas flow was 1.9 ml/min and the nitrogen make-up gas was set at 25 ml/min. The detector was maintained at 250°C. Typical GC conditions were: initial oven temperature of 120°C was maintained for 10 min then raised at 60°C/min to 145°C and held there for 10 min.

In this way, the more volatile esters were eluted within the first ten minute period and the less volatile esters were eluted within the second ten minute period. All the hexafluoroisopropyl esters eluted as sharp, symmetrical peaks with half-height widths of 2–5 s depending on their retention times.

Reagents and standards

Distilled-in-glass grade solvents were purchased from Caledon Labs. (Georgetown, Canada). Diisopropylcarbodiimide (DIC), dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDI), 3-phenoxybenzoic acid (3-PBA), diphenylacetic acid, 1-(4-chlorophenyl)cyclopropanecarboxylic acid, oxalyl chloride and hexafluoroisopropanol (HFIP) were purchased from Aldrich (Milwaukee, WI, U.S.A.). Fenvalerate acid [**VI**, 2-(4-chlorophenyl)-3-methylbutyric acid] was purchased from Chemical Dynamics (South Plainfield, NJ, U.S.A.). Permethrin acid [**II**, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid], as a 56:44 mixture of *cis–trans* isomers, and cyhalothrin acid [**III**, *cis*-3-(*Z*-2-chloro-2-trifluoromethylvinyl)-2,2-dimethylcyclopropanecarboxylic acid] were kindly donated (>98.4% purity) by ICI Americas (Goldsboro, NC, U.S.A.). Fluvalinate acid [**VII**, 2-(2-chloro-4-trifluoromethylanilino)-3-methylbutyric acid] was prepared by saponification of fluvalinate donated by Sandoz (Des Plaines, IL, U.S.A.) and recrystallization of the crude acid in cyclohexane to a constant melting point, m.p. 133–134°C.

HFIP ester standards of acids **II–VI**, **VIII** and **IX** were prepared by gently warming the acid on a hotplate set at 75°C (typically 100–200 mg in a 5-ml vial) with excess oxalyl chloride until effervescence ceased, evaporation of the residual oxalyl chloride with a stream of nitrogen, and mixing the acid chloride with a 3-fold excess of HFIP and pyridine in methylene chloride. The esters were purified by silica column chromatography followed by bulb-to-bulb distillation at 0.5 Torr except for acid **IX** the ester of which was a waxy solid, m.p. 47–48°C. Acid **VII** was condensed with HFIP by carbodiimide (EDI) coupling in methylene chloride and the resulting ester purified as above. Methyl and ethyl esters were made by the Brook–Chan method (alcohol plus trimethylsilyl chloride)[13]. Structures were verified by mass spectrometric fragmentation patterns, precise mass determinations and proton magnetic resonance spectra.

Derivatization procedure

A 1.0-ml volume of a stock solution of carboxylic acid (1–2 ppm in a non-polar solvent such as methylene chloride, hexane or toluene) was added to a 10-ml volumetric flask and diluted to roughly 9 ml with hexane. A 10-μl volume of HFIP was added, swirled into solution, then 15 μl of DIC was added. The flask was filled to the mark with hexane and shaken briefly to mix. After 1 min, an aliquot of the reaction mixture was transferred to a small vial and shaken vigorously with an equal volume of 5% potassium carbonate. The bulk of the top layer was then removed by Pasteur pipette and dried over anhydrous sodium sulphate prior to GC analysis of a 1-μl aliquot.

RESULTS AND DISCUSSION

Initial experiments with 3-phenoxybenzoic acid (**IV**) (0.8 mg/ml in methylene chloride) using a 1.5-fold excess of EDI and a 25-fold excess of HFIP showed that the yield of ester, as determined by GC–FID, was independent of reaction time (15 min to 4 hr). Subsequent work with permethrin acid (**II**) at 0.12 μg/ml (2 mg DCC and 5 μl HFIP in 1 ml cyclohexane) measured the yield of ester after 1, 2, 5, 12 and 25 min. After 1 min, the yield was 98.9% and the mean value of the five samples was

96.0 ± 3.3% (S.D.). In addition, the *cis–trans* ratio was maintained on esterification (by comparison with the ratio determined from NMR of the original acid sample) indicating no rate discrimination due to steric effects.

Further reactions showed that the ester yield was independent of the type of carbodiimide used (DCC, DIC or EDI) or whether a 5-, 10- or 25-fold excess of HFIP was used. Subsequent reactions used an equimolar amount of carbodiimide and alcohol and both in large excess (10 mmolar). The reagents may be mixed up to 90 min before use but background peaks increase with postmix time. In cyclohexane, HFIP was only soluble to approximately 1% but, if a larger percentage was used, the second phase rapidly disappeared once carbodiimide was added. DIC became the carbodiimide of choice because it is a liquid, easily handled by syringe, and readily soluble in all the non-polar solvents used. EDI is non-volatile but insoluble in hydrocarbon solvents, such as cyclohexane.

Choice of solvent is critical[10]. At 1 mg/ml, **IV** gave 107 ± 4, 105, 88, 35, 25 and 14% yield of ester in cyclohexane, 2,4,4-trimethylpentane, toluene, ethyl acetate, ethyl ether and acetonitrile, respectively, after 60 min. Esterification of **II** showed a similar solvent dependence; reaction in cyclohexane, acetonitrile, acetone, or THF gave 103, 3, 0 and 0% yields, respectively, after 10 min. If 5% acetone was added to the cyclohexane, the yield fell to 28%.

Dimethylaminopyridine catalyzes some carbodiimide-coupled reactions and produces higher yields[10]. In the present study, however, at 1 ppm carboxylic acid concentrations and with a large excess of reagents, DMAP had no effect on the yields.

To demonstrate the generality of the reaction, a series of carboxylic acids representing a variety of structures were esterified at trace concentrations (0.1 to 0.2 ppm) using the conditions described in the experimental section. The reactions were done in duplicate and analyzed by GC–ECD in duplicate. The yields of esters were determined by comparison of the integrated area counts to those of standard ester calibration curves. The mean value and the standard deviation of the four results for each acid are tabulated in Table I. The acids include primary, secondary and tertiary structures as well as an amino acid and various pyrethroid metabolites. Maximum

TABLE I

YIELDS OF HFIP ESTERS FROM REPRESENTATIVE ACIDS[a]

Acid	*Yield ± S.D.*[c] *(%)*
Permethrin acid (**II**)	106 ± 3
Cyhalothrin acid (**III**)	101 ± 3
3-Phenoxybenzoic acid (**IV**)	89 ± 4
Diphenylacetic acid (**V**)	96 ± 7
Fenvalerate acid (**VI**)	82 ± 3
Fluvalinate acid (**VII**)	77 ± 2 (101 ± 3)[b]
5-Phenylvaleric acid (**VIII**)	63 ± 7
Cyclopropane acid (**IX**)	78 ± 6

[a] After 1 min at room temperature; acid concentration 0.1–0.2 ppm.
[b] After 25 min.
[c] S.D. = Standard deviation.

yields were reached within 1 min at room temperature except for fluvalinate acid (**VII**) which required 25 minutes to reach quantitative yield. Acid **VII** has a free amino group which may slow the reaction of the carboxylic acid with the carbodiimide as a result of intramolecular hydrogen bonding between the acid proton and the amino group.

The yields from the 0.1–0.2 ppm concentration range in Table I were similar to those from an experiment in the 0.6–1.2 ppm concentration range (data not shown). Limited data at 0.01–0.02 ppm suggests the yields are similar at this concentration also. The lowest detectable levels of the HFIP esters for **II** and **IV** were 0.07 pg (each isomer) and 0.8 pg, respectively, at a signal-to-noise ratio of 2.5.

The rate of esterification with HFIP is exceptionally fast: when **II** was reacted with methanol or ethanol (less sterically hindered alcohols!) under the same conditions, no product peaks were observed even after 30 min. A 1% yield of the methyl ester would have been readily detectable. A competitive experiment in which an equimolar mixture of HFIP and methanol were used gave only the HFIP ester product on GC. The selectivity of the reaction for HFIP should ensure a cleaner product when the derivatization is applied to plant extracts containing co-extractive alcohols as the reaction with these alcohols will be much slower. The greater reaction rate of HFIP is presumably due its much lower pK_a value in comparison to other alcohols. For example, the pK_a values of isopropanol and hexafluoroisopropanol are 17 and 9.3, respectively[14]. Hexafluoroisopropanol has the acidity of a phenol and, indeed, preliminary work shows that phenols also rapidly generate esters under these conditions. It has long been recognized that the rate of the initial reaction of carboxylic acid with carbodiimide to form a 1-O-acyl isourea is dependent on the pK_a of the acid[10]. Only recently, however, did Balcom and Petersen[15] conclude that the further reaction of the intermediate 1-O-acyl isourea with a second molecule of acid to form an anhydride was also pK_a dependent. The observed faster rate of esterification with HFIP over normal alcohols; *i.e.*, non-acidic, is consistent with their conclusion.

The HFIP esters are stable to column chromatography and vacuum distillation and have a number of advantages over other esters (*e.g.*, methyl esters): rapid rate of formation, earlier elution on GC, elution at a lower GC column temperature, faster elution on silica chromatography, and in the case of permethrin acid isomers, complete separation on silica thin-layer chromatography (*RF* values of 0.39 and 0.29 when eluted with cyclohexane).

The solvent restriction and the lower yields in some cases are disadvantages whose significance must be judged on an individual basis. Once the 1-O-acyl isourea is formed, there is a competition between the bimolecular reaction with alcohol and the unimolecular 1,3-shift which produces an unreactive N-acyl urea. Balcom and Peterson[15] showed that the rate of the former reaction declined with increasing solvent polarity whereas that of the latter reaction remained unchanged. As a result, as the solvent polarity increases, the 1,3-shift becomes dominant and the yield of the ester declines. If the 1,3-shift could be suppressed, the reaction developed herein would be more generally useful.

Nevertheless, in spite of these limitations, the present method is simple, rapid and clean, and generates ECD-sensitive derivatives in good to quantitative yield. The application of this method of esterification to the analysis of pyrethroid metabolites in food products is currently being investigated.

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