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(21) International Application Number: PCT/GB94/02147 (22) International Filing Date: 4 October 1994 (04.10.94) (30) Priority Data: 9320563.1 6 October 1993 (06.10.93) GB (71) Applicant (for all designated States except US): ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): HAWKES, Timothy, Robert [GB/GB]; 40 Anneford Place, Priestwood, Bracknell, Berkshire RG12 2ES (GB). (74) Agents: MALLALIEU, Catherine, Louise et al.; Intellectual Property Dept., Zeneca Agrochemicals, Jealott's Hill Research Station, P.O. Box 3538, Bracknell, Berkshire RG12 6YA (GB).		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: ASSAY PROCEDURE AND APPLICATION IN IDENTIFICATION OF HERBICIDES (57) Abstract An assay for detecting inhibitors of aminoacyl-tRNA synthetases, which when reacted with divalent metal cations, a corresponding species of tRNA and an appropriate non-cognate amino acids catalyse the hydrolysis of ATP to pyrophosphate; the assay comprising incubating a divalent metal cation, ATP, the said tRNA, the said non-cognate amino acid, inorganic pyrophosphatase and the said aminoacyl-tRNA synthetase, in at least a partially pure form, both with and without a potential inhibitor and providing detecting means for phosphate and comparing the results obtained.		

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ASSAY PROCEDURE AND APPLICATION IN IDENTIFICATION OF HERBICIDES

The present invention relates to a new assay procedure for detecting compounds which inhibit the activity of certain aminoacyl-tRNA synthetases, to the use of these procedures for identifying compounds which have such activity for use as antibiotics or herbicides, to herbicides derived thereby, and to a novel cDNA sequence encoding *E. coli* isoleucyl-tRNA synthetase.

Aminoacyl-tRNA synthetases are enzymes found in all bacteria, plants and animals and are required to make protein. Inhibitors of the bacterial enzymes are potentially useful as antibiotics and the applicants have discovered that they may also have application as herbicides.

It is vital that the genetic code is accurately translated into protein. To ensure that this happens each aminoacyl-tRNA synthetase must attach the right (cognate) amino acid to the right (cognate) species of tRNA. To ensure that this happens, certain of these enzymes have evolved "editing" mechanisms to hydrolyse (at different stages) inappropriate intermediates complexes and "mischarged" tRNA species. Particular examples are valine, a non-cognate amino acid with respect to isoleucyl-tRNA synthetase (hereinafter referred to as ITRS) (1), threonine (2), a non-cognate amino acid with respect to valyl-tRNA synthetase and homocysteine, a non-cognate amino acid with respect to methionyl-tRNA synthetase.

The applicants have found a means of using these editing mechanisms to develop an assay technique for discovering inhibitors of enzyme activity and consequently of biologically active compounds having industrial applicability.

According to one aspect of the present invention there is provided an assay for detecting inhibitors of an aminoacyl-tRNA synthetase, which when reacted with a divalent metal cation, a corresponding species of tRNA and an appropriate non-cognate amino acid, will result in the hydrolysis of ATP to pyrophosphate; the assay comprising incubating the said divalent metal cation, ATP, the said tRNA, the said non-cognate amino acid, inorganic pyrophosphatase and the said aminoacyl-tRNA synthetase, in at least a partially pure form, both with and without a potential inhibitor and providing detecting means for phosphate and comparing the results obtained.

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According to another aspect of the present invention there is provided an assay for detecting isoleucyl-tRNA synthetase of E. coli comprising (a) incubating magnesium ions, adenosine triphosphate (ATP), a corresponding species of tRNA, isoleucyl-tRNA synthetase and inorganic pyrophosphatase with valine; (b) simultaneously incubating a similar mixture further containing a potential inhibitor of the enzyme; (c) detecting phosphate production from the incubates; and (d) comparing the results.

As used herein, the expression "partially pure" used in relation to enzyme means that the enzyme preparation is substantially free of interfering activities, in particular is substantially free of phosphatases and, for example, in the particular case of the assay for ITRS exemplified, free of valyl-tRNA synthetase.

The tRNA employed in the assay technique may be pure tRNA appropriate for the particular enzyme or a mixture of tRNAs, such as the mixture of tRNA species from E. coli strain W commercially obtainable from Sigma (UK) Ltd, provided that the mixture contains sufficient of the tRNA appropriate for the particular enzyme.

The assay of the invention is applicable for the screening of chemicals for biological activity in a commercial environment. Consequently the term "readily hydrolysable" means that the assay reaction can proceed at a useful rate.

Particular examples of enzymes and amino acids which can be employed in this screen because of the editing mechanism by which the misacylated products are removed are ITRS, valyl-tRNA synthetase and methionyl-tRNA synthetase with valine, threonine and homocysteine respectively.

The divalent metal cation is preferably magnesium or manganese. Magnesium is especially preferred.

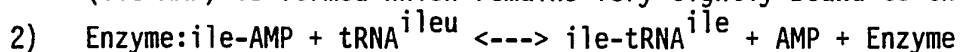
In a preferred embodiment the enzyme employed in the assay is ITRS from a bacterial source, preferably E. coli, and the amino acid is valine.

The assay is based upon the principles exemplified below. The two partial reactions of ITRS (enzyme) involved in the biosynthesis of the aminoacyl-tRNA can be represented as follows:

1) Enzyme (ITRS) + ATP + ile <---> Enzyme:ile-AMP + PPi

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ITRS, in the presence of Mg^{2+} ions catalyses a partial reaction in which pyrophosphate (PPi) is released and an aminoacyl adenylate (ile-AMP) is formed which remains very tightly bound to the enzyme.

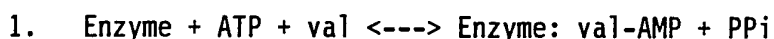


The enzyme-bound aminoacyl adenylate reacts with the cognate tRNA to transfer the ileu to the tRNA and to release adenosine monophosphate (AMP).

As can be seen, pyrophosphate is produced in step 1. Adding inorganic pyrophosphatase converts this to phosphate (which can be measured, for example, colorimetrically by a suitable phosphate determination method such as that involving malachite green described by Lanzetta et al (3) and slightly modified by Howard and Ridley (4)). Consequently it should be possible to detect ITRS activity by detecting phosphate.

However, the above two reactions are tightly coupled. The stoichiometry requires one equivalent of tRNA to react for each pyrophosphate released. Large amounts of tRNA would be needed to generate enough pyrophosphate and subsequently phosphate to detect. This would be too costly to employ on a routine basis and for use in a high throughput screen.

When valine is substituted for isoleucine it also initially reacts to form the aminoacyl AMP bound to the enzyme. The reaction scheme can be represented as follows:



In this case rather than forming the aminoacyl-tRNA, it is rapidly hydrolysed by the ITRS enzyme in the presence of tRNA^{ile} (5). The assay of the invention makes use of this in that valine allows the tRNA to be recycled so that it does not limit the extent of the reaction. Thus, the enzyme catalyses the hydrolysis of ATP to PPi (and, via pyrophosphatase, ultimately, Pi).

Since the enzyme catalyses the hydrolysis of the non-cognate aminoacyl adenylate bound to the enzyme and there is no transfer to the tRNA, only catalytic amounts of tRNA are required. In addition the starting enzyme and valine are also regenerated while generating

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pyrophosphate.

Experiments with the assay conditions have shown that once the reaction with the ITRS enzyme was started, a linear rate could be maintained for at least 40 minutes. A colour change of >0.3 OD units is preferred.

The amount of tRNA which is employed in the reaction is generally low since the tRNA is recycled in the reaction. For example, doses of from about 0.05 mg to about 0.3 mg/200 μ l reaction mixture may be employed. As discussed above, this may be pure or mixed tRNA species from E. coli.

In one preferred embodiment, mixed tRNA species from E. coli from Sigma (UK) Ltd is added in an amount of about 0.1mg/200 μ l reaction mixture.

The amount of ATP present can be from about 0.05 to about 10 mM.

The K_m for valine is about 0.5mM, so at least 0.5mM of valine, suitably from about 0.5mM to about 25 mM of valine, preferably about 5mM valine is used to obtain near maximum rates. In contrast, the K_m for Ile using an ^3H -Ile assay was found to be about 4.3 μ M.

Purified or partially purified enzyme may be prepared by conventional techniques (6) including use of recombinant DNA technology. Using ITRS obtained from E. coli, and partially purified as described below, amounts of from about 0.10 μ g to about 5 μ g are suitably employed. Based on this amount, suitable assay times have been found to be up to about 90 minutes or longer.

We have also now sequenced the gene encoding for E. coli ITRS. Thus, according to another aspect of the present invention there is provided a cDNA sequence as shown in Seq ID No 1, including non-critical allelic variations of that sequence.

According to yet another aspect of the present invention there is provided an amino acid sequence as shown in Seq ID No 2, including variants thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

The present invention includes sequences having at least 70% nucleic acid homology with the sequence shown in Seq ID No 1, and which encode for functionally equivalent proteins. In a preferred embodiment, the nucleic acid sequence has at least 75%, 80%, 85%, 90%, 95%, 97% or 99% homology with the sequence shown in Seq ID No 1.

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The present invention includes functionally equivalent sequences to that shown in Seq ID No 2, having at least 70% homology with said sequence. In a preferred embodiment, the amino acid sequence has at least 75%, 80%, 85%, 90%, 95%, 97% or 99% homology with the sequence shown in ID Seq No 2.

It will be appreciated that the expressed ITRS can be used in the assay of the present invention.

By using the assay technique described above, it is possible to carry out high throughput screens for detecting inhibitors of the enzymes. In a further aspect of the invention there is provided an enzyme inhibitor having biological application detected by an assay method as described above.

In particular the applicants have found that inhibitors of ITRS may have application as herbicides. Such compounds are described in our co-pending International Patent Publication No. W093/19599.

In yet a further aspect of the invention there is provided a herbicidal compound which acts by inhibiting the plant isoleucyl-tRNA synthetase enzyme excluding those compounds of International Patent Publication No. W093/19599 of general formula (I) or (IA) or (IB) where Y represents a group of sub-formula (IC) or (ID or (IE) and wherein R^2 is a group $CO-XR^3$ wherein X is O or S and R^3 is hydrogen or an agrochemically acceptable ester-forming radical; or R^2 is a group $-R^4$ wherein R^4 is an optionally substituted aryl or heterocyclic group; or R^2 is a group $CO-NR^5R^6$ wherein R^5 and R^6 are the same or different and each represent an agrochemically acceptable amide-forming radical; stereoisomers of the compounds of formula (I), (IA) and (IB) and salts of the compound of formula (I), (IA) and (IB) wherein R^2 is $COXR^3$, X is O and R^3 is hydrogen.

The following examples illustrate the invention.

1. Partial Purification of E. coli ITRS

a) Cell breakage:

100g of E. coli cell paste was mixed with 200ml of buffer A (100mM Tris (tris(hydroxymethyl)aminomethane) pH7.4, 30mM KCl, 0.5mM $MgCl_2$, 0.1mM EDTA (ethylenedinitrilotetraacetate), 4mM 2-mercaptoethanol, 6mM DTT (dithiothreitol) and 1mM benzamidine). The cells were broken in a French press at 8,000 psi (5.5×10^4 kPa). The extract was spun at 23,500 x g (12,000rpm) in a 6 x 250ml Sorvall GSA rotor for 20 minutes at 4°C.

b) Precipitation:

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The supernatant was removed and 2.5% protamine sulphate in buffer A was added in a dropwise manner to a final concentration of 0.1%. The extract was centrifuged at 23,500 x g (12,000 rpm) for 20 minutes. The supernatant was removed, and 50% ammonium sulphate added slowly and left to mix on ice for 30 minutes. The extract was spun again at 12,000 rpm for 15 minutes to form a pellet.

c) Gel filtration on a Sephadex G-50 column:

The pellet was resuspended in a small amount of buffer B (25mM Tris pH 7.4, 5mM MgCl₂, 1mM EDTA, 4mM 2-mercaptoethanol). A further spin in a 8 x 50ml Sorvall SS-34 rotor at 39,000 x g (18,000 rpm) for 15 minutes was carried out prior to the extract being added to a Sephadex G-50 column (5cm i.d.) with a bed volume of 250ml. The protein eluted with buffer B was collected and stored at -80°C.

d) Ion-exchange on a Q-Sepharose Column:

A pre-equilibrated Pharmacia Q-Sepharose column (11.5cm x 5cm) in buffer B was prepared. The extract from the Sephadex G-50 column was added, and a 0 to 1M NaCl gradient applied. All the fractions from this column were kept and analysed by the radiolabelled method described below. The active fractions (47 - 57) were pooled, and had 90% ammonium sulphate added and were then spun at 12,000 x g (17,400 rpm) in the 8 x 50ml Sorvall SS-34 rotor for 15 minutes. The supernatant was removed and the resulting pellet was dissolved in buffer B.

e) Gel Filtration on a Superdex-200 Column:

Five ml of the extract from step d) was added to a pre-packed Pharmacia Superdex-200 HiLoad column (2cm i.d.) with a bed volume of 120ml. It was pre-equilibrated in buffer B and 50mM NaCl. All of the fractions were kept separate and tested using the radiolabelled assay. Fractions 25 - 40 were pooled and an equal volume of glycerol was added. When this extract was used for the initial development work on the colorimetric assay it was found to contain contaminating phosphatase activity.

f) Removal of Contaminating Phosphatase Activity:

Using a high resolution Q-Sepharose column with a gradient of 0 - 1M NaCl on an FPLC system, fractions containing ITRS activity free of contaminating phosphatase were eluted at 0.3M NaCl.

2. Measurement of ITRS Activity using a ³H-Isoleucine-Based Radiolabelled Assay

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The activity of ITRS during fractionation was monitored using conditions based on the method described by Steinmetz and Weil (7), and as described below.

Stock reagents used in the method are as follows:

Buffer solution:

500mM Tris-HCl pH7.4, 150mM MgCl₂, 6H₂O, 300mM KCl, 25mM glutathione, and 1% bovine serum albumin (BSA).

ATP:

10mM in 50mM-Tris-HCl pH 7.4.

tRNA (mixed):

Approximately 0.54nmol tRNA^{ile}/mg from the *E. coli* strain W (supplied by Sigma (UK) Ltd). A stock was made of 50mg mixed tRNA/ml 50mM Tris-HCl pH 7.4.

Isoleucine:

L-[4,5-³H]-Isoleucine (obtained from Amersham) at approximately 100Ci/mmol in 2% aqueous ethanol. A 100μM stock was prepared of 50μl ³H-isoleucine, 100μl cold isoleucine, and 850μl 50mM Tris-HCl pH7.4.

Enzyme - ITRS:

Prepared as described above. The specific activity of the enzyme used in the present example was approximately 145nM of product formed/minute/mg, and was about 5% - 10% active. The enzyme was diluted as appropriate. It will be appreciated that the enzyme amounts in the assay can be adjusted according to the purity of the enzyme used.

The assay mixture for a final volume of 200μl was composed of:

- 20μl buffer solution
- 20μl ATP
- 20μl tRNA
- 20μl ³H-Isoleucine solution
- 100μl 50mM Tris-HCl pH 7.4

The standard assay (in 200μl) was carried out in quadruplicate in Eppendorf microcentrifuge tubes. The ingredients were preincubated for 2-3 minutes at 37°C, and the reaction started by addition of approximately 20μl of the appropriately diluted enzyme extract as described above.

The final reaction contained reagents in the following concentrations:

- 50mM Tris-HCl, pH 7.4
- 15mM MgCl₂, 6H₂O

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30mM KCl
2.5mM Glutathione
0.1% bovine serum albumin
1mM ATP
5mg ml⁻¹ tRNA
10μM Isoleucine containing ³H-Ile

The assay was incubated at 37°C for 20 minutes, and stopped by addition of 50μl 20% TCA, and the tubes placed on ice.

200μl of the reaction mixture was pipetted onto 1.5cm² cellulose 3MM filters. The filters were washed (in groups of 4) as follows: 1 x 10% TCA, 2 x 5% TCA, and 2 x ethanol using fresh wash medium for each set of replicates to avoid cross contamination. The dried filters were placed in 20ml scintillation vials and the radioactivity counted in 15ml Optiphase.

2. Measurement of ITRS activity using a Colorimetric Assay based on using Valine as Substrate

Incubations were set up in quadruplicate (as above) with a final volume of 200μl as described below. Stock reagents used in the method were as follows:

Buffer solution:

500mM Tris-HCl pH7.4, 150mM MgCl₂, 6H₂O, 300mM KCl, 25mM glutathione, and 1% bovine serum albumin (BSA).

ATP:

7.5mM in 50mM Tris-HCl pH 7.4.

tRNA (mixed):

Approximately 0.54nmoles tRNA^{ile}/mg from the E. coli strain W (supplied by Sigma (UK) Ltd). A stock was made of 1.2mg mixed tRNA/ml 50mM Tris-HCl pH 7.4.

L-Valine

A 30mM stock in 50mM Tris-HCl pH 7.4.

Inorganic Pyrophosphatase

This was in the form of the HPLC purified grade from Bakers yeast supplied by Sigma (UK) Ltd as a lyophilized powder. This was made up in 50mM Tris-HCl pH 7.4 to a concentration of 10 units/ml.

Enzyme - ITRS:

Prepared as described above. The specific activity of the enzyme used

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in the present example was approximately 145nM of product formed/minute/mg, and was about 5% - 10% active. The enzyme was diluted as appropriate. It will be appreciated that the enzyme amounts in the assay can be adjusted according to the purity of the enzyme used.

The assay mixture for a final volume of 200 μ l was composed of:

20 μ l buffer solution

20 μ l ATP

20 μ l tRNA

20 μ l inorganic pyrophosphatase

100 μ l L valine

The standard assay (in 200 μ l) was carried out in quadruplicate in Eppendorf microcentrifuge tubes. The ingredients were pre-incubated for 2-3 minutes at 37°C and the reaction started by addition of 20 μ l of appropriately diluted enzyme extract (as described above).

Thus the final reaction contained reagents in the following concentrations:

50mM Tris-HCl pH 7.4

10mM MgCl₂

30mM KCl

2.5mM Glutathione

0.75mM ATP

15mM Valine

0.12mg of mixed tRNA species from E. coli (as described above)

HPLC purified from Bakers yeast available from by Sigma (UK) Ltd

1 unit/ μ l of inorganic pyrophosphatase and

an appropriate concentration of ITRS (for example 1-2 g/ml of the enzyme as described above).

The samples were incubated at 37°C for 20 - 60 minutes. The reaction is stopped by the addition of a Malachite Green containing reagent as described by Howard and Ridley (4) and subsequently quenched with the addition of 34% citric acid. The optical absorbance is then measured at a wavelength of 630nm using a spectrophotometer. The K_m value of 0.048mM of ATP was determined using the novel colorimetric assay was in close agreement with the K_m of 0.047mM obtained using the ³H-Ile assay.

The inhibition constants of two compounds were measured and the results compared with the values obtained using the standard ³H-isoleucine

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assay. Compound 1 was tested at 0, 0.25 μM and 1.0 μM concentrations with varying ^3H -Isoleucine concentrations up to 100 μM , and was seen to be competitive with respect to isoleucine.

Results

Concentration giving 50% inhibition (I_{50}) measured using the valine-based assay of the present invention:

	<u>Compound 1</u>	<u>Compound 2</u>
I_{50}	2000nM	38nM

Concentration giving 50% inhibition (I_{50}) measured using the standard ^3H -isoleucine radiometric assay:

	<u>Compound 1</u>	<u>Compound 2</u>
I_{50}	109nM	2.3nM

The two assays both detected the compounds as inhibitors and both assays indicated that the potencies of the two inhibitors were different. The actual values are different because:

- 1) Compound 2 is, in reality, more potent than can be measured and the apparant I_{50} value is mainly determed by the concentration of enzyme in the assay (8). Since the radiometric assay is more sensitive it uses less enzyme and therefore yields a lower apparant I_{50} value than in the assay of the present invention.
- 2) Both compound 1 and compound 2 are competitive with the amino acid. In the case of the radiometric assay, the amino acid, isoleucine, is used at a concentration only approximately 2 fold above the K_m whereas in the assay of the present invention it is some 30 fold greater. Thus, allowing for this fifteen fold difference in the ratio of amino acid concentration to K_m , the two values given by the two assays are in good agreement. It will therefore be appreciated that the valine-based assay described here is useful as a colorimetric method for detecting inhibitors of E. coli ITRS.

3. Sequencing of the E. coli ITRS gene

The gene was sequenced in two stages:

- a) The Promega "Erase-A-Base" kit (Promega Cat. No. E5850) was used. The gene was cloned into the vector pGEM3Zf(-). The Erase-A-Base system allows the contruction of a series of unidirectional nested deletion sets from plasmid or M13 clones using the procedure developed by Henikoff (9). In

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this case the deletion mutants, each containing a different part of the gene, were sequenced with the pUC/M13 reverse sequence primer (5'-AACAGCTATGACCATG-3') using the Sequenase Version 2.0 kit.

b) About 60% of the gene was sequenced using the "Erase-A-Base" system. This sequence information was used to design synthetic oligonucleotide primers (listed below) so that the gaps in the gene sequence could be read. The gaps were filled in and the entire coding nucleotide sequence of the gene obtained.

Primers for top strand

ITS39F: 5'-GGCATCATCCGTGCGGCT-3'
ITS91F: 5'-TATGTGCCTGGCTGGGAC-3'
ITS114F: 5'-GGTGAGAAATTCACCGCC-3'
ITS226F: 5'-TTTGCCGTAAGCAACGTT-3'
ITS346F: 5'-GGTCAGAAATACGGCCTG-3'
ITS393F: 5'-CTGCTGCACGTTGAGAAA-3'
ITS566F: 5'-CAACACCGCGGCTGGTTC-3'
ITS765F: 5'-GCACCAATCCTCTCCTTC-3'
ITS808F: 5'-TTCTGGGACGAGCTGTTG-3'

Primers for bottom strand

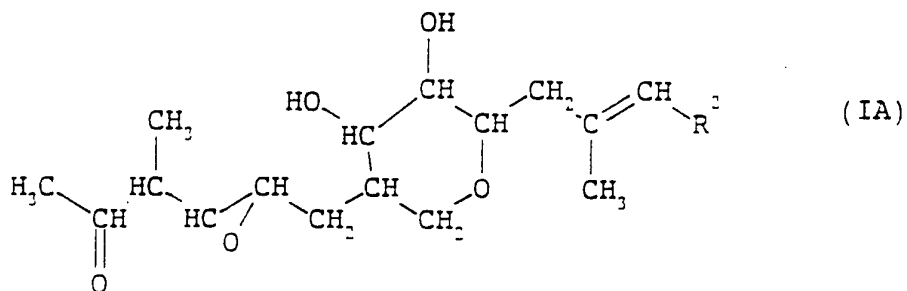
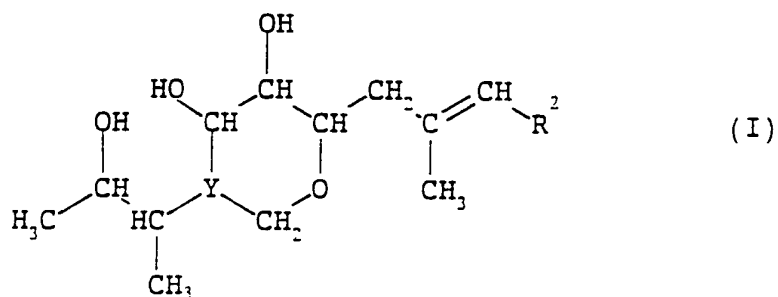
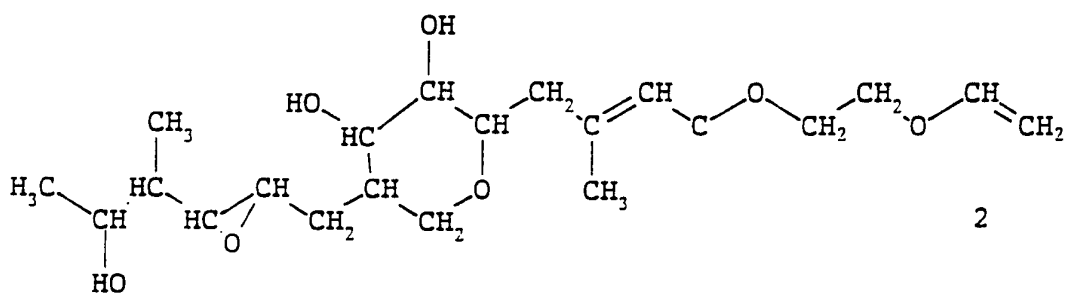
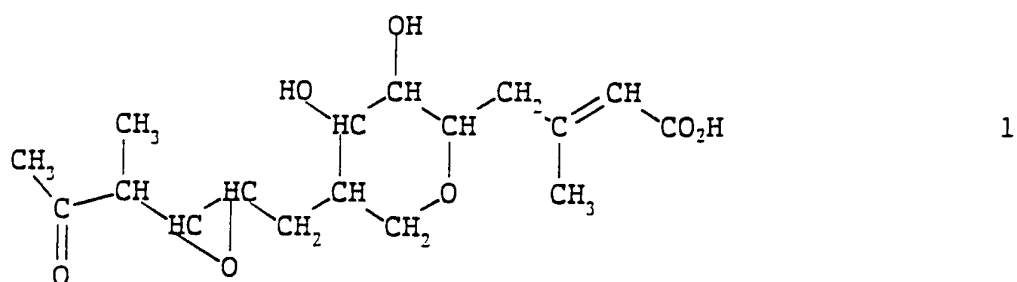
ITS252R: 5'-TTGCGCGGTTGGCAGGCA-3'
ITS336R: 5'-CGCGGTGTGAACGGCACC-3'
ITS526R: 5'-TTTACGTACTGATCAGC-3'

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CHEMICAL FORMULAE

(IN DESCRIPTION)



(IN DESCRIPTION)



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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Zeneca limited
- (B) STREET: 15 Stanhope Gate
- (C) CITY: London
- (E) COUNTRY: UK
- (F) POSTAL CODE (ZIP): W1Y 6LN

(ii) TITLE OF INVENTION: Assay Procedure and Application in
Identification of Herbicides

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2820 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2814

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Ser Lys Gly Leu Ser Gly Tyr Asp Ser Pro Tyr Val Pro Gly Trp Asp	
85 90 95	
TGC CAC GGT CTG CCG ATC GAG CTG AAA GTC GAG CAA GAA TAC GGT AAG	336
Cys His Gly Leu Pro Ile Glu Leu Lys Val Glu Gln Glu Tyr Gly Lys	
100 105 110	

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CCG GGT GAG AAA TTC ACC GCC GCC GAG TTC CGC GCC AAG TGC CGC GAA Pro Gly Glu Lys Phe Thr Ala Ala Glu Phe Arg Ala Lys Cys Arg Glu 115 120 125	384
TAC GCG GCG ACC CAG GTT GAC GGT CAA CGC AAA GAC TTT ATC CGT CTG Tyr Ala Ala Thr Gln Val Asp Gly Gln Arg Lys Asp Phe Ile Arg Leu 130 135 140	432
GGC GTG CTG GGC GAC TGG TCG CAC CCG TAC CTG ACC ATG GAC TTC AAA Gly Val Leu Gly Asp Trp Ser His Pro Tyr Leu Thr Met Asp Phe Lys 145 150 155 160	480
ACT GAA GCC AAC ATC ATC CGC GCG CTG GGC AAA ATC ATC GGC AAC GGT Thr Glu Ala Asn Ile Ile Arg Ala Leu Gly Lys Ile Ile Gly Asn Gly 165 170 175	528
CAC CTG CAC AAA GGC GCG AAG CCA GTT CAC TGG TGC GTT GAC TGC CGT His Leu His Lys Gly Ala Lys Pro Val His Trp Cys Val Asp Cys Arg 180 185 190	576
TCT GCG CTG GCG GAA GCG GAA GTT GAG TAT TAC GAC AAA ACT TCT CCG Ser Ala Leu Ala Glu Ala Glu Val Glu Tyr Tyr Asp Lys Thr Ser Pro 195 200 205	624
TCC ATC GAC GTT GCT TTC CAG GCA GTC GAT CAG GAT GCA CTG AAA GCA Ser Ile Asp Val Ala Phe Gln Ala Val Asp Gln Asp Ala Leu Lys Ala 210 215 220	672
AAA TTT GCC GTA AGC AAC GTT AAC GGC CCA ATC TCG CTG GTA ATC TGG Lys Phe Ala Val Ser Asn Val Asn Gly Pro Ile Ser Leu Val Ile Trp 225 230 235 240	720
ACC ACC CGC CGT GGA CTC TGC CTG CCA ACC GCG CAA TCT CTA TTG CAC Thr Thr Arg Arg Gly Leu Cys Leu Pro Thr Ala Gln Ser Leu Leu His 245 250 255	768
CAG ATT TCG ACT ATG CGC TGG TGC CAG ATC GAC GGT CAG GCC GTG ATT Gln Ile Ser Thr Met Arg Trp Cys Gln Ile Asp Gly Gln Ala Val Ile 260 265 270	816
CTG GCG AAA GAT CTG GTT GAA AGC GTA ATG CAG CGT ATC GGC GTG ACC Leu Ala Lys Asp Leu Val Glu Ser Val Met Gln Arg Ile Gly Val Thr 275 280 285	864
GAT TAC ACC ATT CTC GGC ACG GTA AAA GGT GCG GAT GTC GAG CTG CTG Asp Tyr Thr Ile Leu Gly Thr Val Lys Gly Ala Asp Val Glu Leu Leu 290 295 300	912
CGC TTT ACC CAT CCG TTT ATG GGC TTC GAC GTT CCG GCA ATC CTC GGC Arg Phe Thr His Pro Phe Met Gly Phe Asp Val Pro Ala Ile Leu Gly 305 310 315 320	960
GAT CAC GTT ACC CTG GAT GCG GGT ACC GGT GCC GTT CAC ACC GCG CCT Asp His Val Thr Leu Asp Ala Gly Thr Gly Ala Val His Thr Ala Pro 325 330 335	1008
GGC CAC GGC CCG GAC GAC TAT GTG ATC GGT CAG AAA TAC GGC CTG GAA Gly His Gly Pro Asp Asp Tyr Val Ile Gly Gln Lys Tyr Gly Leu Glu 340 345 350	1056
ACC GCT AAC CCG GTT GGC CCG GAC GGC ACT TAT CTG CCG GGC ACT TAT Thr Ala Asn Pro Val Gly Pro Asp Gly Thr Tyr Leu Pro Gly Thr Tyr 355 360 365	1104
CCG ACG TTG GAT GGC GTG AAC GTC TTC AAA GCG AAC GAC ATC GTC GTT Pro Thr Leu Asp Gly Val Asn Val Phe Lys Ala Asn Asp Ile Val Val 370 375 380	1152
GCG CTG CTG CAG GAA AAA GGC GCT CTG CTG CAC GTT GAG AAA ATG CAG	1200

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Ala 385	Leu	Leu	Gln	Glu	Lys 390	Gly	Ala	Leu	Leu	His 395	Val	Glu	Lys	Met	Gln 400	
CAC	AGC	TAT	CCG	TGC	TGC	TGG	CGT	CAC	AAA	ACG	CCG	ATC	ATC	TTC	CGC	1248
His	Ser	Tyr	Pro	Cys 405	Cys	Trp	Arg	His	Lys 410	Thr	Pro	Ile	Ile	Phe	Arg	
GCG	ACG	CCG	CAG	TGG	TTC	GTC	AGC	ATG	GAT	CAG	AAA	GGT	CTG	CGT	GCG	1296
Ala	Thr	Pro	Gln 420	Trp	Phe	Val	Ser	Met 425	Asp	Gln	Lys	Gly	Leu 430	Arg	Ala	
CAG	TCA	CTG	AAA	GAG	ATC	AAA	GGC	GTG	CAG	TGG	ATC	CCG	GAC	TGG	GGC	1344
Gln	Ser	Leu 435	Lys	Glu	Ile	Lys	Gly 440	Val	Gln	Trp	Ile	Pro 445	Asp	Trp	Gly	
CAG	GCG	CGT	ATC	GAG	TCG	ATG	GTT	GCT	AAC	CGT	CCT	GAC	TGG	TGT	ATC	1392
Gln	Ala	Arg	Ile	Glu	Ser	Met 455	Val	Ala	Asn	Arg	Pro 460	Asp	Trp	Cys	Ile	
TCC	CGT	CAG	CGC	ACC	TGG	GGT	GTA	CCG	ATG	TCA	CTG	TTC	GTG	CAC	AAA	1440
Ser	Arg	Gln	Arg	Thr	Trp 470	Gly	Val	Pro	Met	Ser 475	Leu	Phe	Val	His	Lys 480	
GAC	ACG	GAA	GAA	CTG	CAT	CCG	CGT	ACC	CTT	GAA	CTG	ATG	GAA	GAA	GTG	1488
Asp	Thr	Glu	Glu 485	Leu	His	Pro	Arg	Thr 490	Leu	Glu	Leu	Met	Glu	Glu 495	Val	
GCA	AAA	CGC	GTT	GAA	GTC	GAT	GGC	ATC	CAG	GCG	TGG	TGG	GAT	CTC	GAT	1536
Ala	Lys	Arg	Val 500	Glu	Val	Asp	Gly 505	Ile	Gln	Ala	Trp	Trp	Asp 510	Leu	Asp	
GCG	AAA	GAG	ATC	CTC	GGC	GAC	GAA	GCT	GAT	CAG	TAC	GTG	AAA	GTG	CCG	1584
Ala	Lys	Glu 515	Ile	Leu	Gly	Asp 520	Glu	Ala	Asp	Gln	Tyr	Val 525	Lys	Val	Pro	
GAC	ACA	TTG	GAT	GTA	TGG	TTT	GAC	TCC	GGA	TCT	ACC	CAC	TCT	TCT	GTT	1632
Asp	Thr	Leu	Asp	Val	Trp 535	Phe	Asp	Ser	Gly	Ser 540	Thr	His	Ser	Ser	Val	
GTT	GAC	GTG	CGT	CCG	GAA	TTT	GCC	GGT	CAC	GCA	GCG	GAC	ATG	TAT	CTG	1680
Val	Asp	Val	Arg	Pro	Glu 550	Phe	Ala	Gly	His	Gla 555	Ala	Asp	Met	Tyr	Leu 560	
GAA	GGT	TCT	GAC	CAA	CAC	CGC	GGC	TGG	TTC	ATG	TCT	TCC	CTA	ATG	ATC	1728
Glu	Gly	Ser	Asp 565	Gln	His	Arg	Gly	Trp 570	Phe	Met	Ser	Ser	Leu 575	Met	Ile	
TCC	ACC	GCG	ATG	AAG	GGT	AAA	GCG	CCG	TAT	CGT	CAG	GTA	CTG	ACC	CAC	1776
Ser	Thr	Ala	Met 580	Lys	Gly	Lys	Ala	Pro 585	Tyr	Arg	Gln	Val	Leu 590	Thr	His	
GGC	TTT	ACC	GTG	GAT	GGT	CAG	GGC	CGC	AAG	ATG	TCT	AAA	TCC	ATC	GGC	1824
Gly	Phe	Thr 595	Val	Asp	Gly	Gln 600	Gly	Arg	Lys	Met	Ser 605	Lys	Ser	Ile	Gly	
AAT	ACC	GTT	TCG	CCG	CAG	GAT	GTG	ATG	AAC	AAA	CTG	GGC	GCG	GAT	ATT	1872
Asn	Thr	Val	Ser	Pro	Gln 615	Asp	Val	Met	Asn	Lys 620	Leu	Gly	Ala	Asp	Ile	
CTG	CGT	CTG	TGG	GTG	GCA	TCA	ACC	GAC	TAC	ACC	GGT	GAA	ATG	GCC	GTT	1920
Leu	Arg	Leu	Trp 625	Val	Ala 630	Ser	Thr	Asp	Tyr	Thr 635	Gly	Glu	Met	Ala	Val 640	
TCT	GAC	GAG	ATC	CTG	AAA	CGT	GCT	GCC	GAT	AGC	TAT	CGT	CGT	ATC	CGT	1968
Ser	Asp	Glu	Ile 645	Leu	Lys	Arg	Ala	Ala 650	Asp	Ser	Tyr	Arg	Arg	Ile	Arg 655	
AAC	ACC	GCG	CGC	TTC	CTG	CTG	GCA	AAC	CTG	AAC	GGT	TTT	GAT	CCA	GCA	2016
Asn	Thr	Ala	Arg	Phe	Leu	Leu	Ala	Asn	Leu	Asn	Gly	Phe	Asp	Pro	Ala	

AAA Lys	GAT Asp	ATG Met 675	GTG Val	AAA Lys	CCG Pro	GAA Glu	GAG Glu 680	ATG Met	GTG Val	GTA Val	CTG Leu	GAT Asp 685	CGC Arg	TGG Trp	GCC Ala	2064
GTA Val	GGT Gly 690	TGT Cys	GCG Ala	AAA Lys	GCG Ala	GCA Ala 695	CAG Gln	GAA Glu	GAC Asp	ATC Ile	CTC Leu 700	AAG Lys	GCG Ala	TAC Tyr	GAA Glu	2112
GCA Ala 705	TAC Tyr	GAT Asp	TTT Phe	CAC His	GAA Glu 710	GTG Val	GTA Val	CAG Gln	CGT Arg	CTG Leu 715	ATG Met	CGC Arg	TTC Phe	TGC Cys	TCC Ser 720	2160
GTT Val	GAG Glu	ATG Met	GTT Val	TCC Ser 725	TTC Phe	TAC Tyr	CTC Leu	GAC Asp	ATC Ile 730	ATC Ile	AAA Lys	GAC Asp	CGT Arg	CAG Gln 735	TAC Tyr	2208
ACC Thr	CCA Pro	AAG Lys	CGG Arg 740	ACA Thr	GTG Val	TGG Trp	GCG Ala	CGT Arg 745	CGT Arg	AGC Ser	TGC Cys	CAG Gln	ACT Thr 750	GCG Ala	CTA Leu	2256
TAT Tyr	CAC His	ATC Ile 755	GCA Ala	GAA Glu	GCG Ala	CTG Leu	GTG Val 760	CGC Arg	TGG Trp	ATG Met	GCA Ala 765	CCA Pro	ATC Ile	CTC Leu	TCC Ser	2304
TTC Phe	ACC Thr 770	GCT Ala	GAT Asp	GAA Glu	GTG Val	TGG Trp 775	GGC Gly	TAC Tyr	CTG Leu	CCG Pro	GGC Gly 780	GAA Glu	CGT Arg	GAA Glu	AAA Lys	2352
TAC Tyr 785	GTC Val	TTC Phe	ACC Thr	GGT Gly	GAG Glu 790	TGG Trp	TAC Tyr	GAA Glu	GGC Gly	CTG Leu 795	TTT Phe	GGC Gly	CTG Leu	GCA Ala	GAC Asp 800	2400
AGT Ser	GAA Glu	GCG Ala	ATG Met	AAC Asn 805	GAT Asp	GCG Ala	TTC Phe	TGG Trp	GAC Asp 810	GAG Glu	CTG Leu	TTG Leu	AAA Lys	GTG Val 815	CGT Arg	2448
GGC Gly	GAA Glu	GTG Val	AAC Asn 820	AAA Lys	GTC Val	ATT Ile	GAG Glu	CAA Gln 825	GCG Ala	CGT Arg	GCC Ala	GAC Asp	AAG Lys 830	AAA Lys	GTG Val	2496
GGT Gly	GGC Gly	TCG Ser 835	CTG Leu	GAA Glu	GCG Ala	GCG Ala	GTA Val 840	ACC Thr	TTG Leu	TAT Tyr	GCA Ala	GAA Glu 845	CCG Pro	GAA Glu	CTG Leu	2544
TCG Ser	GCG Ala 850	AAA Lys	CTG Leu	ACC Thr	GCG Ala	CTG Leu 855	GGC Gly	GAT Asp	GAA Glu	TTA Leu	CGA Arg 860	TTT Phe	GTC Val	CTG Leu	TTG Leu	2592
ACC Thr 865	TCC Ser	CGC Arg	CGC Arg	TAC Tyr	GTT Val 870	GCA Ala	GAC Asp	TAT Tyr	AAC Asn	GAC Asp 875	GCA Ala	CCT Pro	GCT Ala	GAT Asp	GCT Ala 880	2640
CAG Gln	CAG Gln	AGC Ser	GAA Glu	GTA Val 885	CTC Leu	AAA Lys	GGG Gly	CTG Leu	AAA Lys 890	GTC Val	GCG Ala	TTG Leu	AGT Ser	AAA Lys 895	GCC Ala	2688
GAA Glu	GGT Gly	GAG Glu	AAG Lys 900	TGC Cys	CCA Pro	CGC Arg	TGC Cys	TGG Trp 905	CAC His	TAC Tyr	ACC Thr	CAG Gln	GAT Asp 910	GTC Val	GGC Gly	2736
AAG Lys	GTG Val	GCG Ala 915	GAA Glu	CAC His	GCA Ala	GAA Glu	ATC Ile 920	TGC Cys	GGC Gly	CGC Arg	TGT Cys	GTC Val 925	AGC Ser	AAC Asn	GTC Val	2784
GCC Ala	GGT Gly 930	GAC Asp	GGT Gly	GAA Glu	AAA Lys	CGT Arg 935	AAG Lys	TTT Phe	GCC Ala	TGATGA						2820

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 938 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Asp Tyr Lys Ser Thr Leu Asn Leu Pro Glu Thr Gly Phe Pro
 1 5 10 15
 Met Arg Gly Asp Leu Ala Lys Arg Glu Pro Gly Met Leu Ala Arg Trp
 20 25 30
 Thr Asp Asp Asp Leu Tyr Gly Ile Ile Arg Ala Ala Lys Lys Gly Lys
 35 40 45
 Lys Thr Phe Ile Leu His Asp Gly Pro Pro Tyr Ala Asn Gly Ser Ile
 50 55 60
 His Ile Gly His Ser Val Asn Lys Ile Leu Lys Asp Ile Ile Val Lys
 65 70 75 80
 Ser Lys Gly Leu Ser Gly Tyr Asp Ser Pro Tyr Val Pro Gly Trp Asp
 85 90 95
 Cys His Gly Leu Pro Ile Glu Leu Lys Val Glu Gln Glu Tyr Gly Lys
 100 105 110
 Pro Gly Glu Lys Phe Thr Ala Ala Glu Phe Arg Ala Lys Cys Arg Glu
 115 120 125
 Tyr Ala Ala Thr Gln Val Asp Gly Gln Arg Lys Asp Phe Ile Arg Leu
 130 135 140
 Gly Val Leu Gly Asp Trp Ser His Pro Tyr Leu Thr Met Asp Phe Lys
 145 150 155 160
 Thr Glu Ala Asn Ile Ile Arg Ala Leu Gly Lys Ile Ile Gly Asn Gly
 165 170 175
 His Leu His Lys Gly Ala Lys Pro Val His Trp Cys Val Asp Cys Arg
 180 185 190
 Ser Ala Leu Ala Glu Ala Glu Val Glu Tyr Tyr Asp Lys Thr Ser Pro
 195 200 205
 Ser Ile Asp Val Ala Phe Gln Ala Val Asp Gln Asp Ala Leu Lys Ala
 210 215 220
 Lys Phe Ala Val Ser Asn Val Asn Gly Pro Ile Ser Leu Val Ile Trp
 225 230 235 240
 Thr Thr Arg Arg Gly Leu Cys Leu Pro Thr Ala Gln Ser Leu Leu His
 245 250 255
 Gln Ile Ser Thr Met Arg Trp Cys Gln Ile Asp Gly Gln Ala Val Ile
 260 265 270
 Leu Ala Lys Asp Leu Val Glu Ser Val Met Gln Arg Ile Gly Val Thr
 275 280 285
 Asp Tyr Thr Ile Leu Gly Thr Val Lys Gly Ala Asp Val Glu Leu Leu
 290 295 300

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Arg Phe Thr His Pro Phe Met Gly Phe Asp Val Pro Ala Ile Leu Gly
 305 310 315 320
 Asp His Val Thr Leu Asp Ala Gly Thr Gly Ala Val His Thr Ala Pro
 325 330 335
 Gly His Gly Pro Asp Asp Tyr Val Ile Gly Gln Lys Tyr Gly Leu Glu
 340 345 350
 Thr Ala Asn Pro Val Gly Pro Asp Gly Thr Tyr Leu Pro Gly Thr Tyr
 355 360 365
 Pro Thr Leu Asp Gly Val Asn Val Phe Lys Ala Asn Asp Ile Val Val
 370 375 380
 Ala Leu Leu Gln Glu Lys Gly Ala Leu Leu His Val Glu Lys Met Gln
 385 390 395 400
 His Ser Tyr Pro Cys Cys Trp Arg His Lys Thr Pro Ile Ile Phe Arg
 405 410 415
 Ala Thr Pro Gln Trp Phe Val Ser Met Asp Gln Lys Gly Leu Arg Ala
 420 425 430
 Gln Ser Leu Lys Glu Ile Lys Gly Val Gln Trp Ile Pro Asp Trp Gly
 435 440 445
 Gln Ala Arg Ile Glu Ser Met Val Ala Asn Arg Pro Asp Trp Cys Ile
 450 455 460
 Ser Arg Gln Arg Thr Trp Gly Val Pro Met Ser Leu Phe Val His Lys
 465 470 475 480
 Asp Thr Glu Glu Leu His Pro Arg Thr Leu Glu Leu Met Glu Glu Val
 485 490 495
 Ala Lys Arg Val Glu Val Asp Gly Ile Gln Ala Trp Trp Asp Leu Asp
 500 505 510
 Ala Lys Glu Ile Leu Gly Asp Glu Ala Asp Gln Tyr Val Lys Val Pro
 515 520 525
 Asp Thr Leu Asp Val Trp Phe Asp Ser Gly Ser Thr His Ser Ser Val
 530 535 540
 Val Asp Val Arg Pro Glu Phe Ala Gly His Ala Ala Asp Met Tyr Leu
 545 550 555 560
 Glu Gly Ser Asp Gln His Arg Gly Trp Phe Met Ser Ser Leu Met Ile
 565 570 575
 Ser Thr Ala Met Lys Gly Lys Ala Pro Tyr Arg Gln Val Leu Thr His
 580 585 590
 Gly Phe Thr Val Asp Gly Gln Gly Arg Lys Met Ser Lys Ser Ile Gly
 595 600 605
 Asn Thr Val Ser Pro Gln Asp Val Met Asn Lys Leu Gly Ala Asp Ile
 610 615 620
 Leu Arg Leu Trp Val Ala Ser Thr Asp Tyr Thr Gly Glu Met Ala Val
 625 630 635 640
 Ser Asp Glu Ile Leu Lys Arg Ala Ala Asp Ser Tyr Arg Arg Ile Arg
 645 650 655
 Asn Thr Ala Arg Phe Leu Leu Ala Asn Leu Asn Gly Phe Asp Pro Ala
 660 665 670

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Lys Asp Met Val Lys Pro Glu Glu Met Val Val Leu Asp Arg Trp Ala
 675 680 685
 Val Gly Cys Ala Lys Ala Ala Gln Glu Asp Ile Leu Lys Ala Tyr Glu
 690 695 700
 Ala Tyr Asp Phe His Glu Val Val Gln Arg Leu Met Arg Phe Cys Ser
 705 710 715 720
 Val Glu Met Val Ser Phe Tyr Leu Asp Ile Ile Lys Asp Arg Gln Tyr
 725 730 735
 Thr Pro Lys Arg Thr Val Trp Ala Arg Arg Ser Cys Gln Thr Ala Leu
 740 745 750
 Tyr His Ile Ala Glu Ala Leu Val Arg Trp Met Ala Pro Ile Leu Ser
 755 760 765
 Phe Thr Ala Asp Glu Val Trp Gly Tyr Leu Pro Gly Glu Arg Glu Lys
 770 775 780
 Tyr Val Phe Thr Gly Glu Trp Tyr Glu Gly Leu Phe Gly Leu Ala Asp
 785 790 795 800
 Ser Glu Ala Met Asn Asp Ala Phe Trp Asp Glu Leu Leu Lys Val Arg
 805 810 815
 Gly Glu Val Asn Lys Val Ile Glu Gln Ala Arg Ala Asp Lys Lys Val
 820 825 830
 Gly Gly Ser Leu Glu Ala Ala Val Thr Leu Tyr Ala Glu Pro Glu Leu
 835 840 845
 Ser Ala Lys Leu Thr Ala Leu Gly Asp Glu Leu Arg Phe Val Leu Leu
 850 855 860
 Thr Ser Arg Arg Tyr Val Ala Asp Tyr Asn Asp Ala Pro Ala Asp Ala
 865 870 875 880
 Gln Gln Ser Glu Val Leu Lys Gly Leu Lys Val Ala Leu Ser Lys Ala
 885 890 895
 Glu Gly Glu Lys Cys Pro Arg Cys Trp His Tyr Thr Gln Asp Val Gly
 900 905 910
 Lys Val Ala Glu His Ala Glu Ile Cys Gly Arg Cys Val Ser Asn Val
 915 920 925
 Ala Gly Asp Gly Glu Lys Arg Lys Phe Ala
 930 935

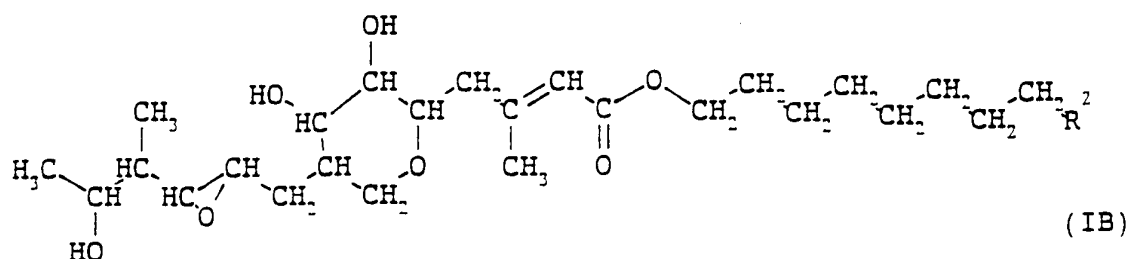
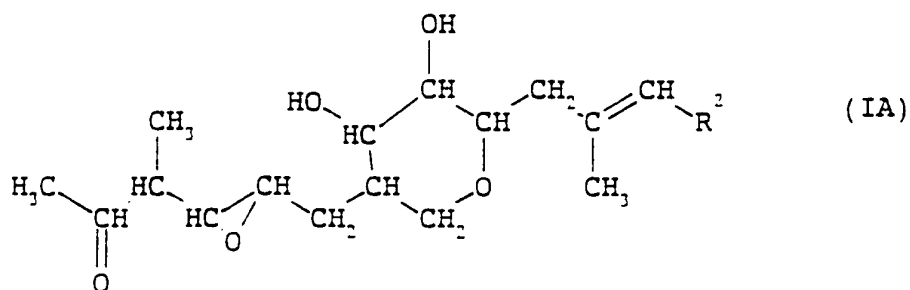
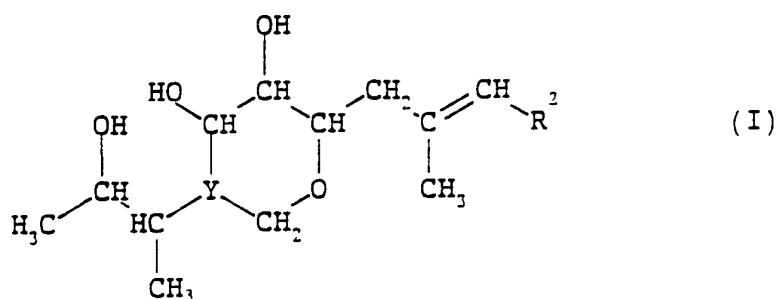
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CLAIMS

1. An assay for detecting an inhibitor of an aminoacyl-tRNA synthetase, which when reacted with a divalent metal cation, a corresponding species of tRNA and an appropriate non-cognate amino acid catalyses the hydrolysis of ATP to pyrophosphate; the assay comprising incubating a divalent metal cation, ATP, the said tRNA, the said non-cognate amino acid, inorganic pyrophosphatase and the said aminoacyl-tRNA synthetase, in at least a partially pure form, both with and without a potential inhibitor, and providing detecting means for phosphate, and comparing the results obtained.
2. An assay according to claim 1 wherein the aminoacyl-tRNA synthetase is isoleucyl-tRNA, valyl-tRNA synthetase or methionyl-tRNA synthetase, and the corresponding non-cognate amino acid is valine, threonine or homocysteine respectively.
3. An assay for detecting isoleucyl-tRNA synthetase of E. coli comprising
 - (a) incubating magnesium ions, adenosine triphosphate (ATP), an appropriate species of tRNA, isoleucyl-tRNA synthetase and inorganic pyrophosphatase with valine;
 - (b) simultaneously incubating a similar mixture further containing a potential inhibitor of the enzyme;
 - (c) detecting phosphate production from the incubates; and
 - (d) comparing the results.
4. An assay according to any one of claims 1 to 3 wherein phosphate is detected colorimetrically.
5. An assay according to any one of the preceding claims which is operated as a high throughput assay.
6. An enzyme inhibitor identified by the assay of any preceding claim.

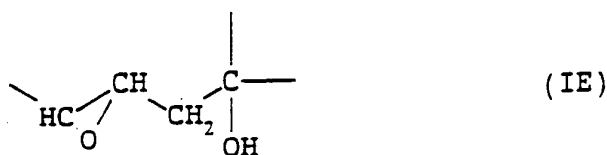
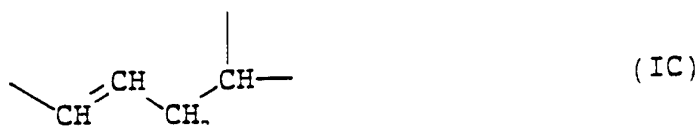
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7. An inhibitor of tRNA synthetase identified by the assay of any one of claims 1 to 5.
8. A herbicidal compound comprising an inhibitor according to either of claims 6 and 7.
9. A herbicidal compound which acts by inhibiting the plant isoleucyl tRNA synthetase enzyme excluding those compounds of general formula (I) or (IA) or (IB)



where Y represents a group of sub-formula (IC) or (ID or (IE)

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and wherein R^2 is a group $CO-XR^3$ wherein X is O or S and R^3 is hydrogen or an agrochemically acceptable ester-forming radical; or R^2 is a group $-R^4$ wherein R^4 is an optionally substituted aryl or heterocyclic group; or R^2 is a group $CO-NR^5R^6$ wherein R^5 and R^6 are the same or different and each represent an agrochemically acceptable amide-forming radical; stereoisomers of the compounds of formula (I), (IA) and (IB) and salts of the compound of formula (I), (IA) and (IB) wherein R^2 is $COXR^3$, X is O and R^3 is hydrogen.

10. A cDNA sequence as shown in Seq ID No 1, including non-critical variations of that sequence.
11. A cDNA sequence according to claim 10 wherein there is at least 70% homology with the cDNA sequence as shown in Seq ID No 1.
12. An amino acid sequence as shown in Seq ID No 2, including variants thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

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13. An amino acid sequence according to claim 13 wherein there is at least 70% homology with the amino acid sequence as shown in Seq ID No 2.