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Note

Separation of enantiomers of fungicides and some analogues by capillary gas chromatography using Chirasil Val

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The analytical separation of optical isomers of fungicides is of increasing interest in agriculture since it has been reported[1–4] that optically related isomers, enantiomers, of some fungicides have large differences in biological activities. This type of analysis is needed in metabolic, translocation and residue research work and possibly other studies as well. Existing methods have involved the conversion of the enantiomers into diastereoisomers, using chiral reagents, which were then separated by conventional chromatography. There are examples in the literature of this type of conversion and the method has been reviewed[5] for different functional groups. However, there are a number of disadvantages to this approach: (1) the chiral reagent would have to have absolute optical purity, (2) the derivatisation may not be quantitative and (3) racemisation could occur during the derivatisation procedure. Another method involves the conversion of the enantiomers to a suitable derivative using a non-chiral reagent and then separating the derivatised enantiomers by chiral gas chromatography (GC)[6]. Again a problem would be preventing racemisation during the reaction. From the above it is therefore obvious that a direct method of analysis with no derivatisation would be best, provided sufficient resolution of the individual enantiomers for quantitation was possible. This paper reports the results obtained in the attempted separations of triazole, pyrimidine, imidazole and morpholine fungicides and some analogues using Chirasil Val.

EXPERIMENTAL

Materials

All samples (see Figs. 1 and 2 for structures) were kindly donated by the manufacturers and for analysis were dissolved in methanol. The Chirasil Val and RSL-007 columns were purchased from Alltech Associates.

Methods

Gas chromatographic analyses were done on a Chirasil Val fused-silica capillary column (25 m × 0.24 mm I.D.) using a split/splitless injector in the splitless mode, with hydrogen as carrier gas. The optimum GC conditions are given in Fig. 3 for the compounds where some resolution was achieved. The same GC conditions were used for analyses with the RSL-007 chiral column (25 m × 0.25 mm I.D.).

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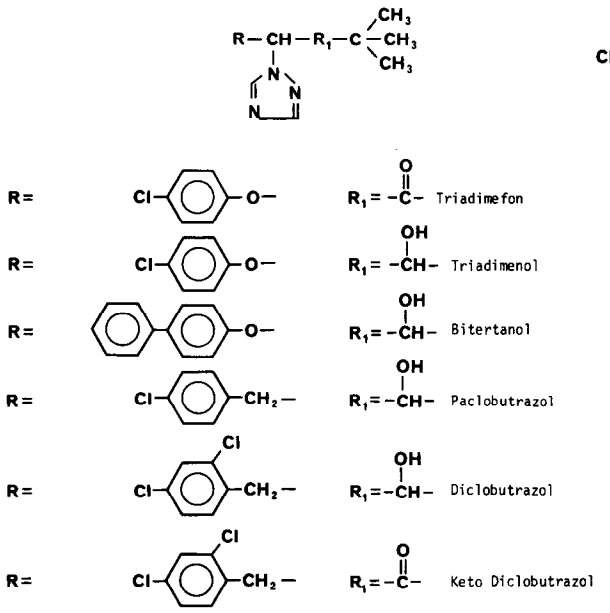

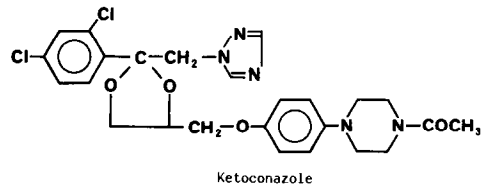

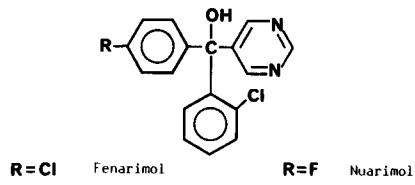

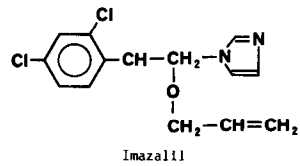

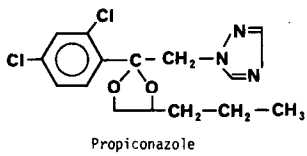

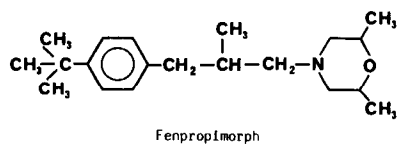

Fig. 1. Structures of the triazole xenobiotics.

Fig. 2. Structures of the imidazole, pyrimidine and morpholine xenobiotics.

RESULTS AND DISCUSSION

For each compound all parameters such as flow-rate, temperature programme rate and initial temperature were systematically varied to obtain the best separation. The elution order of enantiomers was obtained for triadimenol, paclobutrazol and diclobutrazol by comparison of their retention times to those of authentic single enantiomers. The order for bitertanol was assumed to be the same as for triadimenol.

Due to the low thermal stability of Chirasil Val (210°C maximum allowable operating temperature) ketoconazole could not be eluted from the column at a safe temperature. The enantiomers of compounds containing a keto as opposed to a hydroxyl group could not be separated, and only compounds containing a triazole group were resolved at all. Compounds which were resolved to some degree were triadimenol, bitertanol, paclobutrazol, diclobutrazol, and only the separation of these is discussed. All contain two chiral centres and hence exist as two diastereoisomers and four enantiomers. Both diclobutrazol and paclobutrazol are specifically the 2*RS*,3*RS* diastereoisomers. However, in this study each of the samples used contained

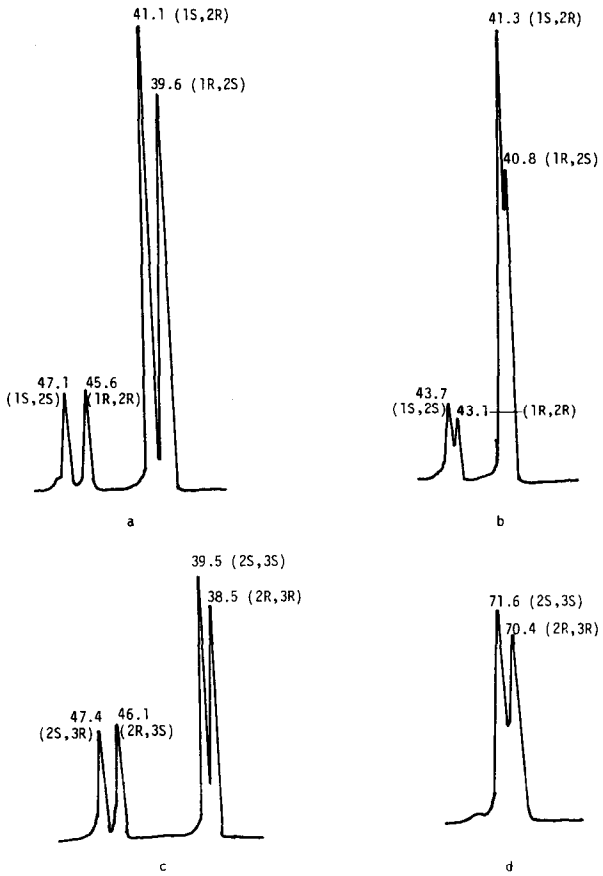

Fig. 3. Gas chromatograms of (a) triadimenol (60 to 150°C at 10°C min^{-1}, isothermal at 150°C, 8 p.s.i.); (b) bitertanol (60 to 190°C at 10°C min^{-1}, isothermal at 190°C, 12 p.s.i.); (c) paclobutrazol [same conditions as (a)]; (d) diclobutrazol (60 to 140°C at 10°C min^{-1}, isothermal at 140°C, 8 p.s.i., only first pair of enantiomers shown). All retention times are given in minutes.

different amounts of their other diastereomer (2*RS*,3*SR*) also; but in the text and figures they are still referred to as diclobutrazol and paclobutrazol. The separation of the first diastereoisomeric pair of enantiomers of each compound was improved by decreasing the upper temperature of the GC temperature programme. However, only one set of conditions is reported for each compound in which the best overall separation was obtained. For each compound, these optimum conditions are given in Fig. 3 which also includes the part of the chromatogram where the peaks elute.

It can be seen from the chromatogram of triadimenol (80:20 ratio of 1*RS*,2*SR* to 1*RS*,2*RS*) that virtually baseline resolution was obtained for all four enantiomers. A similar result was obtained for paclobutrazol (50:50 ratio of 2*RS*,3*RS* to 2*RS*,3*SR*) which is structurally related to triadimenol, the difference being a $-CH_2-$ group is substituted for an oxygen. As can be seen from Fig. 3 the conditions and chromatogram for paclobutrazol were almost identical to those for triadimenol. However, the introduction of a second chlorine atom into the aromatic ring of paclobutrazol, to

give diclobutrazol (87:13 ratio of 2*RS*,3*RS* to 2*RS*,3*SR* used) considerably decreased resolution and increased the retention time, as shown in Fig. 3. In order to resolve the first pair of enantiomers of diclobutrazol the retention time was over 60 min. Under these conditions the peaks due to the second pair of enantiomers were very broad and this was only improved by a higher final temperature of the GC programme which decreased the resolution of the first pair of enantiomers. The separation of the enantiomers was insufficient for the method to be used quantitatively.

Bitertanol (80:20 ratio of 2*RS*,3*SR* to 2*RS*,3*RS*) is an analogue of triadimenol where the chlorine atom has been replaced by a phenyl group. This substitution also considerably increased the retention characteristics of the compound and decreased the resolution of the enantiomers. The conditions of separation and the chromatogram are shown in Fig. 3. When the acetate of triadimenol was analysed no separation of the enantiomers was obtained and the significance of this result is discussed below.

For the results it appears that a hydroxyl group is important for resolution, probably because it provides differential binding of enantiomers to the Chirasil Val via hydrogen bonding. The conversion of the hydroxyl group of triadimenol to the acetate resulted in the loss of resolution of the enantiomers although the diastereoisomers were still well separated.

The compounds which gave separation on Chirasil Val were also analysed on an RSL-007 chiral column. No resolution of these compounds into their enantiomers was achieved. In both phases the chiral moiety is L-valine-*tert.*-butylamide and it might be expected that resolution would be similar between them. However, in Chirasil Val the chiral entity is coupled to the carboxyl group of a copolymer of dimethylsiloxane and carboxyalkylmethylsilicone[7]. In the case of RSL-007 it is coupled to a modified GC stationary phase, polycyanopropylmethylphenylmethylsilicone (OV-225)[4]. The difference in the backbone of the chiral stationary phase would alter the conformation of the phase. This would change the specific interactions between the solute and stationary phase, suich as hydrogen bonding, dipole–dipole interactions and dispersion forces, and therefore resolution would be affected.

A quantitative method for the analysis of the enantiomers of the fungicide triadimenol and its analogue paclobutrazol was obtained. This method of analysis has been used in the authors' laboratory in metabolism studies of triadimenol[8–10]. Although some resolution of the enantiomers of other compounds was obtained it was not sufficient for satisfactory quantitative analysis.

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