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Note

Interference by warfarin in the detection of carbamate insecticides on thin-layer chromatograms by an esterase inhibition technique

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Many enzyme inhibition methods for the detection of esterase-inhibiting pesticides on thin-layer chromatograms have been described in the literature[1,2]. Some of these methods, developed in this[3] and other[4] laboratories, have been used routinely for the detection of organophosphorus pesticide residues in wildlife casualties investigated by the Pest Infestation Control Laboratory[5] for several years. The recent introduction of the oxime carbamates into agricultural use has necessitated the development of a method sensitive enough to detect small amounts of these pesticides on thin-layer chromatograms. This was achieved by combining the enzyme source used by Winterlin *et al.*[6] with the substrate and dye-coupling reagent of Ackermann[7]. When the specificity of this esterase inhibition reaction was investigated, it was observed that in addition to directly esterase-inhibiting organophosphorus and carbamate pesticides being detected, the anticoagulant rodenticide warfarin gave an apparent inhibition spot. Although we are not aware of any previous report of warfarin causing esterase inhibition, Reiner and Simeon[8] have shown that other coumarin derivatives are inhibitors of these enzymes. The relative sensitivity of this procedure to a number of carbamate compounds as compared to warfarin has now been determined.

EXPERIMENTAL

Glass plates (200 × 200 mm) coated with either silica gel G or silica gel GF_{254} (E. Merck, Darmstadt, G.F.R.) to a thickness of 200 or 400 μm were used. Warfarin (Sorex, London, Great Britain) was applied in ethyl acetate solutions of various concentrations to the plate using disposable capillaries (Microcaps; Drummond, Broomall, Pa., U.S.A.). Several carbamate pesticides were similarly spotted for comparison. The developing solvent (*n*-hexane–acetone, 60:40) was allowed to ascend the plate to a distance of 150 mm above the origin in a closed tank saturated with solvent vapour. The plate was then removed from the tank and, after evaporation of the solvent (30 min), it was sprayed with a solution of outdated human blood plasma (2 ml, National Blood Transfusion Service) in aqueous tris(hydroxymethyl)methylamine buffer solution, 0.05 *M*, pH 7.5, (20 ml). The plate was left at room temperature for 20 min to allow inhibition to occur before spraying with substrate spray reagent.

This reagent was prepared by mixing substrate solution (2-naphthyl acetate, 30 mg, dissolved in absolute ethanol, 8 ml) with coupling reagent solution (diazonium salt of *o*-dianisidine [fast blue B salt], 50 mg, dissolved in distilled water, 32 ml) immediately prior to spraying.

RESULTS AND DISCUSSION

The magenta background colour appeared on the plate within 20 min after spraying the substrate spray reagent. The position of esterase-inhibiting compounds was shown by white spots where hydrolysis of 2-naphthyl acetate to 2-naphthol had not taken place. The lowest detectable amount of warfarin was 0.5 μg compared to 0.5 ng for aldicarb (a potent inhibitor). R_F values for warfarin varied according to the layer material and thickness, ranging from 0.33 on 400 μm silica gel G to 0.44 on 200 μm silica gel GF_{254}. Preliminary kinetic studies have indicated that the detection of warfarin by this method involves inhibition of the enzyme and not direct reaction with the substrate or interference with the coupling reaction.

This work has demonstrated that the detection of a spot on a thin-layer chromatogram by esterase inhibition should not be taken as unequivocal evidence of the presence of an organophosphorus or carbamate pesticide; the anticoagulant rodenticide warfarin can also be detected by such a method to a lower limit of 0.5 μg. Although this detection limit is high when compared to that for certain organophosphorus and carbamate compounds, it compares favourably with that of existing methods for the detection of warfarin on thin-layer chromatograms.

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