Tetrahedron: *Asymmetry* 25 (2014) 529–533

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Chemoenzymatic resolution of *rac*-malathion

David M. Hitt [a,b], Yamina Belabassi [a], Joyce Suhy [c], Clifford E. Berkman [d], Charles M. Thompson [a,*]

[a] *ATERIS Technologies, 901 N Orange Street, Missoula, MT 59802, USA*
[b] *Department of Natural Sciences, Carroll College, 1601 N. Benton Ave., Helena, MT 59625, USA*
[c] *Synarc 7707 Gateway Boulevard, 3rd Floor, Newark, CA 94560, USA*
[d] *Department of Chemistry, Washington State University, Pullman, WA, USA*

ARTICLE INFO

Article history:
Received 21 January 2014
Accepted 26 February 2014

ABSTRACT

Malathion, diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate, is an organophosphate used to control insect pests. Malathion contains a diethyl succinate moiety that is a known functional group susceptible to desymmetrizing enzymes such as esterases that selectively react with a single enantiomer. Purified *rac*-malathion was subjected to hydrolysis at the diethyl succinate moiety of malathion under various conditions using wild type pig liver esterase to form (*S*)-malathion (12% ee) and ~3:2 mixture of α- and β-monoacids of (*R*)-malathion. Technical malathion could not be enriched due to the presence of esterase inhibitors. Further investigation of this resolution using a panel of six PLE isoenzymes also demonstrated the formation of (*S*)-malathion, however, an improvement of up to 56% ee was obtained.

1. Introduction

Malathion **1** (Scheme 1) is one of the most widely-recognized organophosphate (OP) insecticides. There has been a steady domestic and global demand for malathion covering four principle applications: (a) reduction or eradication of disease vectors (e.g., mosquitoes; malaria); (b) domestic or residential insect pest control including fleas; (c) in broadcast crop protection (e.g., cotton); and (d) as a pharmaceutical pediculicide (head lice, etc.).[1–6]

Malathion is one of the few OP insecticides that is recommended by the World Health Organization (WHO) for indoor use for eradicating the mediterranean fruit fly 'MedFly', and mosquitoes carrying West Nile Virus. For decades, malathion and lindane (chlorinated hydrocarbon) were the only FDA-approved agents for pediculosis. For the most part, malathion is in continued use because it is relatively easy to prepare, inexpensive, minimally toxic to humans, and biodegradable.

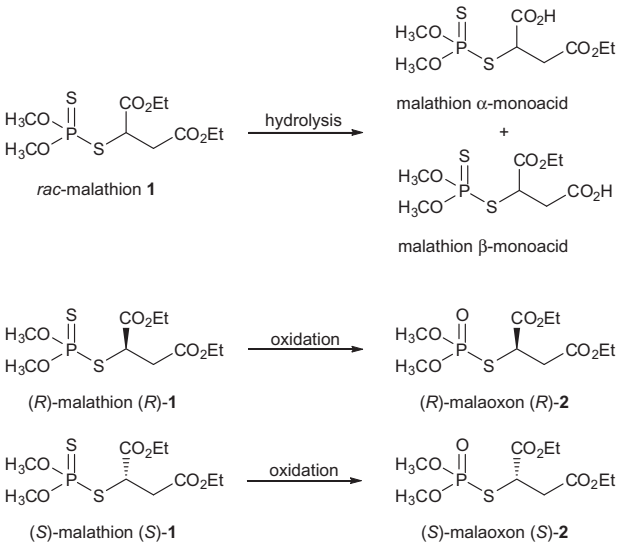
Despite numerous advantages, the continued use of malathion is accompanied by concerns with the central nervous system (CNS) toxicity typically associated with OP compounds. *rac*-Malathion is relatively non-toxic to humans because it is preferentially hydrolyzed (detoxified) by carboxylesterases to form the innocuous malathion alpha- and beta-monoacids (Scheme 1). However, malathion can be oxidatively converted into malaoxon **2**,[7] which is a potent inhibitor of acetylcholinesterases (AChEs) and carboxyesterases (CaEs).[8,9] Moreover, (*R*)-malaoxon (Scheme 1) had demonstrated up to 22-fold greater inhibition of mammalian AChE than the corresponding (*S*)-isomer,[10–13] (*R*)-malaoxon (88% ee; LD_{50} 23.1 mg/kg) is twice as toxic as the (*S*)-isomer (90% ee; LD_{50} 48.0 mg/kg) to rats.[13,14] Based on this data, (*S*)-malathion (*S*)-**1** is expected to be safer to humans since it metabolizes into (*S*)-malaoxon. The enantiomers of malathion and malaoxon (Scheme 1) were first synthesized in the author's lab[12,15,16] although *O*,*O*-diethyl phosphate analogs of malathion and malaoxon have been previously prepared.[17]

The first synthetic approaches to asymmetric malathion provided 90% ee, but were lengthy, used expensive starting materials and highly reactive reagents, and were conducted at low temperature.[12,16] Other groups have improved upon these synthetic approaches,[13,14] and introduced chiral HPLC as a useful alternative for separating racemic malathion into the (*R*)- and (*S*)-isomers.[18,19] However, these approaches may not be amenable for large scale preparation. The ease of preparation and very low cost of technical grade, racemic malathion suggest its possible use as a starting material.

As indicated, malathion is a substrate of mammalian esterases forming α- and β-monoacids although only the α-monoacid was initially found in the urine from rats[20] and sheep.[21,22] Malathion resembles substrates of esterases that are known to stereospecifically hydrolyze one ester from a racemate,[23–25] although the succinate diethyl ester may be less preferred as a substrate. Assuming malathion is a substrate, enzymatic resolution would form a

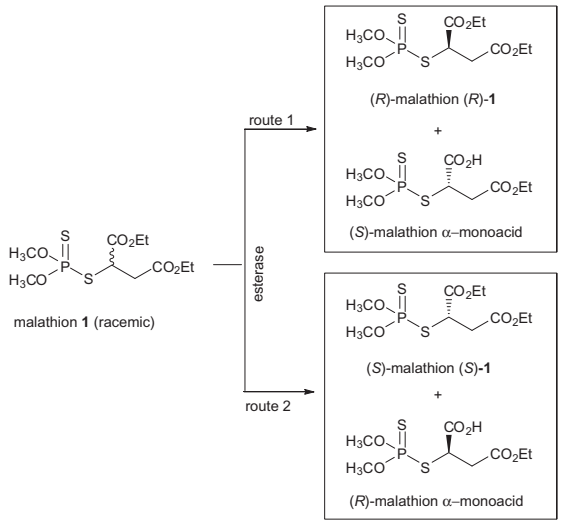
* Corresponding authors. Tel.: +1 4062434643; fax: +1 4062435228.
E-mail address: charles.thompson@umontana.edu (C.M. Thompson).

http://dx.doi.org/10.1016/j.tetasy.2014.02.013
0957-4166/



Scheme 1. Hydrolysis of malathion to form non-toxic monoacids and oxidation of malathion stereoisomers to form the more toxic malaoxon stereoisomers.

separable, carboxylic acid of one enantiomer and an unreacted malathion enantiomer (Scheme 2). Although esterases have been used in organic synthesis for decades,[24] to the best of our knowledge, malathion has not been examined as a possible substrate. Thus, if racemic malathion can be resolved via enzymes, the individual enantiomers could be isolated following separation and esterification (Scheme 2).



Scheme 2. Enzymatic resolution of malathion.

As shown in Scheme 2, the (*R*)- (route 1) or (*S*)-malathion (route 2) could be directly obtained from an esterase[26,27] along with the opposite enantiomer as the malathion monoacid. Using either route, the (*R*)- or (*S*)-malathion monoacid antipode could also be separated. Based on the in vivo data,[20,28] malathion α-monoacid[29,30] could be hypothesized to be the major or sole regioisomer formed.

Herein, we report the first preparation of enantiomerically enriched malathion and malathion monoacids via kinetic resolution using pig liver esterase (PLE). In addition to wild type PLE, a number of PLE isoforms have been engineered with distinctive ligand binding properties that have the potential to optimize the conversion. As noted in Scheme 2 (only the α-monoacid is shown), the resolution of malathion by PLE would produce the oppositely configured monoacid enantiomer and a malathion enantiomer that are easily separable by conventional extraction (sodium carbonate vs ether). For validation, the spectroscopic and optical characteristics of the malathion products can be compared with known authentic samples. Herein, the resolution of malathion was conducted by wild type and isoforms of PLE.

2. Results and discussion

2.1. Hydrolysis of malathion using wild type pig liver esterase

The resolution of racemic malathion into an enantiomer of malathion and a corresponding monoacid (Scheme 2) using wild-type pig liver esterase (PLE; Sigma–Aldrich) was examined. A solution of technical malathion (>90% purity) in acetone was added to a solution of PLE (90 U/mmol) in phosphate buffered saline (PBS; 50 mM, pH 7.5) and when needed the reaction periodically adjusted to pH 7.8 using 0.01 M NaOH. Under these reaction conditions, malathion was hydrolyzed to form the monoacids as initially evidenced by thin layer chromatography (lower R_f on silica TLC).[31] Unfortunately, only ~20% of the malathion underwent conversion based on the isolation of monoacids and recovery of malathion. Suspecting possible inhibition for the low conversion, the enzyme activity was monitored as a function of time under the reaction conditions. In control studies (absence of malathion) a 16% reduction in PLE activity was observed over 24 h. However, when malathion was present at the onset of the reaction, the enzyme activity decreased by 70% of the initial rate within 1 h and the substrate turnover rate had diminished to slightly above background at 23 h ($\Delta A/\Delta t = 0.036\ \text{min}^{-1}$; Table 1). The enzyme activity was determined at various time points based on the hydrolysis of *p*-nitrophenylacetate (*p*NPA).[32]

Table 1
Hydrolysis of *rac*-malathion by PLE (90 U/mmol) in acetone:PBS (pH 7.5; 50 mM) 1:11

Time	$\Delta A/\Delta t$ (min^{-1})	U/mg	Activity (rel)
0[a]	1.301	17.3	1.00
1 h[b]	0.328	4.71	0.27
2 h[b]	0.195	2.80	0.16
23 h[b]	0.044	0.63	0.04

[a] Assay performed with stock enzyme solution.
[b] Assay performed with reaction aliquots.

Based on these results, the observed reduction in enzyme activity was either due to malathion, the malathion monoacid products, and/or impurities in the malathion source. To resolve this issue, technical malathion was analyzed by ^{31}P NMR. The spectrum contained the expected major peak at 96.2 ppm (malathion), but also a number of minor impurities including a peak at 28.3 ppm, which corresponds to malaoxon. As a result of this finding, technical malathion was chromatographed (95:5 hex/EtOAc) to afford >99% purity, and resubjected to the same PLE-hydrolysis conditions. At 24 h, enzyme activity decreased to only 60% of the original level and by 43 h, residual activity remained but no additional conversion to the monoacid was observed. The improvement in retained PLE activity may be due to the removal of one or more minor amounts of the contaminants including malaoxon **2**, which is a known esterase inhibitor. However, the immediate inhibition of PLE suggests that malaoxon is likely to be an impurity in technical malathion rather than being produced from malathion under the reaction conditions. Therefore, the direct conversion of technical malathion