



(43) International Publication Date
27 September 2012 (27.09.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/129142 A2

- (51) **International Patent Classification:**
A01N 57/12 (2006.01) *A01P 7/04* (2006.01)
- (21) **International Application Number:**
PCT/US2012/029594
- (22) **International Filing Date:**
18 March 2012 (18.03.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/454,470 18 March 2011 (18.03.2011) US
- (71) **Applicant** (*for all designated States except US*): **ATERIS TECHNOLOGIES, LLC** [US/US]; 8581 St. Vrain Way, Missoula, MT 59808 (US).
- (72) **Inventor; and**
- (75) **Inventor/Applicant** (*for US only*): **THOMPSON, Charles, M.** [US/US]; 8581 St. Vrain Way, Missoula, MT 59808 (US).
- (74) **Agent:** **KIM, Katherine, J.**; Molecule Law, 22 W. Washington Street, Suite 1500, Chicago, IL 60602 (US).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) **Title:** STEREOSELECTIVE PROCESS FOR PREPARING ASYMMETRIC MALATHION AND ANALOGS FOR PHARMACEUTICAL AND AGRICULTURAL USE

(57) **Abstract:** The preparation of enantiomeric excess of a first enantiomer of malathion or an analog thereof from a solution of racemic malathion or an analog thereof, by stereoselectively hydrolyzing the first enantiomer to produce one or more monoacid enantiomers of the first enantiomer using an effective amount of a hydrolyzing agent and then subjecting the monoacid enantiomers to esterification.



WO 2012/129142 A2

STEREOSELECTIVE PROCESS FOR PREPARING ASYMMETRIC MALATHION AND
ANALOGS THEREOF FOR PHARMACEUTICAL AND AGRICULTURAL USE

5

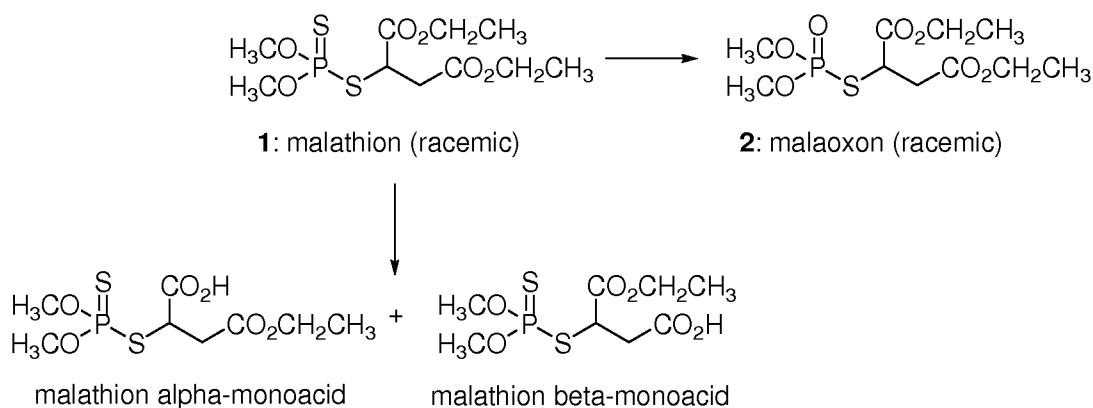
TECHNICAL FIELD

This invention is directed to the preparation and separation of enantiomers, and in particular to the preparation and separation of malathion or malathion methyl enantiomers and the preparation of stereoisomeric malathion or malathion methyl analogs and derivatives for pharmaceutical and insecticidal uses.

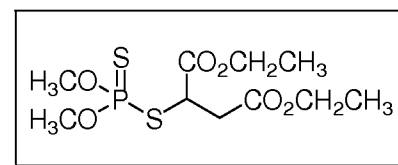
10

BACKGROUND OF THE INVENTION

Malathion **1** is an organophosphate insecticide for use in agriculture, pharmaceutical applications and other treatments. In pure form malathion has low human and mammalian toxicity and short environmental and *in-vivo* half-lives in comparison to other insecticides. Although purified malathion is relatively nontoxic to humans, impurities (isomalathion, trimethyl phosphorothionates, etc.) may be present resulting in greater toxicity of the product. In insects, malathion is readily converted to malaoxon, which is 40-times more toxic than malathion as an anti-cholinesterase agent. In humans, however, conversion of malathion to malaoxon occurs at a slower rate, and a margin of safety is conferred.

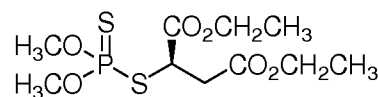


The structure of malathion (racemic) **1** contains a single asymmetric center at a carbon atom that affords a mixture of R and S-enantiomers; a mixture that constitutes the current commercial form of malathion. Each R- and S-malathion stereoisomer undergoes the aforementioned oxidation and hydrolysis reactions.

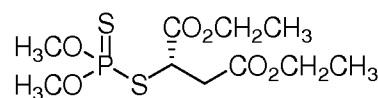


1: malathion (racemic)

R)-Malaoxon has been shown to be a more potent inhibitor (up to 22-fold greater) of mammalian acetylcholinesterases than the corresponding (S)-isomer. Because the (R)-stereoisomer of malathion corresponds with oxidative conversion to (R)-malaoxon, (R)-malathion is expected to be more toxic to mammals than the corresponding (S)-isomer of malathion (expected to be of lower toxicity to mammals). It follows that



(R)-malathion



(S)-malathion

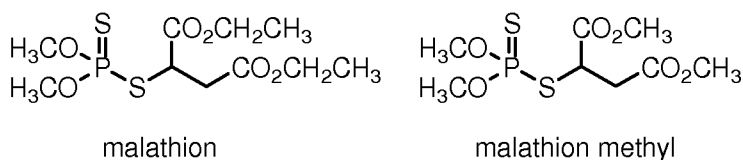
pharmaceutical, agricultural and other preparations should use a single enantiomer of malathion to reduce the overall toxicity risk to mammals. However, existing syntheses of the (R)- or (S)-malathion isomers are poor yielding, large amounts of impurities are produced, the approach can lead to racemization (loss of asymmetry) limiting the potential for single enantiomer use, and do not use readily available, inexpensive racemic malathion as a starting material.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic showing a process for producing an enantiomeric excess of malathion in accordance with an aspect of the present invention.

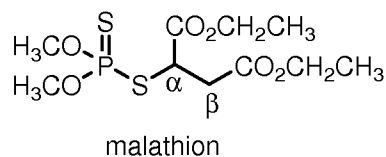
DETAILED DESCRIPTION OF THE INVENTION

A novel process for the preparation of (R)- and/or (S)-malathion from racemic malathion using enzymes, cell culture or tissue homogenates is demonstrated along with methods of synthesizing new classes of stereoisomeric organophosphate analogs from the intermediates produced. Likewise, the preparation of (R)- and/or (S)-malathion- methyl from racemic malathion methyl using enzymes, cell culture or tissue homogenates is described along with methods of synthesizing new classes of stereoisomeric organophosphate analogs from intermediates produced.

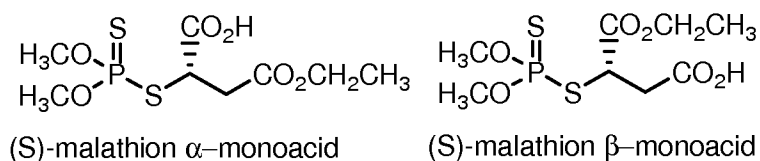


Definitions

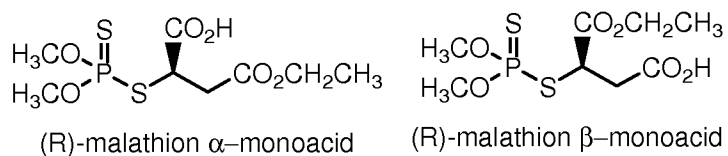
[1] Malathion [2-(dimethoxyphosphinothioylthio) butanedioic acid diethyl ester] is:



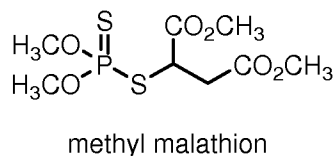
5 [2] (S)-malathion α -monoacid and (S)-malathion β -monoacid are:



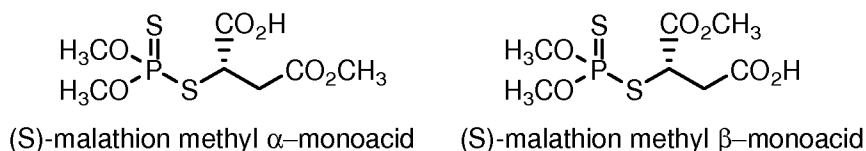
[3] (R)-malathion α -monoacid and (R)-malathion β -monoacid are:



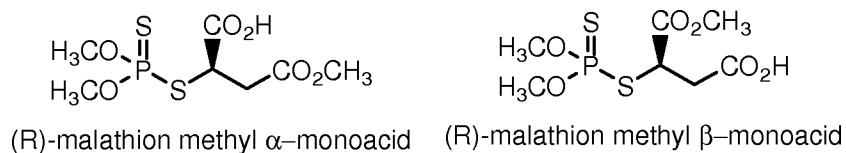
10 [4] Malathion methyl, the dimethyl carboxyester analog of malathion [2 (dimethoxyphosphinothioylthio) butanedioic acid dimethyl ester] is:



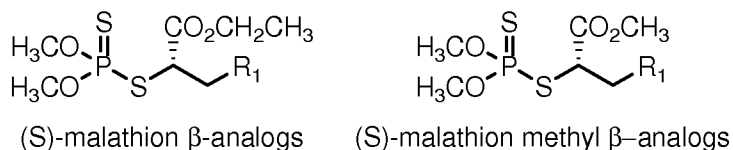
[5] (S)-malathion methyl α -monoacid and (S)-methyl malathion β -monoacid are:



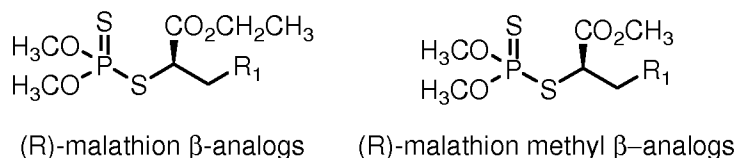
[6] (R)-malathion methyl α-monoacid and (R)-malathion methyl β-monoacid are:



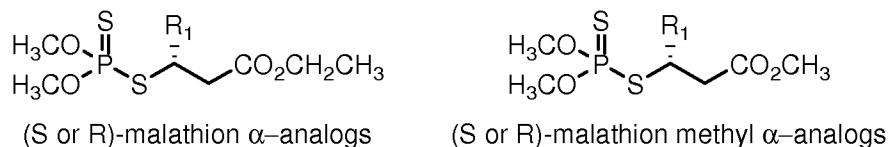
5 **[7]** (S)-malathion β-analogs and (S)-malathion methyl β-analogs are:



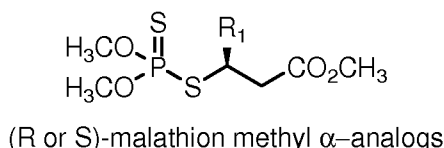
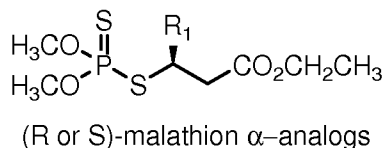
[8] (R)-malathion β-analogs and (R)-malathion methyl β-analogs are:



10 **[9]** (S or R)-malathion α-analogs and (S or R)-malathion methyl α-analogs contain an R₁ substituent positioned behind the plane (note: configuration assignment at carbon depends on the nature of the R₁ group) are:



15 **[10]** (R or S)-malathion α-analogs and (R or S)-malathion methyl α-analogs contain an R₁ substituent positioned in front of the plane (note: configuration assignment at carbon depends on the nature of the R₁ group):



In the structures defined in [7] through [10]:

R^1 may be selected from the group consisting of esters defined by $C(O)OR^2$, where R^2 is any carbon atom which may be optionally substituted;

5

In another embodiment, R^1 may be selected from the group consisting of amides of the formulas $C(O)NH_2$, $C(O)NHR$, and NR^2R^3 , where R^2 and/or R^3 may be any carbon atom of which may be optionally substituted;

10

In another embodiment, R^1 may be selected from the group consisting of thioesters of the formula $C(O)SR^4$, where R^4 may be any carbon atom of which may be optionally substituted but R^4 may not be hydrogen;

15

In another embodiment, R^1 may be selected from the group consisting of CH_2X , where $X = F, Cl$ or Br but not I (iodine);

In another embodiment, R^1 may be selected from the group consisting of any CH_2XR^5 , where $X = O, N$, or S and R^5 may be hydrogen or any carbon atom of which may be optionally substituted;

20

In another embodiment, R^1 may be selected from the group consisting of any CH_2XR^5 , where $X = O(C)R^5$, $NC(O)R^5$ -, or $SC(O)R^5$ - and R^5 may be hydrogen or any carbon atom of which may be optionally substituted;

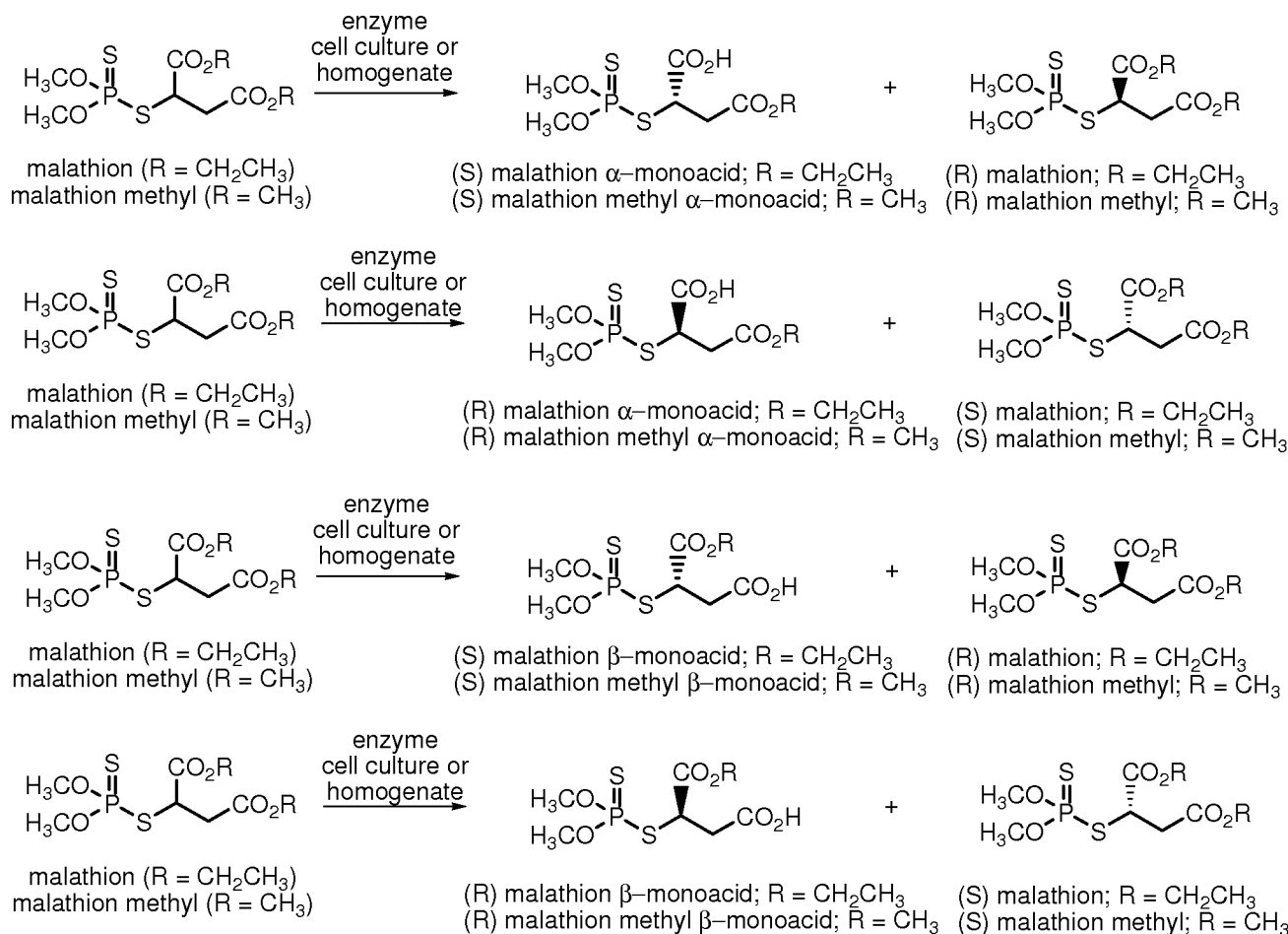
25

In another embodiment, R^1 may be selected from the group consisting of any carbon atom of which may be optionally substituted;

R^1 may include aryl, heteroaryl, alkyl, cycloalkyl, araalkyl, alkenyl, and alkynyl in certain embodiments.

In certain aspects of the present invention, the use of enzymes, cell culture, tissue homogenates ('homogenates') or other catalysts may be used to convert racemic malathion or malathion methyl into asymmetric, non-racemic monoacids and the corresponding asymmetric products are defined as:

5



As used herein, "enzyme" is a chemical catalyst that conducts a hydrolysis with or without the formation of asymmetric products. Examples of enzymes may be but are not limited to: hydrolase, esterase, diesterase, phosphatase, or other biological catalyst capable of hydrolytic action (naturally occurring, recombinant or genetic variants therein).

As used herein, "cell culture" is defined as single cells, isolates or cultures of cells capable of conducting the hydrolysis with or without the formation of asymmetric products.

As used herein, tissue homogenates ("homogenates") are cell-, tissue-, biofluid-, or organism-based mixtures capable of conducting the hydrolyses with or without the formation of asymmetric products.

As used herein, "stereoselectively" means through the use of stereoselectivity.

5 Stereoselectivity is the property of a chemical reaction in which a single reactant forms an unequal mixture of stereoisomers during the non-stereospecific creation of a new stereocenter or during the non-stereospecific transformation of a pre-existing one. The selectivity arises from differences in steric effects and electronic effects in the mechanistic pathways leading to the different products.

10 As used herein, the term "analog" refers to a chemical compound that is structurally similar to another compound as described herein, but differs slightly in composition (as in the replacement of an atom by an atom of a different element or the replacement of one functional group by another functional group). There may be a single substitution or multiple substitutions in the subject compound.

15 As used herein, "administering" or "administration of" a compound or composition herein to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. The administering or administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, or
20 topically. Administering or administration includes self-administration and the administration by another.

As used herein, "composition", "pharmaceutical composition" or "therapeutic agent" refers to the nature and proportions of the elements comprising a chemical compound. Optionally, the "composition," "pharmaceutical composition" or "therapeutic agent" further comprises
25 pharmaceutically acceptable incipients, diluents or carriers. In the case of an interfering molecule, for example, the interfering molecule may be combined with one or more pharmaceutically acceptable diluents.

As used herein, the term "effective amount" or the like, is meant an amount at concentrations, dosages and/or for periods of time necessary to achieve the desired result.

30 As used herein, "hydrolase" refers to an enzyme that catalyzes the hydrolysis of a chemical bond. Hydrolysis is a chemical reaction during which molecules of water (H_2O) are split into hydrogen cations (H^+) and hydroxide anions (OH^-) in the process of a chemical mechanism.

As used herein, "hydrolyze" means undergo hydrolysis.

As used herein, "alpha carbon" refers to the first carbon bonded to a functional group.

As used herein, "beta carbon" refers to the carbon bonded to the alpha carbon or the second carbon bonded to a functional group.

5 As used herein, "esters" are chemical compounds derived by reacting an oxoacid with hydroxyl compound such as an alcohol or phenol. Esters are usually derived from an inorganic acid or organic acid in which at least one –OH (hydroxyl) group is replaced by an –O-alkyl (alkoxy) group, and most commonly from carboxylic acids and alcohols.

10 As used herein, "esterification" is the reaction between alcohols and carboxylic acids to make esters.

As used herein, "monoacid" refers to a carboxylic acid with a single carboxyl group.

As used herein, "racemic mixture", also known as "racemate", is one that has equal amounts of left- and right-handed enantiomers of a chiral molecules. Racemate is optically inactive.

15 As used herein, "isomer" is one of several chemical species that have the same molecular formula but different structural formula or different stereochemical formula and hence potentially different physical and/or chemical properties.

20 As used herein, "stereoisomers" are isomers which have their atoms connected in the same sequence but differ in the way the atoms are oriented in space. Stereoisomers can be classed as cis- or trans- isomers or optical isomers.

As used herein, "enantiomer" is one of the two stereoisomers that are mirror images of each other that are not identical, much as one's left and right hands are the same except for opposite orientation.

25 As used herein, "non-miscible" refers to the property of liquids that do not form a solution in certain proportions.

As used herein, "organic solvents" are organic (hydrophobic) liquids in which something is dissolved.

As used herein, "homogenate" refers to a material that is obtained through homogenization, consisting of similar elements or ingredients, or a uniform quality throughout.

30 As used herein, the term "preventing" means causing the clinical symptoms of the disease state not to develop, e.g., inhibiting the onset of disease, in a subject that may be exposed to or predisposed to the disease state, but does not yet experience or display symptoms of the disease state.

As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment.

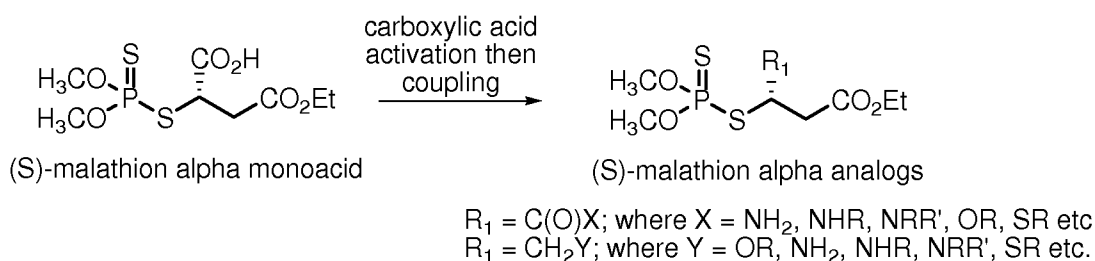
As used herein, the terms "treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder.

Aspects of the present invention are directed to a novel process to produce malathion and analogs of malathion (new chemical entities) in enantiomeric excess or high enantiomeric purity derived from racemic malathion or a racemic mixture of malathion analogs. In one embodiment, there is provided a novel process for preparing an enantiomeric excess of a first enantiomer of malathion or an analog thereof from a solution of racemic malathion or an analog thereof, the process comprising stereoselectively hydrolyzing the selected enantiomer using an effective amount of a hydrolyzing agent. In one embodiment, using biotransformation (e.g., enzymes) to stereospecifically hydrolyze one of the carbon esters, the formation of enantioenriched carboxylic monoacids can be accomplished along with the oppositely-configured malathion [unhydrolyzed] enantiomer.

Depending on the enzyme, cell culture or homogenate conditions used, the malathion *alpha*-monoacid or the *beta*-monoacid may be produced as the hydrolyzed enantiomer in the process. The monoacid product may be physically separated from the unhydrolyzed isomer using, for example, a separatory funnel or related separation method containing aqueous base and a non-miscible organic solvent. The malathion monoacid isomer (diagram indicates malathion *alpha*-monoacid) is soluble in the aqueous base and separated from the unhydrolyzed malathion isomer that is soluble in the organic phase. The process results in the organic solvent containing a single enantiomer of malathion and the aqueous layer containing the opposite enantiomer (at the alpha carbon) as the malathion monoacid. The organic solvent containing the unhydrolyzed malathion is separated from the aqueous layer and the solvent removed to afford a single malathion stereoisomer. To the remaining aqueous layer is added a non-miscible organic solvent and the aqueous layer made acidic (pH < 7.0) and the malathion monoacid single enantiomer is extracted into the organic phase. The organic solvent is removed to provide the malathion monoacid stereoisomer. Repeated extractions to separate malathion from malathion monoacid improves the extraction efficiency. The malathion

monoacid stereoisomer can be converted to the diethyl ester using a traditional esterification procedure thereby completing the synthesis of both enantiomers of malathion [by enzymatic resolution]. This invention also represents a novel process to produce malathion methyl and analogs of malathion methyl (new chemical entities) in high enantiomeric purity derived from racemic malathion using the process identified above.

In certain aspects of the present invention, new chemical entities derived from the malathion monoacid enantiomers are prepared by functionalization (chemical changes) to the carboxylic acid including esterification to other R groups, formation of amides, formation of thioesters, formation of acylhydrazides or other carboxylic acid derivatives via activation of the carboxylic acid. Malathion monoacid can be converted to the corresponding alcohol or other functional group for analog preparation at either the alpha or beta-monoacid position depending on the initial hydrolysis pathway chosen.



The process is shown as an example of the hydrolytic resolution of the alpha-monoacid from unreacted malathion to afford stereoisomeric products. The separation of malathion stereoisomer products using enzymes that hydrolyze the alpha ester or beta-ester or both are possible as is the formation of either malathion enantiomer as unreacted starting material. Moreover, using malathion methyl as the starting material makes possible the production of stereoisomer, malathion methyl analogs using the same processes.

In accordance with another aspect of the present invention, there is provided a process for preparing an enantiomeric excess of an enantiomer of malathion or an analog thereof from a solution of racemic malathion or an analog thereof, the process comprising stereoselectively hydrolyzing the first enantiomer using an effective amount of a hydrolyzing agent. The hydrolyzing agent is selected from the group consisting of an enzyme, a cell culture, a homogenate, and combinations thereof. In one embodiment, the hydrolyzing agent is effective

to solely hydrolyze the first enantiomer of malathion or an analog thereof. In a particular embodiment, the first enantiomer comprises an S-enantiomer of malathion or an analog thereof.

5 In certain embodiments, the hydrolyzing agent comprises an esterase. Examples of esterases that could be used include but are not limited to acetylcholinesterase (from electric eel, torpedo californica, human erythrocyte, recombinant) butyrylcholinesterases, lipases, pig liver esterase (or hog liver esterase or variants), or carboxylesterases.

In certain embodiments, the hydrolyzing agent hydrolyzes the first enantiomer at an alpha or beta carbon thereof.

10 In a particular embodiment, one or more hydrolyzed malathion monoacids of the selected enantiomer may be produced. After hydrolyzation, a non-miscible organic solvent is added to the racemic malathion solution, wherein the adding results in an aqueous layer comprising the one or more hydrolyzed malathion monoacids and an organic layer comprising an unhydrolyzed enantiomer of malathion. Examples of organic solvents may be but are not limited to ethyl acetate, diethyl ether, methylene chloiride, chloroform or toluene. The organic layer comprising
15 the unhydrolyzed enantiomer is separated from the aqueous layer comprising the one or more malathion monoacids by traditional separation methods such as a separatory funnel. One or more hydrolyzed malathion monoacids are subjected to esterfication to produce the desired enantiomer in enantiomeric excess.

20 In another embodiment, there is provided a process for preparing a first enantiomer of malathion from a solution of racemic malathion. The method comprises hydrolyzing the first enantiomer of malathion in a solution of racemic malathion using a hydrolyzing agent, wherein one or more malathion monoacids of the first enantiomer are formed. Thereafter, the method comprises adding a non-miscible organic solvent to the solution to produce an aqueous layer comprising the hydrolyzed enantiomer and an organic layer comprising an unhydrolyzed
25 enantiomer, subjecting the solution to separation, wherein the organic layer comprising the unhydrolyzed enantiomer is separated from the aqueous layer comprising the hydrolyzed enantiomer, and subjecting the hydrolyzed enantiomer of the aqueous layer to esterfication to produce the first enantiomer.

30 In another embodiment of the present invention, there is provided a process for preparing an enantiomeric excess of a first enantiomer of malathion methyl or an analog thereof from a

solution of racemic malathion methyl or an analog thereof comprises hydrolyzing the selected enantiomer of malathion methyl or an analog thereof using a hydrolyzing agent .

In yet another embodiment, there is provided a composition comprising an enantiomeric excess of a single enantiomer of malathion or analog thereof and one or more pharmaceutically acceptable carriers. In certain embodiments, the composition is effective for use in treating head lice.

The compounds described herein may be formulated as a pharmaceutical composition suitable for administration to a subject. Such compositions typically comprise an enantiomeric excess of malathion or an analog thereof and a pharmaceutically acceptable carrier. Exemplary pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa., which is incorporated herein by reference). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

In another embodiment, the composition may be formulated for agricultural use. In this embodiment, the composition typically comprises an enantiomeric excess, e.g., a greater amount of one enantiomer vs. the other, of an enantiomer of malathion or an analog thereof along with an agriculturally acceptable carrier. By "agriculturally acceptable carrier," it is meant an agent that does not have a substantial detrimental effect on the activity of the active ingredients described herein.

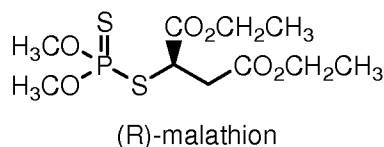
Suitable solid agriculturally acceptable carriers include, but are not limited to, soybean flour, grain flour, wood flour, bark flour, sawing flour, tobacco stalk flour, walnut shell flour, bran, cellulose powder, a residue after plant extraction, a synthetic polymer such as a synthetic resin

powder, clay (e.g., kaoline, bentonite, or acid white clay), talc (e.g., talc or pyrophyllite), silica (e.g., diatomite, silica powder, mica, activated carbon, sulfur powder, pumice, calcined diatomite, brick powder, fly ash, sand, inorganic mineral powders such as calcium carbonate and calcium phosphate, chemical fertilizers such as ammonium sulfate, ammonium phosphate, ammonium nitrate, urea, and ammonium chloride, and compost.

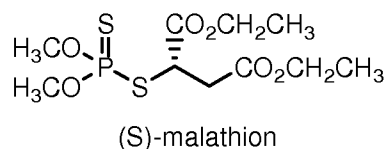
Suitable liquid carriers may be one having a solvent ability or a material having no solvent ability for the components, including dodecanoic acid, but having an ability to assist in the dispersion of the active ingredient compound, e.g., dodecanoic acid. Exemplary liquid carriers include but are not limited to, alcohols (e.g., methanol, ethanol, isopropanol, butanol, and ethylene glycol); ketones (e.g., acetone, methylethyl ketone, methyl isobutyl ketone, diisobutyl ketone, and cyclohexanone); ethers (e.g., diethyl ether, dioxane, cellosolve, diisopropyl ether, and tetrahydrofuran); aliphatic hydrocarbons (e.g., kerosine and mineral oil); aromatic hydrocarbons (e.g. benzene, toluene, xylene, solvent naphtha, and alkylnaphthalene); halogenated hydrocarbons (e.g., dichloromethane, chloroform, carbon tetrachloride, and chlorobenzene); esters (e.g., ethyl acetate, butyl acetate, ethyl propionate, diisobutyl phthalate, dibutyl phthalate, and dioctyl phthalate); amides (e.g., dimethylformamide, diethylformamide, and dimethylacetamide); and nitriles (e.g., acetonitrile). In one particular embodiment, the agriculturally acceptable carrier comprises mineral oil or a vegetable oil such as canola oil, sunflower oil, cottonseed oil, palm oil, soybean oil, and the like.

In another embodiment, there is provided a method of treating pediculosis comprising administering to a subject in need thereof an effective amount of a composition as described herein.

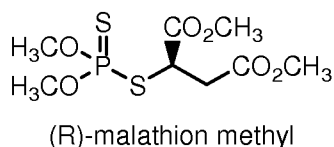
In another embodiment, the preparation of (R)-malathion, useful as an insecticide in pharmaceutical, agricultural and domestic applications.



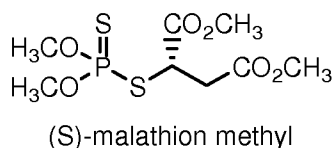
In another embodiment, there is provided the preparation of (S)-malathion, useful as an insecticide in pharmaceutical, agricultural and domestic applications:



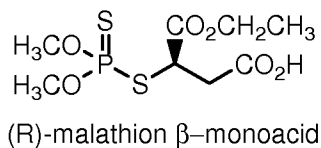
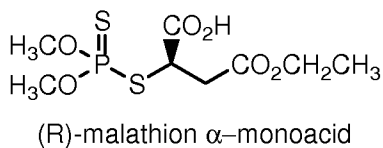
In yet another embodiment, there is provided the preparation of (R)-malathion methyl, useful as an insecticide in pharmaceutical, agricultural and domestic applications:



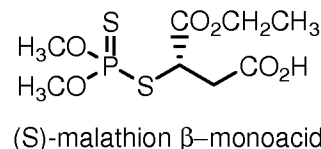
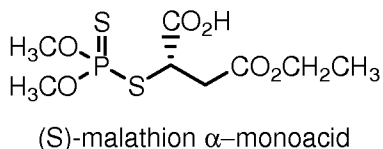
5 In yet another embodiment, there is provided the preparation of (S)-malathion methyl, useful as an insecticide in pharmaceutical, agricultural and domestic applications:



10 In yet another embodiment, there is provided the preparation of (R)-malathion α -monoacid and (R)-malathion β -monoacid.

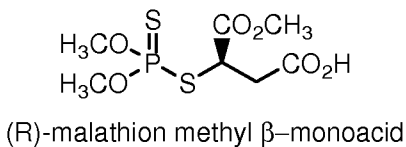
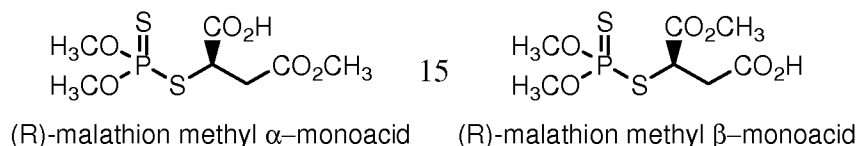


In yet another embodiment, there is provided the preparation of (S)-malathion α -monoacid and (S)-malathion β -monoacid.

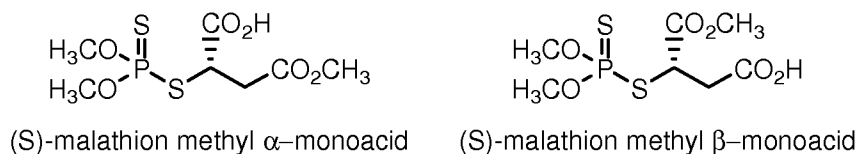


15

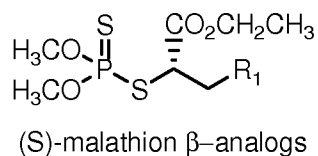
In yet another embodiment, there is provided the preparation of (R)-malathion methyl α -monoacid and (R)-malathion methyl β -monoacid.



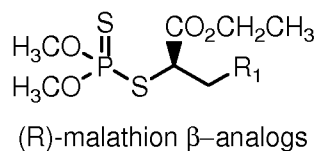
In yet another embodiment, there is provided the preparation of (S)-malathion methyl α -monoacid and (S)-malathion methyl β -monoacid.



5 In yet another embodiment, there is provided a class of β -analogs derived from (S)-malathion β -monoacid for insecticidal and pharmacologic use wherein:

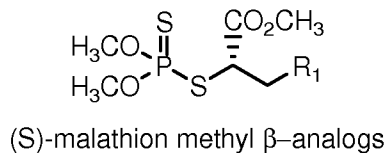


In yet another embodiment, there is provided a class of β -analogs derived from (R)-malathion β -monoacid for insecticidal and pharmacologic use wherein:

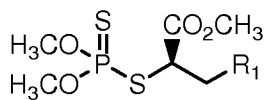


10

In yet another embodiment, there is provided a class of β -analogs derived from (S)-malathion methyl β -monoacid for insecticidal and pharmacologic use wherein:

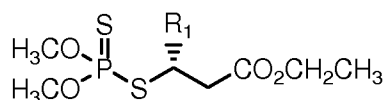


In yet another embodiment, there is provided a class of β -analogs derived (R)-malathion methyl β -monoacid for insecticidal and pharmacologic use wherein:



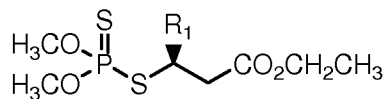
(R)-malathion methyl β -analogs

5 In yet another embodiment, there is provided a class of α -analogs derived from (S)-malathion α -monoacid for insecticidal and pharmacologic use wherein:



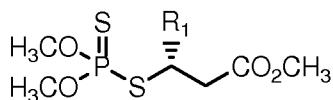
(S)-malathion α -analogs

In yet another embodiment, there is provided a class of α -analogs derived from (R)-malathion α -monoacid for insecticidal and pharmacologic use wherein:



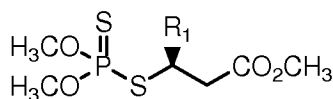
(R)-malathion α -analogs

10 In yet another embodiment, there is provided a class of α -analogs derived from (S)-malathion methyl α -monoacid for insecticidal and pharmacologic use wherein:



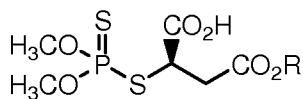
(S)-malathion methyl α -analogs

15 In yet another embodiment, there is provided a class of α -analogs derived from (R)-malathion methyl α -monoacid for insecticidal and pharmacologic use wherein:

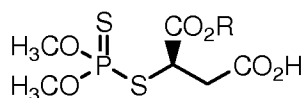


(R)-malathion methyl α -analogs

In another embodiment, there is provided the use of esterases/hydrolases (from enzymes, cell culture of homogenates) to convert racemic malathion or racemic malathion methyl into (R)-malathion or (R)-malathion methyl α - and β -monoacids.



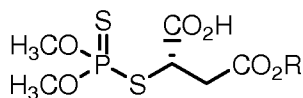
(R)-malathion α -monoacid; R = CH₂CH₃
 (R)-malathion methyl α -monoacid; R = CH₃



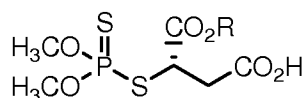
(R)-malathion β -monoacid; R = CH₂CH₃
 (R)-malathion methyl β -monoacid; R = CH₃

5

In yet another another embodiment, there is provided the use of esterases/hydrolases (from enzymes, cell culture of homogenates) to convert racemic malathion or malathion methyl into (S)-malathion or (S)-malathion methyl α - and β -monoacids.



(S)-malathion α -monoacid; R = CH₂CH₃
 (S)-malathion methyl α -monoacid; R = CH₃



(S)-malathion β -monoacid; R = CH₂CH₃
 (S)-malathion methyl β -monoacid; R = CH₃

10

In yet another embodiment, there is provided the use of extraction (liquid-liquid, etc.) following enzyme, cell culture or homogenate hydrolysis to separate a single malathion stereoisomer from a single stereoisomer/regioisomer of malathion monoacid.

15

In yet another embodiment, there is provided the use of extraction (liquid-liquid, etc.) following enzyme, cell culture or homogenate hydrolysis to separate a single malathion methyl stereoisomer from a single stereoisomer/regioisomer of malathion methyl monoacid.

20

In yet another embodiment, there is provided the use of chromatographic methods following enzyme, cell culture or homogenate hydrolysis to separate a single malathion stereoisomer from a single stereoisomer/regioisomer of malathion monoacid.

25

In yet another embodiment, there is provided the use of chromatographic methods following enzyme, cell culture or homogenate hydrolysis to separate a single malathion methyl stereoisomer from a single stereoisomer/regioisomer of malathion methyl monoacid.

Malathion is a useful insecticide with a number of important pharmacologic and agricultural applications, however, it is used as a 50:50 racemic mixture of isomers. Use of a single enantiomer will likely afford a greater margin of safety and reduce risks associated with contamination by the more toxic enantiomer. Methods for producing (R)-malathion or (S)-malathion from racemic malathion are presented using enzymes, cell culture or homogenates that convert racemic malathion into an extractable, water-soluble malathion monoacid enantiomer that is physically separated from an unreacted, malathion enantiomer. Following isolation, the monoacid enantiomer is esterified to form the opposite malathion enantiomer. The carboxymethyl ester analog, malathion methyl, may be converted to the corresponding enantiomeric products using like procedures.

Methods for producing stereoisomeric analogs from the water-soluble, monoacid enantiomer intermediates are also presented. The enantiomeric, malathion-like or malathion methyl-like analogs are new chemical entities.

Aspects of the present invention use enzyme, cell culture or homogenate hydrolysis to stereoselectively hydrolyze one of the carbon esters in malathion or malathion methyl, resulting in the formation of an enantioenriched malathion and the oppositely-configured monoacid enantiomer. The monoacid product may be physically separated from the unhydrolyzed malathion or malathion methyl isomer by extraction using water (> pH 7.0) and a non-miscible organic solvent. The enantioenriched monoacid stereoisomer can be converted to a single enantiomer of malathion (the diethyl ester) or malathion methyl (dimethyl ester) using an esterification procedure thereby producing both enantiomers of malathion or malathion methyl. A panel of malathion-like or malathion methyl-like, single stereoisomer analogs may thus be prepared from the malathion monoacid stereoisomers.

EXAMPLES

A racemic (*R/S* or *d/l*) mixture of malathion was converted to the enantioenriched form of malathion and the opposite enantiomer of malathion monoacid using an esterase enzyme and physically separating the two stereoisomers by liquid-liquid extraction; a process that
5 incorporates all the essential concepts and functionality described in the written description of this provisional patent application. The conversion was effected in the following manner:

To a solution of malathion (100 mg, 0.30 mmol, 1 equiv) in a solution mixture of phosphate buffer (0.1M, pH = 7.2, 11 mL) and acetone (1 mL), was added the esterase in 3.2M (NH₄)₂SO₄ (pH = 8; 7 μL) at room temperature. The reaction mixture was monitored by TLC until
10 completion. The crude mixture was brought to pH 8-9 by the addition of 0.1M NaOH and the mixture was extracted with ethyl acetate (3 x 100 mL). The aqueous layer was acidified to pH 2-3 by the addition of 1.2 M HCl and extracted with ethyl acetate (3 x 100 mL). The combined organic fractions were dried over Na₂SO₄, filtered and concentrated under reduced pressure. Purification by column chromatography over silica gel (chloroform-methanol, 100:0 to 90:10, v/v)
15 afforded the enantioenriched (*S*)-malathion (43 mg) and the (*R*)-malathion monoacids as colorless oils (46 mg).

(S)-malathion. ¹H NMR (CDCl₃, 500 MHz) δ 4.12-4.24 (m, 5H), 3.80 (dd, *J* = 4 Hz, *J* = 15 Hz, 6H), 3.02 (dd, *J* = 9 Hz, *J* = 17 Hz, 1H), 2.86 (dd, *J* = 5 Hz, *J* = 17 Hz, 1H), 1.27 (t, *J* = 7 Hz, 3H), 1.23 (t, *J* = 7 Hz, 3H); ¹³C NMR (CDCl₃, 125.67 MHz) δ 169.9, 169.8, 62.3, 61.4, 54.5 (d, *J*_{POC} = 5Hz), 44.7 (d, *J*_{POC} = 5Hz), 37.6, 37.4, 14.1, 13.9; ³¹P NMR (CDCl₃, 202.32 MHz) δ 95.6 (s). HRMS (ES⁺) calcd. for C₁₀H₁₉O₆PS₂, [M + H]⁺ 331.3580, found 331.1580.
20

α- and β-monoacids. ¹H NMR (CDCl₃, 500 MHz) δ 4.23 (q, *J* = 7 Hz, 2H), 4.15-3.83 (m, 1H), 3.82 (dd, *J* = 3 Hz, *J* = 15Hz, 6H), 3.14 (dd, *J* = 8 Hz, *J* = 17 Hz, 1H), 2.96 (dd, *J* = 5 Hz, *J* = 17 Hz, 1H), 1.24 (t, *J* = 7 Hz, 3H), 1.22 (t, *J* = 7 Hz, 3H); ³¹P NMR (CDCl₃, 202.32 MHz) δ 92.2. HRMS (ES⁺) calcd. for C₈H₁₅O₆PS₂, ([M]⁺) 302.3049, found 302.8977.
25

Other modes of the invention include using a hydrolyzing agent such as but not limited to acetylcholinesterase (from electric eel, torpedo californica, human erythrocyte, recombinant)
30 butyrylcholinesterases, lipases, pig liver esterase (or hog liver esterase or variants), or carboxylesterases. The hydrolyzing agent hydrolyzes the selected enantiomer at an alpha or

beta carbon thereof. One or more hydrolyzed malathion monoacids of the selected enantiomer may be produced. After hydrolyzation, a non-miscible organic solvent is added to the racemic malathion solution, wherein the adding results in an aqueous layer comprising the one or more hydrolyzed malathion monoacids and an organic layer comprising an unhydrolyzed enantiomer of malathion. Examples of organic solvents may be but are not limited to ethyl acetate, diethyl ether, methylene chloiride, chloroform or toluene. The organic layer comprising the unhydrolyzed enantiomer is separated from the aqueous layer comprising the one or more hydrolyzed malathion monoacids by traditional separation methods such as a separatory funnel. One or more hydrolyzed malathion monoacids are subjected to esterfication to produce the selected enantiomer.

While various embodiments of the present invention have been shown and described herein, it will be obvious that such embodiments are provided by way of example only. Numerous variations, changes and substitutions may be made without departing from the invention herein. Accordingly, it is intended that the invention be limited only by the spirit and scope of the appended claims.

The present invention can be applied in the industries of agricultural and pharmaceutical use. In one embodiment, the compounds or compositions described herein are useful in the treatment of head lice, ringworm, and the like.

REFERENCES

- Bommarius, A.S. and Riebel-Bommarius, B.R (2004) *Biocatalysis: Fundamentals and Applications*. Wiley-VCH; 1st edition; 634 pages.
- Bornscheuer, U.T. and Kazlauskas, R.J.(2006) *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*. Wiley-VCH; 2nd edition; 368 pages.
- Faber, K. (2004) *Biotransformations in Organic Chemistry: A Textbook*. Springer; 5th Edition; 454 pages.

CLAIMS

1. A process for obtaining an enantiomeric excess of a first enantiomer of malathion or an analog thereof from a solution of racemic malathion or an analog thereof, the process comprising stereoselectively hydrolyzing the first enantiomer using an effective amount of a hydrolyzing agent.
2. The process of claim 1, wherein the hydrolyzing agent is selected from the group consisting of an enzyme, a cell culture, a homogenate, and combinations thereof.
3. The process of claim 2, wherein the hydrolyzing agent is effective to solely hydrolyze the first enantiomer of malathion or an analog thereof.
4. The process of claim 2, wherein the hydrolyzing agent comprises an esterase.
5. The process of claim 2, wherein the hydrolyzing agent hydrolyzes the first enantiomer at an alpha or beta carbon thereof.
6. The process of claim 1, wherein the first enantiomer comprises an S-enantiomer of malathion or an analog thereof.
7. The process of claim 1, further comprising forming one or more malathion monoacids of the first enantiomer.
8. The process of claim 7, further comprising adding a non-miscible organic solvent to the solution, wherein the adding results in an aqueous layer comprising the one or more malathion monoacids and an organic layer comprising an unhydrolyzed enantiomer of malathion.
9. The process of claim 8, further comprising separating the organic layer comprising the unhydrolyzed enantiomer from the aqueous layer comprising the one or more malathion monoacids.
10. The process of claim 8, wherein the one or more hydrolyzed malathion monoacids are subjected to esterification to produce the first enantiomer.

11. A process for obtaining an enantiomeric excess of a first enantiomer of malathion or an analog thereof from a solution of racemic malathion or an analog thereof comprising:
 - (a) stereoselectively hydrolyzing the first enantiomer in the solution using a hydrolyzing agent, wherein one or more monoacids of the first enantiomer are formed;
 - (b) after step (a), adding a non-miscible organic solvent to the solution to produce an aqueous layer comprising the hydrolyzed enantiomer and an organic layer comprising an unhydrolyzed enantiomer;
 - (c) separating the organic layer comprising the unhydrolyzed enantiomer from the aqueous layer comprising the hydrolyzed enantiomer;
 - (d) subjecting the hydrolyzed enantiomer of the aqueous layer to esterification to produce the first enantiomer.
12. The process of claim 11, wherein the first enantiomer comprises an S-enantiomer of malathion or an analog thereof.
13. A process for obtaining an enantiomeric excess of a first enantiomer of malathion methyl or an analog thereof from a solution of racemic malathion methyl or an analog thereof comprising stereoselectively hydrolyzing the first enantiomer of malathion methyl or an analog thereof using a hydrolyzing agent.
14. A pharmaceutical composition for topical administration comprising an enantiomeric excess of a single enantiomer of malathion or an analog thereof and a pharmaceutically acceptable carrier.
15. The pharmaceutical composition of claim 14, wherein the single enantiomer comprises an S-enantiomer of malathion or an analog thereof.
16. A composition comprising an enantiomeric excess of a single enantiomer of malathion methyl or analog thereof and formulation pharmaceutically acceptable carrier.
17. The composition of claim 14 or 16 wherein said composition is effective for use as a pediculicide or as an insecticide.

18. A method of treating pediculosis comprising administering to a subject in need thereof an effective amount of the composition of claim 14.
19. An insecticidal composition comprising an enantiomeric excess of a single enantiomer of malathion or an analog thereof and an agriculturally acceptable carrier.
20. A method for treating or preventing infestation of a plant by an insect comprising administering to the plant the composition of claim 19.

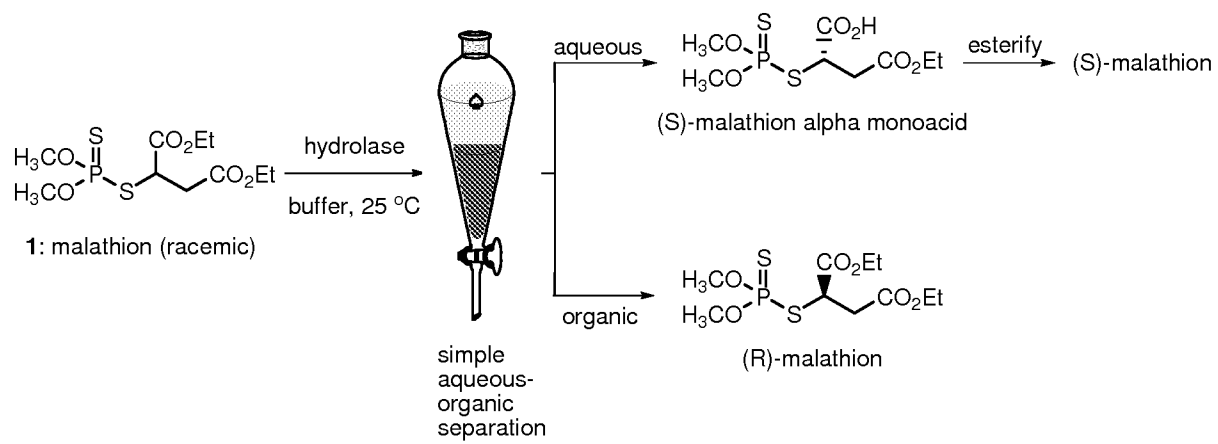


FIGURE 1