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Note

Separation of geometrical isomers of oxime O-ethers by high-performance liquid chromatography: use of extended multiple recycle on high-efficiency columns

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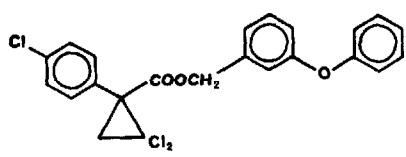
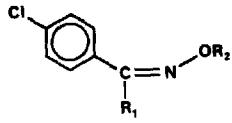
In the course of our studies on structure–activity relationships of insect neurotoxicants, *e.g.* I, which combine part structures of DDT-type and pyrethroid insecticides[1], we decided to modify the ester linkage in this group of compounds. Ester cleavage, either oxidative or hydrolytic, is known[2] to be a major pathway for biochemical degradation in the insect. In order to produce a longer-acting group of new insecticides, a series of analogues were synthesised[3], in which the ester group was replaced with the more enzymatically stable[4] oxime O-ether linkage.

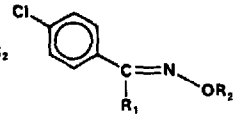
Synthesis[3] of the oxime O-ethers generally gave a mixture of the two geometric isomers (II and III) which required separation before insecticidal testing and detailed neurophysiological studies could be carried out. It has been shown[5], that less active isomers of pyrethroid-type insecticides may not act simply as inert diluents of the most active isomer, but can act as both competitive and non-competitive inhibitors of insecticidal action. Thus, for critical studies of their mode of action, absolute isomeric purity of test compounds is required.



I



II



III

a $R_1 = CF_3$
R_2 = 3-Phenoxybenzyl

b $R_1 = CF_3$
R_2 = Pentafluorobenzyl

We developed a rapid assay of isomeric composition using capillary gas chromatography (GC) and identified the geometric form (*E* or *Z*) by proton and carbon-13 nuclear magnetic resonance spectrometry[3]. However, separation of the quantities required for our test program presented certain problems, engendered mainly by a tendency of the compounds to isomerise in polar solvents. Thus, preparative high-performance liquid chromatography (HPLC) was restricted to normal phase

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using minimum polarity solvents. In most cases in the series, satisfactory separation of the geometric isomers was achieved by preparative HPLC using a limited number of recycle passes. We wish to report the technique used to separate the isomers of two oxime O-ethers (*e.g.* IIa from IIIa, IIb from IIIb) which could not be purified by these methods.

EXPERIMENTAL

GC

Gas chromatography was performed on a Hewlett-Packard Model 5880A Level 4 instrument with flame ionisation detection. Analyses were carried out on a wall-coated open tubular (WCOT) fused-silica capillary column (12 m × 0.2 mm I.D.), stationary phase BP10 (cyanopropylsilicone), nominal phase thickness 0.25 μm, from Scientific Glass Engineering, using helium as carrier gas at a linear velocity of 24 cm/sec. The column was operated isothermally at 230°C for isomer mixture IIa–IIIa and at 180°C for isomer mixture IIb–IIIb.

HPLC

Preparative system 1: a Prep LC/System 500 (Waters Assoc.) fitted with two silica gel cartridges (5.7 cm × 30 cm) plus a Model 100-40 UV spectrophotometer (Hitachi) with a preparative 0.5-mm path length flow cell (Altex) operating at 290 nm from a 20:1 effluent flow splitter.

Preparative system 2: a M6000A solvent delivery system (Waters Assoc.), fitted with extended-flow range heads, U6K injector (Waters Assoc.) a silica gel (250 × 21.2 mm) column (DuPont, SIL) plus a Model 100-40 UV spectrophotometer (Hitachi) with an analytical 10-mm path length flow cell (Altex) operating at 290 nm. This system was also used in analytical mode by substitution of a SIL (250 × 4.6 mm) column (DuPont) for the preparative one.

Petroleum spirit (60–80°C) (Ajax Chemicals, Univar) was fractionally distilled from lithium aluminium hydride ($LiAlH_4$). Dichloromethane (Ajax Chemicals, Univar) was distilled over phosphorus pentoxide or used directly (Burdick and Jackson). All solvent mixtures were degassed and filtered through a 0.5-μm Millipore PTFE filter before use.

RESULTS AND DISCUSSION

Preparative separation of mixtures of components with separation factor (α) approaching unity is usually carried out by repetitive small sample injections onto high efficiency semi-preparative columns with packings of small (10 μm or less) mean particle size or by repetitive chromatography using peak shaving techniques. Some elegant separations have been achieved[6,7] using recycle and peak shaving on radially compressed cartridge columns of high sample capacity, relatively large packing particle size (75 μm) and consequent lower efficiency (preparative system 1). However for the oxime O-ethers IIa–IIIa and IIb–IIIb, with the restrictions on solvent polarity imposed by sample instability, the best result obtained on preparative system 1 was an enrichment of each isomer in the fractions taken. The number of recycles was limited by the degree of peak spreading induced by the large particle size of the

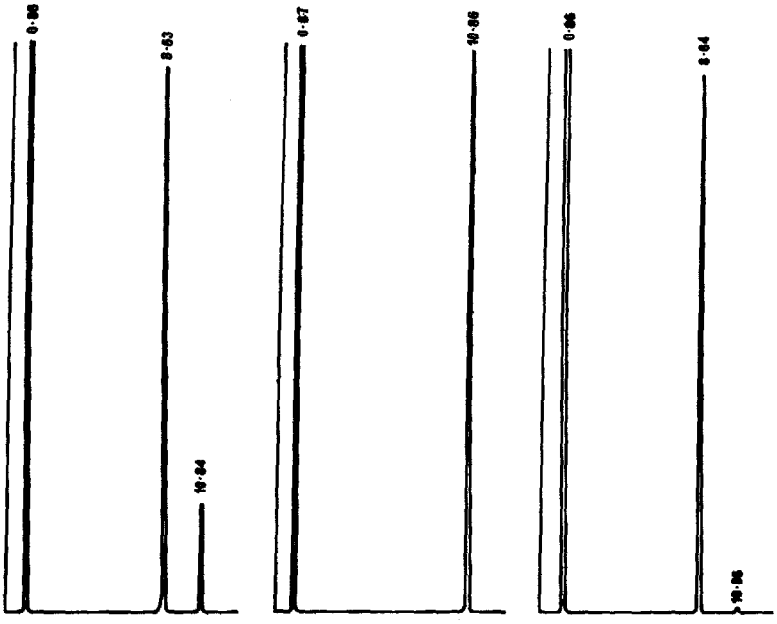

Fig. 1. Gas chromatogram of IIa–IIIa. (a) The starting mixture (80:20). (Major component *E* isomer, 8.63 min, minor component *Z* isomer, 10.84 min). (b) HPLC-separated *Z* isomer. Peak area: 1150 units. No *E* isomer detected. (c) HPLC-separated *E* isomer. Peak area: 1080 units. Peak area of *Z* isomer impurity: 1.62 units. *E* isomer purity 99.85%. Column: fused-silica capillary (12 m × 0.2 mm I.D.), stationary phase BP10 cyanopropylsilicone (SGE). Carrier gas: helium at 24 cm/sec. Injector temperature 250°C. Oven temperature 230°C, isothermal.

support. The use of a more polar solvent mixture sharpened the recycled peaks but separation was lost. Even with preparative system 2 in analytical mode, the oxime O-ether IIa–IIIa (an 80:20 mixture of isomers by GC, Fig. 1), gave a single symmetrical peak with solvent mixtures producing values of k' which ranged from 2.0 to 10.5. Only after three recycles in analytical mode at $k' = 2.0$ was peak asymmetry induced, with appearance of a valley on the sixth recycle at a sample size of 10 μg. Thus, the preparative separation of pure isomers of these compounds, in the required amounts, could only be achieved by the use of an extended recycle technique on a high-efficiency preparative column.

In the case of the isomer mixture IIa–IIIa, a typical preparative run on preparative system 2 is shown in Fig. 2. Using a solvent mixture which gave a k' value of 2.5 for the unresolved compound peak, an 80-mg injection of the 80:20 IIa–IIIa mixture gave the result shown. It should be noted that the order of elution of the isomer peaks on HPLC is the reverse of that obtained on GC; *i.e.* on HPLC the minor component, the *Z* isomer, elutes first. Although the detector was used at 290 nm to minimise differences in response factor between isomers, the higher extinction coefficient of the *Z* isomer (3300 *vs.* 600 for the *E* isomer at 290 nm) gives a detector response which shows peak areas in favour of the minor component. This separation yielded 72 mg of isomers of better than 99.8% purity by GC and 8 mg of a 15:85 *E*–*Z* mixed fraction. For the isomer mixture IIb–IIIb separation was somewhat easier

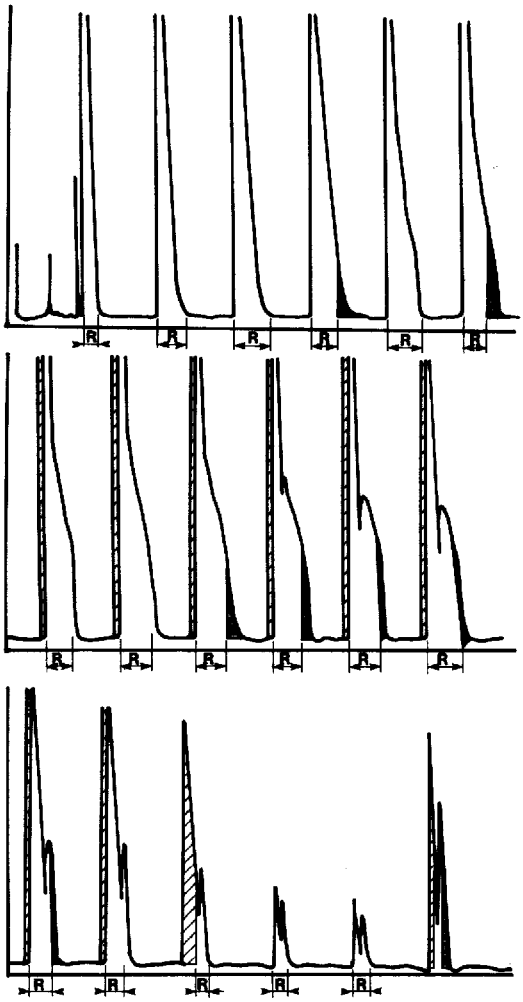

Fig. 2. Preparative liquid chromatogram of an 80:20 isomer mixture IIa–IIIa. Column: SIL (250 × 21.2 mm) (DuPont). Detector: UV at 290 nm and 2.0 a.u.f.s. Solvent: light petrolum (60–80°C)–dichloromethane (85:15) at 15 ml/min. Hatched areas indicate portions of peak collected on each pass. R indicates portion of peak recycled. Attenuation change (to 0.5 a.u.f.s.) after 17th recycle.

with only ten recycles on preparative system 2 required to achieve separation of a similarly sized sample.

These results demonstrate the utility of extended recycle on preparative columns of high efficiency and medium capacity for the separation of labile components with separation factors (α) approaching unity.

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