Dimethylphosphorothioates

Reaction with Malathion and Effect on Malathion Toxicity

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Abstract. Five dimethylphosphorothioates were tested for their toxicity to rats, potentiation of malathion toxicity in rats, inhibition of carboxylesterase in vitro, and reaction with malathion in vitro. The compounds were: potassium salts of (CH₃S)₂P(O)O⁻(I), (CH₃O)(CH₃S)P(O)S⁻(II), (CH₃O)₂P(O)S⁻(III), (CH₃O)₂P(S)S⁻(IV), and (CH₃O)(CH₃S)P(O)O⁻(V).

The dimethylphosphorothioates are not toxic to rats (up to 1 g/kg, orally), they do not potentiate malathion toxicity in rats, and do not inhibit carboxylesterase activity in vitro (up to 1 mM concentrations). However, when the S-acid diesters (II, III, IV) are incubated with malathion for several days at room temperature or for several hours at 50° C they become methylated forming the trimethylphosphorothioates OSS-trimethyl phosphorodithioate, OOS-trimethyl phosphorothioate and OOS-trimethyl phosphorodithioate respectively, which potentiate malathion toxicity. Furthermore, these same acid diesters increase the rate of isomerization of malathion into OS-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate (isomalathion) particularly, diester IV.

The formation of the trimethylphosphorothioates and isomalathion from the interaction of the S-acid diesters with malathion was determined by thin layer chromatography (TLC), gas chromatography and mass spectrometry and could be detected by in vitro inhibition of carboxylesterase. TLC methods can detect 1 mg of the trimethylphosphorothioates and isomalathion per gram malathion.

Key words: Dimethylphosphorothioates – Trimethylphosphorothioates – Malathion – Thin layer chromatography – Carboxylesterase

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Introduction

In an epidemic of malathion poisoning in 1976 the enhanced toxicity of many water dispersable powder samples of this insecticide was shown to be best correlated with their isomalathion content (Baker et al. 1978; Miles et al. 1979; Aldridge et al. 1979; WHO 1978, 1979a). This increase in toxicity is undoubtedly due to the inhibition of a carboxylesterase (malathionase) responsible for the detoxification of malathion by removal of one of the ethyl groups from the diethylmercaptosuccinate moiety. Other impurities which can potentiate the toxicity of this insecticide in rats are the various trimethylphosphorothioates, which include OSS-trimethylphosphorodithioate OOS-trimethylphosphorodithioate and OOS-trimethyl phosphorodithioate (Pellegrini and Santi 1972; Umetsu et al. 1977; Aldridge et al. 1979). However, some samples of malathion stored in hot climates have appeared to be more toxic than would be expected from their content of isomalathion. Dimethylphosphorothioates which are also present in malathion and were increased on accelerated storage in the laboratory (Miles et al. 1980) might contribute in some way to the increased mammalian toxicity.

The toxicity of these dimethylphosphorothioates alone and together with malathion has therefore been examined. During the course of this work it was observed that mixtures of some dimethylphosphorothioates and malathion when stored increased in toxicity. The relationship between the composition and toxicity of these mixtures has been examined and some observations of their inhibitory potency on a carboxylesterase are presented.

Materials and Methods

Organophosphorus Compounds (Table 1). All compounds were supplied by Dr. J. Miles and Mr. D. Mount (Centre for Disease Control, Atlanta, Georgia, USA) except malathion which was supplied by Dr. M. Thomson (Cheminova, Copenhagen, Denmark). Isomalathion was 90% pure and concentrations were appropriately corrected. The purity of all other organophosphorus compounds was 98%.

Experiments in vivo. Female Porton derived Wistar rats (LAC : P) 180–200 g, 8–10 weeks of age, were used throughout. Prior to dosing rats were deprived of food overnight (approximately 16 h) and then dosed by oesophageal intubation; the carrier solvent was glycerol formal (Sanderson 1959) unless otherwise stated. After dosing the rats were fed and kept under observation for up to 5 days. Calculation of the LD$_{50}$ was by the method of Weil (1952) using groups of four rats.

Thin Layer Chromatography. The procedure described by Abbot et al. (1965) was applied. Aluminium sheets precoated with silica gel 60F 254 to a thickness of 0.2 mm (E. Merck, Darmstadt, FRG) were used throughout. Three solvent systems were used (a) 100% benzene for OOS-trimethylphosphorodithioate, (b) 60% hexane + 40% acetone for OOS-trimethylphosphorothioate, OSS-trimethylphosphorodithioate and isomalathion, and (c) 10% hexane + 90% acetone for the separation of compounds, other than those named above, produced in the reaction of malathion with diesters (cf. text). Visualisation of compounds was by spraying with brilliant green C.I. 42040 (0.1% in acetone) followed by colour development in bromine vapour. Location and determination of compounds was by comparison of $R_f$ values against known standards (Table 2). Quantitation was by visual comparison with standards of known concentrations.