dylcholine is also a major phosphoglyceride in wheat flour, but in this case failure to find more than trace amounts can be attributed to the use of solvents which do not extract this lipid efficiently. In our experience water-saturated *n*-butanol is the best solvent for this purpose[1].

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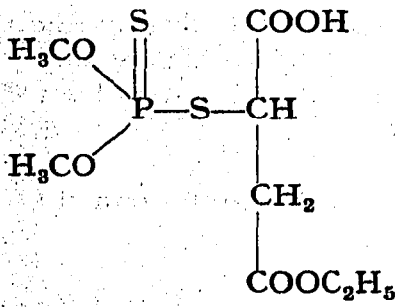
Separation of isomers of malathion monocarboxylic acid by thin-layer chromatography

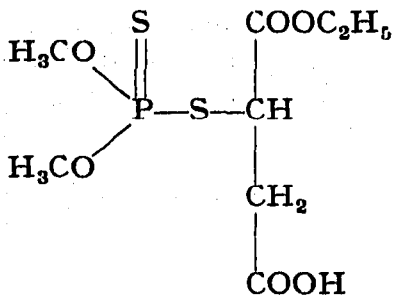
The insecticide malathion (O,O-dimethyl-S-[1,2-di(ethoxycarbonyl)ethyl] phosphorodithioate) has been shown to be degraded by carboxyesterases of mammals and insects to malathion monoacid. The latter compound exists in two isomeric forms, denoted as α- and β-monoacid (cf. p. 282).

We encountered this effect in a study of the malathion resistance mechanism of a certain strain of housefly. A simple chromatographic technique, which may be of wider application, was developed to separate the α- and β-monoacids.

Thin-layer chromatography was carried out on silica gel (DC-Fertigplatten, Kieselgel F_{254}, Merck A.G., Darmstadt, G.F.R.). Before use the plates (10 × 20 cm) were washed with acetone and activated for 15 min at 90°. They were developed in



Malathion α-monoacid



Malathion β-monoacid

a sandwich chamber (width 1.5 mm) with acetone to which 0.5 vol. % acetic acid was added. After development the chromatograms were dried, sprayed first with 0.2 *N* HCl in acetone[1], dried again, sprayed with a 0.5% solution of 2,6-dibromoquinone-4-chloroimide (DQC) in cyclohexane[2], and heated at 110° for 5 min. DQC reacts with compounds containing P=S bonds, giving red to orange-red spots. About 1–2 μg of compound can be detected.

Crystalline malathion monoacid (a gift from Dr. R. BLINN, American Cyanamid Company, Princeton, N.J., U.S.A.) behaves as a single compound with R_F 0.57, when it is chromatographed in this way. CHEN *et al.*[3] have shown that this crystalline monoacid is the β-isomer. A sample of malathion monoacid (oil) prepared according to MARCH *et al.*[4] gives two spots with R_F values of 0.39 and 0.57. The two components were isolated from the thin-layer plate and subjected to rechromatography under the same conditions. Both were found to be chromatographically pure substances with the expected R_F values.

The identity of the two components was confirmed by IR spectroscopy. About 400 μg of the mixture of both components were applied as a band on the thin-layer plate. After separation, a 3-cm wide strip was cut off to permit the compounds to be located with DQC. On the remaining part of the plate, the two components were each concentrated into areas of about 1 cm^2 by displacement with 96% ethanol in a direction perpendicular to that of separation. The material from these areas was scraped off and the silica gel powder was stored for several days *in vacuo* over KOH pellets to remove residual acetic acid as far as possible.

Each sample of isolated material was filled into a small vertical column (I.D. 2.5 mm, height 3 cm), tapered to a point at the bottom. About 50 μl of acetone were injected into the top of the column. The first drops of the eluent were collected in a small vessel placed under the column. The collected eluent was transferred by the method of CURRY *et al.*[5] to 4 mg of KBr powder from which a pellet was then formed. In this way IR spectra of the two spots with R_F values of 0.39 and 0.57 were obtained. The two spectra were nearly identical, except for the parts between 1500 cm^{-1} and 1300 cm^{-1}, which are reproduced in Fig. 1.

The IR spectrum of the spot with R_F 0.39 had a pronounced absorption band at 1350 cm^{-1}, indicative of the α-monoacid[3]. This band was absent from the IR spectrum of the spot with R_F 0.57. The latter spectrum was identical with that of the synthetic β-monoacid.

We presume that the chromatographic separation of α- and β-monoacid is brought about by a pH gradient created during development. This would explain

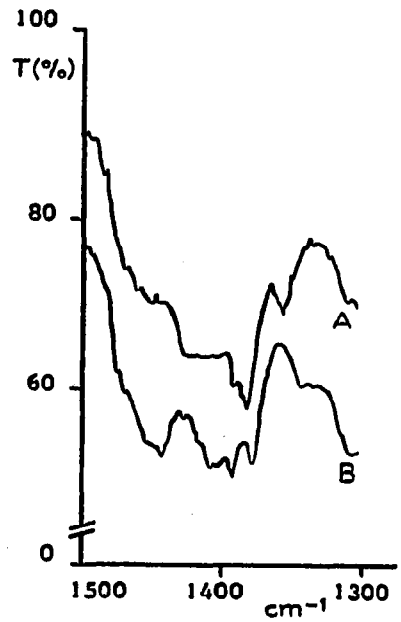


Fig. 1. Part of the IR spectra of the spots with R_F values of 0.39 (A) and 0.57 (B).

why the isomer with the larger pK_a (3.74 for β-, 3.62 for α-monoacid[3]) has the higher R_F value. The reproducibility of the R_F values is about 5%; with non-activated plates the variation is larger. The concentration of acetic acid in the developer affects both the R_F value and the separation. At lower acetic acid concentrations the R_F values are lower but the spots tend to become elongated, resulting in decreased resolution. We have also observed that some batches of precoated plates give higher R_F values than others.

Our technique may be useful in the separation of other compounds differing slightly in pK_a that are otherwise difficult to separate.

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