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(54) Title: PLANT FATTY ACID HYDROXYLASE GENES

(57) Abstract

Several cytochrome P450-dependent fatty acid hydryoxylases from different plant sources have been identified by recombinant cloning technology and characterized structurally and functionally. These clones represent novel plant hydroxylases which are active when expressed in a heterologous yeast system. These hydroxylase enzymes hydroxylate fatty acid substrates at different, well-defined postions in acid substrates of various chain lengths. The hydroxylases catalyze epoxidation of fatty acids, natural and synthetic, bearing a double bond at the site of attack.

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PLANT FATTY ACID HYDROXYLASE GENES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the identification of plant fatty acid hydroxylase genes; their use in genetic engineering and the modification of the fatty acid content of a cell, preferably a transfected cell; and products thereof, such as nucleic acids, recombinant vectors, polypeptides, host cells, transgenic plants, plant products with altered hydroxylated fatty acid content.

2. Description of the Related Art

Two laurate hydroxylases exist in plant microsomes that catalyze either the terminal hydroxylation or the in-chain hydroxylation of the fatty acid substrate. These two hydroxylases, which are both cytochrome P-450 enzymes, were not found to coexist in the same plant (Salaün et al., 1982).

Lauric acid is hydroxylated in Jerusalem artichoke tubers, tulip bulbs, maize seedlings, and several other plants by an in-chain hydroxylase producing a mixture of ω-2, ω-3, and ω-4 monohydroxylaurates. A laurate omega-hydroxylase is present in other plants, mainly leguminosae, which hydroxylates the methyl terminus of the fatty acid substrate. These two activities are found in different P450 species and did not coexist in 12 plant species that were analyzed (Salaün et al., 1982).

The level of cytochrome P450 in most plants is significantly increased by exposure to various xenobiotics, endobiotic substrates, fungal infections, light irradiation, wounding of the tissues and subsequent aging. A synthetic plant hormone, 2,4-dichloro-phenoxyacetic acid (2,4-D), increased the spectrophotometrically detectable amount of cytochrome P450

in Jerusalem artichoke tuber tissues (Adele et al., 1981). Similarly, cytochrome P450 content and, more specifically, the activities of the lauric acid in-chain hydroxylase and omega-hydroxylase were substantially induced by phenobarbitol in various plants (Salaün et al., 1981; 1982).

Clofibrate (ethyl 2-[4-chlorophenoxy]-2-methylpropanoate) is a hypolipidemic drug causing a proliferation of mitochondria, smooth endoplasmic reticulum, and peroxisomes in mammalian liver. Induction of cytochrome P450 and, more specifically, lauric acid omega-hydroxylase activity was observed in liver tissue from clofibrate treated test animals (Gibson et al., 1982). Similar effects are elicited by di-(2-ethylhexyl)-phthalate (DEHP), a widely used industrial plasticizer.

The induction of cytochrome P450 has allowed the purification of plant fatty acid hydroxylases from enriched sources, determination of a protein sequence from a plant fatty acid hydroxylase, and cloning of a family of hydroxylases as disclosed herein. The identity of the cloned genes as fatty acid hydroxylases is confirmed by functional assay.

SUMMARY OF THE INVENTION

An object of the invention is to provide cytochrome P450 genes encoding plant fatty acid hydroxylases. In particular, plant genes for terminal (omega or ω) hydroxylases having a peptide sequence which is a unique signature of plant fatty acid omega-hydroxylases (for example, cytochrome P450 subtype CYP94) and an in-chain (ω-1, ω-2, ω-3, and ω-4) hydroxylase (cytochrome P450 subtype CYP81) are provided.

Yet another object of the invention is to provide products derived from the plant fatty acid hydroxylase genes. Such products include, for example, nucleic acids, polypeptides, host cells, and transgenic plants.

A further object of the invention is to provide processes of making and using the plant fatty acid hydroxylase genes. In particular, genetic engineering allows making structural and functional variants of the plant fatty acid hydroxylases using the disclosed nucleotide and amino acid sequences. Moreover, plant products with altered hydroxylated fatty acid content are obtained by producing plants with a transgene that affects fatty acid metabolism.

In one embodiment of the invention, nucleic acids (e.g., DNA, RNA, variants thereof), recombinant polynucleotides comprised of the nucleic acids (e.g., recombinant and expression vectors), polypeptides encoded by the nucleic acids (e.g., enzymes with fatty acid hydroxylase activity), host cells (e.g., bacteria, yeast, plant) containing the aforementioned molecules, and whole plants containing wild-type/mutant genes (or wild-type/mutant gene products) which are generated by using the disclosed fatty acid hydroxylase sequences are provided.

A second embodiment of the invention is a process of making recombinant polypeptide by expressing a plant fatty acid hydroxylase gene sequence. The polypeptide may be isolated from a host cell expressing the gene sequence and used as an enzyme in an industrial process, or the polypeptide may act within a host cell or plant to hydroxylate suitable fatty acid substrates.

Variant plant fatty acid hydroxylases could be produced by genetic mutation. Random or site-directed mutation, domain shuffling, rational design based on structural contacts between enzyme and substrate, and correlation between protein structure-enzyme activity are examples of methods to produce variant genes and their cognate proteins. Variant plant fatty acid hydroxylases could be selected for desirable properties such as, for example, modification of substrate specificity. Suitable examples of such

modifications include hydroxylation of shorter or longer fatty acid chains, or fatty acids with odd carbon numbers; hydroxylation of FA with in-chain hydroxy or epoxy groups; hydroxylation of thia-FA, which is a FA having a methylene group replaced by a sulfur atom; hydroxylation of an ether-FA , which is a FA having a methylene group replaced by an oxygen atom; hydroxylation of modified fatty acids such as esters or amides, instead of the usual fatty acids with a free carboxylic group. Other desirable properties for selection are substrate affinity, modification of the rate of catalysis, enzyme lability or stability, and cofactor requirements.

In a third embodiment of the invention, metabolites of the fatty acid hydroxylases and plant fractions enriched for such metabolites are provided. Processes of making transfected host cells and transgenic plants are provided to increase or decrease specific fatty acid hydroxylases using the disclosed sequences and expression vectors. Such transfected host cells and transgenic plants provide a useful starting source for obtaining the desired metabolites from enriched or depleted fractions. Hydroxylated fatty acids are preferably produced as storage lipids in transgenic seeds.

For example, hydroxylated fatty acids are generally present in minor amounts in the phospholipid fractions (cellular membranes) of plants. Overexpression of plant fatty acid hydroxylase genes in a transgenic plant can elevate the content of hydroxylated fatty acids in triglycerides of the transgenic plant. The use of seed-specific promoters may allow accumulation of high amounts of hydroxylated fatty acids as storage lipids. The accumulated fatty acids can be recovered by extracting oil from the transgenic seed and isolating the fatty acids stored therein.

A fourth embodiment of the invention are processes providing for identification of additional fatty acid hydroxylase genes by hybridization (e.g., low or high stringency), nucleic acid amplification (e.g.,

LCR, PCR), or by screening databases using the omega-FA hydroxylase signature defined hereinbelow, and making fatty acid hydroxylase variants by chemical modification of the enzyme or genetic mutagenesis of the hydroxylase sequence (e.g., point mutation, deletion, insertion, domain shuffling).

The fatty acid substrate may be, for example, capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), oleic (C18:1), linoleic (C18:2 and enantiomers (9*E*, 12*Z*) and (9*Z*, 12*E*)), and linolenic (C18:3) fatty acid. Capric, lauric, and myristic fatty acids are considered medium-chain fatty acids, while palmitic, oleic, linoleic, and linolenic fatty acids are considered long-chain fatty acids.

Hydroxylated and epoxidated fatty acids produced by the invention will provide oils with novel properties that may be used for the manufacture of lubricants, anti-slip agents, plasticizers, coating agents, detergents, and surfactants.

Apart from industrial considerations such as mass production as storage lipids in seeds, there are other reasons to over or under produce omega-hydroxylated fatty acids in plants. The involvement of the omega hydroxylases in cuticle synthesis suggests that manipulation of the expression of these genes will affect the resistance of plants towards drought, or attack by insects and other pathogens. Furthermore, hydroxylated fatty acids are *per se* activators (elicitors) which trigger the mechanisms of plant defense against pathogens.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Nucleotide sequence of CYP94A1 (Clone A) and deduced protein translation (SEQ ID NOS:3-4, respectively). Nucleotides of

the open reading frame are shown in capital letters. The typical heme-binding domain, which constitutes the P450 signature, is underlined.

Figure 2: Carbon monoxide difference spectrum of yeast microsomes expressing CYP94A1 (Clone A). Microsomes (10 mg protein/ml) prepared as described by Pompon et al. (1996) were diluted 5-fold, reduced with a few grains of sodium dithionite, and divided into two cuvettes. A baseline was recorded between 400 and 500 nm using a Shimadzu MP2000 double-beam spectrophotometer. Carbon monoxide was bubbled into the sample cuvette and the P450-CO complex spectrum was recorded. Based on a millimolar absorbance of 91/cm, the amount of CYP94A1 was 176 pmole/mg protein.

Figure 3: Chemical structure of sulfur-containing lauric acid analogs and sulfoxide metabolites.

Figure 4: Radiochromatogram of the reaction products formed from capric (C10:0), lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids by CYP94A1 (Clone A). After incubation of microsomes from transformed yeast with fatty acids, the reaction mixtures were extracted as described and analyzed by TLC. A: in the presence of NADPH; B: in the absence of NADPH; and S: residual substrate.

Figure 5: Radiochromatogram of the reaction products formed from oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids by CYP94A1 (Clone A). After incubation of microsomes from transformed yeast with unsaturated fatty acids, the reaction mixtures were extracted as described and analyzed by TLC. A: in the presence of NADPH; B: in the absence of NADPH (control); and S: residual substrate.

Figure 6: Radiochromatogram of the reaction products formed from 9Z, 12*E*-octadecadienoic (C18:2-9E,12Z); 9*E*, 12Z-octadecadienoic (C18:2-9Z,12E); 8-propylsulfinyloctanoic (8S-LAU); and 10-

methylsulfinyldecanoic (10S-LAU) acids by CYP94A1 (Clone A). After incubation of microsomes from transformed yeast with unsaturated fatty acids, the reaction mixtures were extracted as described and analyzed by TLC (C18:2-9E,12Z and C18:2-9Z,12E) or HPLC (8S-LAU and 10S-LAU). A: in the presence of NADPH; B: in the absence of NADPH (control); and S: residual substrate.

Figure 7: Nucleotide sequence of VAGH811 (incomplete at 5' end), also called Clone B (complete cDNA encoding CYP94A2), and deduced protein translation (ω-MAH or CYP94A2) (SEQ ID NOS:5-6, respectively). The consensus heme-binding domain which constitutes the P450 signature is underlined.

Figure 8: Carbon monoxide difference spectrum of yeast microsomes expressing ω-MAH (Clone B). Microsomes (10 mg protein/ml) prepared as described by Pompon et al. (1996) were diluted 5-fold, reduced with a few grains of sodium dithionite, and divided into two cuvettes. A baseline was recorded between 400 and 500 nm using a Shimadzu MP2000 double-beam spectrophotometer. Carbon monoxide was bubbled into the sample cuvette and the P450-CO complex spectrum was recorded. Based on a millimolar absorbance of 91/cm, the amount of ω-MAH was 80 pmole/ml microsomes.

Figure 9: Radiochromatogram of the reaction product formed from capric acid by ω-MAH. After incubation with ^{14}C capric acid, the reaction mixture was extracted as described and analyzed by TLC. A: in presence of NADPH, B: in absence of NADPH.

Figure 10: Radiochromatogram of the reaction product formed from lauric acid by ω-MAH. After incubation with ^{14}C lauric acid, the reaction mixture was extracted as described and analyzed by TLC. A: in presence of NADPH, B: in absence of NADPH.

Figure 11: Radiochromatogram of the reaction product formed from myristic acid by ω-MAH. After incubation with ^{14}C myristic acid, the reaction mixture was extracted as described and analyzed by TLC. A: in presence of NADPH, B: in absence of NADPH.

Figure 12: Radiochromatogram of the reaction product formed from palmitic acid by ω-MAH. After incubation with ^{14}C palmitic acid, the reaction mixture was extracted as described and analyzed by TLC. A: in absence of NADPH, B: in presence of NADPH.

Figure 13: Radiochromatogram of the reaction product formed from stearic acid by ω-MAH. After incubation with ^{14}C stearic acid, the reaction mixture was extracted as described and analyzed by TLC. A: in presence of NADPH, B: in absence of NADPH.

Figure 14: Radiochromatogram of the reaction product formed from oleic acid by ω-MAH. After incubation with ^{14}C oleic acid, the reaction mixture was extracted as described and analyzed by TLC. A: in presence of NADPH, B: in absence of NADPH.

Figure 15: Nucleotide sequence of CYP94A3 (Clone C) and deduced protein translation (SEQ ID NOS:7-8, respectively); compared to Clone B, nine nucleotides were missing at the 5' end.

Figure 16: Carbon monoxide difference spectrum of yeast microsomes expressing CYP94A3 (Clone C). Microsomes (10 mg protein/ml) prepared as described by Pompon et al. (1996) were diluted 5-fold, reduced with a few grains of sodium dithionite, and divided into two cuvettes. A baseline was recorded between 400 and 500 nm using a Shimadzu MP2000 double-beam spectrophotometer. Carbon monoxide was bubbled into the sample cuvette and the P450-CO complex spectrum was recorded. Based on a millimolar absorbance of 91/cm, the amount of CYP94A3 was 550 pmole/ml microsomes.

Figure 17: Radiochromatogram of the reaction products formed from capric (C10:0) and lauric (C12:0) acids by CYP94A3 (Clone C). After incubation of microsomes from transformed yeast with fatty acids, the reaction mixtures were extracted as described and analyzed by TLC. A: in the presence of NADPH; B: in the absence of NADPH (control); and S: residual substrate.

Figure 18: Radiochromatogram of the reaction products formed from myristic (C14:0) and lauric (C16:0) acids by CYP94A3 (Clone C). After incubation of microsomes from transformed yeast with fatty acids, the reaction mixtures were extracted as described and analyzed by TLC. A: in the presence of NADPH; B: in the absence of NADPH (control); and S: residual substrate.

Figure 19: Radiochromatogram of the reaction products formed from oleic (C18:1) and linoleic (C18:2) acids by CYP94A3 (Clone C). After incubation of microsomes from transformed yeast with fatty acids, the reaction mixtures were extracted as described and analyzed by TLC. A: in the presence of NADPH; B: in the absence of NADPH (control); and S: residual substrate.

Figure 20: Nucleotide sequence of CYP81B1 (Clone D) and deduced protein translation (SEQ ID NOS:15-16, respectively). The consensus heme-binding domain which constitutes the P450 signature is underlined.

Figure 21: Carbon monoxide difference spectrum of yeast microsomes expressing CYP81B1 (Clone D). Microsomes (10 mg protein/ml) prepared as described by Pompon et al. (1996) were diluted 5-fold, reduced with a few grains of sodium dithionite, and divided into two cuvettes. A baseline was recorded between 400 and 500 nm using a Shimadzu MP2000 double-beam spectrophotometer. Carbon monoxide was

bubbled into the sample cuvette and the P450-CO complex spectrum was recorded. Based on a millimolar absorbance of 91/cm, the amount of CYP81B1 was 202 pmoles/mg protein.

Figure 22: TLC analysis of the metabolites obtained after 45 min of incubation at 27°C of 100 µM (^{14}C)-radiolabeled C10:0 (a), C12:0 (b), C14:0 (c) with microsomes from *Helianthus tuberosus* (H. tub., 1.2 mg protein) or from transgenic yeast (CYP81B1, 0.1 mg protein) and 600 µM NADPH. After stopping the reaction with one volume of acetonitrile-acetic acid (99.8/0.2), the incubation medium was directly spotted on TLC silica plates (60 F254, Merck) and developed with a mixture of ether-petroleum benzin-formic acid (70/30/0.2). Reaction products were localized using a radiometer thin-layer scanner (Berthold LB 2723).

Figure 23: Radio-HPLC analysis of the metabolites obtained after 45 min of incubation at 27°C of 100 µM C12:0(a), and C14:0(b) with microsomes of transgenic yeast (0.1 mg protein) and 600mM NADPH. After stopping the reaction with one volume of acetonitrile-acetic acid (99.8/0.2, v/v), the incubation medium was extracted twice with ether. Ether was evaporated under argon and the metabolites were separated on a Beckman HPLC ODS 5µm 1.6 mm x 15 cm column using H_2O-acetonitrile-acetic acid (75/25/0.2 by vol for C12:0, 68/32/0.2 by vol for C14:0) as eluent. The flow rate was 2 ml/min. Radioactivity of HPLC effluent was monitored with a computerized on-line solid scintillation counter (Ramona-D, Isomess).

Figure 24: Nucleotide sequence of CYP94A4 (Clone E) and deduced protein translation (SEQ ID NOS: 9-10, respectively) prepared from tobacco mosaic virus-infected tobacco leaves.

Figure 25: Nucleotide sequence of CYP94A5 (Clone F) and deduced protein translation (SEQ ID NOS: 11-12, respectively) prepared

from tobacco mosaic virus-infected tobacco leaves.

Figure 26: Nucleotide sequence of CYP94A6 (Clone G) and deduced protein translation (SEQ ID NOS: 13-14, respectively) prepared from tobacco mosaic virus-infected tobacco leaves.

DETAILED DESCRIPTION OF THE INVENTION

Plants are characterized by the presence of distinct cytochrome P450 isoforms (Salaün and Helvig, 1995). Some of these isoforms appear to be tissue, organ and species specific. Others, such as CYP73 (CA4H = cinnamic acid 4-hydroxylase), are widely distributed in the plant kingdom. As in mammals, the involvement of multiple forms of cytochrome P450s in medium- and long-chain fatty acid (FA) oxidation in plants is well established. Interestingly, there are several similarities between mammals and plants in the catalytic mechanisms and the induction of enzyme activities by various xenobiotics. The plant P450s involved in FA oxidation not been isolated to date because these membrane-bound enzymes are generally present in tissues at very low concentrations. During the past decade, several cytochrome P450 products encoded by the CYP4 gene family (mainly fatty acid hydroxylases) have been purified and cDNAs have been isolated and sequenced from mammalian and insect cDNA libraries. Even though more than fifty cDNAs encoding plant P450s have been sequenced to date, none significantly matches the genes of the CYP4 family from mammals and insects.

The biological roles and the substrate specificity of cytochromes P450 isoforms involved in fatty acid and eicosanoid oxidation are poorly understood. Oxygenated FA from plants are mainly found in polar lipids such as triglycerides and phospholipids, and as monomers in polymeric layers.

Cutins and suberins are polymers mainly composed of hydroxylated fatty acids, especially omega-hydroxylated fatty acids. They protect plants against water loss, chemical penetration, attack by pathogens (e.g., microbes, insects), and other environmental stresses. Some of these

are potent inducers of fungal cutinase and some show anti-fungal properties. These contrasting and apparently opposite effects may be due to the great diversity of defense mechanisms found in plants, and also to strategies developed by the fungi to infect the plant host.

On the other hand, and as reported in mammals, hydroxylated fatty acids may play a role in responding to various stresses by giving rise to reactions similar to inflammatory processes, as a defense mechanism. Moreover, the presence of large amounts of hydroxy derivatives of the C18 family in the plant stigma suggests that they may play a role in recognition of the stigma by pollen.

Long-chain fatty acid omega and in-chain hydroxylases may play an important role in the synthesis of plant cuticles by generating hydroxy functions which appear essential to polymerization of constitutive cutin monomers. Cuticle monomers are often present as complex mixtures with species-specific profiles. In addition to hydroxylated FA, epoxidated derivatives are also found as monomers of cuticles in a few plant species. Moreover, vicinal diol derivatives resulting from chemical and enzymatic ring-opening of epoxides have not been detected in cuticles from the C16 fatty acid family, suggesting that an epoxide function is not essential for polymerization of the cuticle matrix. In this case, it was suggested that the introduction of an internal hydroxyl group involves a direct hydroxylation mechanism catalyzed by cytochrome P450-dependent fatty acid hydroxylases.

Fatty acids and their derivatives are subjected to many types of oxidation reactions including hydroxylation, epoxidation, dehydration and reduction. Several forms of cytochrome P450 are suspected of being involved in these reactions. For example, previous studies have demonstrated that at least three distinct P450 isoforms are present in

microsomes from various plant species when incubated with a model substrate such as lauric acid. An interesting feature is that these P450 systems catalyze alternatively hydroxylation and epoxidation of unsaturated laurate analogs with a regio-specificity strongly dependent on the position and stereo-specificity dependent on the configuration of the double bond in the aliphatic chain.

Cytochrome P450-dependent reactions are involved in oxidation of fatty acids and derivatives in plants. The reactions are grouped below according to type of reaction and the position of the carbon attacked. Some examples of induction by chemicals and inactivation by suicide substrates of the cytochrome P450 activities under consideration are discussed.

A lauric acid omega-hydroxylase (ω-LAH), producing exclusively 12-hydroxylauric acid, has been described in *Pisum sativum*, *Vicia sativa* and other leguminosae. In addition to laurate hydroxylation, the microsomal fraction from clofibrate-treated *V. sativa* seedlings also catalyzed the omega-hydroxylation of capric (C10:0) and myristic (C14:0) acids. A free carboxyl group appears essential for the binding of substrates to the enzyme. Induction and inhibition studies suggest that a single cytochrome P450 is capable of omega-hydroxylating these fatty acids.

To explore the catalytic capabilities of the ω-LAH, a series of (1-^{14}C) radiolabeled unsaturated lauric acid analogs (7-, 8-, 9- and 10-dodecenoic acids) was incubated with the microsomal fraction from clofibrate-treated *V. sativa* seedlings. This subcellular fraction was able to catalyze the omega-oxidation of the analogs when O_2 and NADPH were present. The cis and trans forms of the four in-chain unsaturated analogs were 12-hydroxylated with similar efficiency. It is also important to note that allylic oxidation (i.e., 12-hydroxylation of 10-dodecenoate) occurred

with complete retention of the stereo-chemistry of the double bond and that allylic transposition was never observed. In contrast, the terminal olefin (11-dodecenoic acid) was epoxidized by the enzyme preparation. The formation of each metabolite was inhibited to the same extent when microsomes were incubated in the presence of CO, anti-cytochrome P450 reductase antibodies and suicide substrates, suggesting that a single P-450 isoenzyme is able to omega-hydroxylate lauric acid, unsaturated analogs with a double bond or 1,4-pentadiene motif and to epoxidize the terminal olefin, 11-dodecenoic acid. The fact that ω-LAH activity was not inhibited by oleic acid (C18:1) at a concentration 10 times higher than that of laurate suggests that it is more specific for short- and medium-chain FA.

Early work by Soliday and Kolattukudy demonstrated the omega-hydroxylation of palmitic acid (C16:0) by a microsomal fraction from *V. faba*. Inhibition of the reaction by CO suggested the involvement of a cytochrome P-450 monooxygenase but no reversal of CO inhibition by light was obtained. More recently, microsomes from etiolated *Vicia sativa* seedlings incubated with (1-^{14}C)oleic acid (Z9-octadecenoic acid), (1-^{14}C)9,10-epoxystearic acid or (1-^{14}C)9,10-dihydroxystearic acid catalyzed the NADPH-dependent formation of hydroxylated metabolites. The chemical structure of these compounds was established by GC-MS analysis to be 18-hydroxyoleic acid, 18-hydroxy-9,10-epoxystearic acid and 9,10,18-tribydroxystearic acid, respectively. The reactions were inhibited by CO. Inhibition could be partially reversed by light and all three reactions were inhibited by antibodies raised against NADPH-cytochrome P450 reductase from Jerusalem artichoke. The possibility that a single P450 is involved in the omega-oxidation of both oleic and linoleic acids (C18:2) is suggested by the competitive inhibition of oleic acid hydroxylation by linoleic acid, and vice versa.

In microsomes from Jerusalem artichoke tubers (*Helianthus tuberosus*), a lauric acid in-chain hydroxylase (IC-LAH) catalyzes hydroxylation of carbons 10, 9 and 8 in a 24:63:13 ratio, respectively. The activity undetectable in dormant tuber tissues, was induced by wounding and exposure to chemicals. Several other plant species, such as maize and tulip, catalyze this type of reaction but, in wheat seedlings, lauric acid is mainly converted to the 11-hydroxy derivative. The lauric acid (ω-1)-hydroxylase ((ω-1)-LAH) from wheat generates a mixture of monohydroxylaurate in the proportion of 65%, 31% and 4% for 11-hydroxy, 10-hydroxy and 9-hydroxylaurate, respectively. Capric and myristic acids were also converted to (ω-1) and (ω-2) hydroxylated products. Additional minor metabolites hydroxylated at (ω-3) and (ω-4) were also detected when myristic acid was the substrate. Whatever the length of FA (C10 to C14) incubated, no omega-hydroxylated products were detected. In addition, results from our laboratory suggest that the (ω-1)-LAH from wheat catalyzes the hydroxylation of the herbicide diclofop.

Biosynthesis of plant cuticles involves distinct P450 systems. The in-chain hydroxylation of omega-hydroxypalmitic acid by *V. faba* microsomes gives rise to 9 (or 10),16-dihydroxypalmitic acid. The reactions have been attributed to a cytochrome P450 which differs from those involved in omega-hydroxylation of palmitic acid by effective reversal by light of CO inhibition.

The most abundant constituents found in the cutin of wheat caryopses are omega-hydroxylated oleic and 9,10-epoxystearic acids. Surprisingly, incubation of the microsomal fraction from etiolated wheat shoots (*Triticum aestivum* L.) with (1-^{14}C)oleic acid led to the formation of 18-, 17- and 16-hydroxyoleic acids, identified by GC-MS analysis. They were generated in a molar ratio of 1.4:4.6:4, respectively. The involvement

of cytochrome P450 was demonstrated by the dependence of these hydroxylations upon O_2 and NADPH, and by their light-reversible inhibition by CO. This reaction was selectively inhibited by a suitably designed mechanism-based inhibitor (see below), while lauric acid and cinnamic acid hydroxylations were not affected.

The capability of *V. sativa* microsomes to catalyze the oxidation of two sulphur-containing lauric acid analogs has been examined. Two sulphides synthesized in radiolabeled form, (1-^{14}C) 10-methylsulphinyldecanoic acid (10S-LAU) and (1-^{14}C)8-propylsuphinyloctanoic acid (8S-LAU), were incubated with *V. sativa* microsomes under conditions promoting either P450 or peroxidase reactions. In addition to the expected peroxidative oxidation, both 8- and 10-thio fatty acids were actively converted to the sulphoxide by at least two distinct membrane bound enzymes. Based on the NADPH requirement, reversal of CO inhibition and inactivation of the NADPH-dependent reactions by the mechanism-based inhibitor 11-dodecynoic acid (11-DDYA) targeted to inhibit the ω-LAH (see below), it is suggested that the sulphoxidation of 10S-LAU and 8S-LAU were catalyzed by the same or similar P450 forms which hydroxylate lauric acid. The second membrane-bound enzyme which appears to be NADPH-independent was not fully characterized. However, the presence of beta-mercaptoethanol in the incubation medium had no effect on the sulphoxidation of either 8S-LAU or 10S-LAU, suggesting that the peroxidase present in these membranes was not involved.

A remarkable property of living organisms is their ability to induce the activity of P450 monooxygenases in response to chemical or physical stresses. Cytochrome P450 activities from plants are induced by light, UV-irradiation, wounding, ripening, fungal infection, elicitors, endogenous compounds and numerous chemicals, including safeners,

herbicides, drugs and pollutants.

Plant P450 systems involved in omega-hydroxylation of lauric acid (ω-LAH) and oleic acid (ω-OAH) are induced by clofibrate in a dose dependent manner. Clofibrate is a well known hypolipidemic drug which induces peroxisome proliferation in both mammals and plants. Clofibrate and related arylphenoxy compounds, such as 2,4-dichlorophenoxy-acetic acid (2,4-D), which selectively induce fatty acid omega-hydroxylase activity, have little or no effect on the activity of IC-LAH from *H. tuberosus* tubers and (ω-l)-LAH from wheat seedlings. *V. sativa* microsomes contain exclusively fatty acid omega-hydroxylases. In-chain hydroxylated fatty acids have never been detected in microsomes from either untreated or clofibrate- or phenobarbital-treated *Vicia* seedlings, although exposure to these xenobiotics produces a dramatic increase of omega-hydroxylase activity: circa 20 times with phenobarbital and over 30-50 times with clofibrate. It is noteworthy that in mammalian systems, clofibrate induces the omega-hydroxylase selectively, while phenobarbital enhances (ω-l)-hydroxylation of lauric acid. The microsomal (ω-1)-LAH activity of etiolated wheat shoot was stimulated by treatment with naphthalic anhydride (NA) or phenobarbital (PB). Coating the seeds with the safener NA resulted in a 4.5-fold increase of (ω-1)-LAH activity and a 1.5-fold increase in P-450 content, while the activity of cinnamate hydroxylase (CA4H), a P450 involved in lignin synthesis, was reduced. The herbicide metabolizing activity of diclofop arylhydroxylase (DIAH) was stimulated 4-fold. A much higher stimulation of the (ω-1)-LAH and DIAH was observed when the seedlings were aged on a 5 mM PB solution. Coating the seeds with NA and subsequently aging on PB resulted in a synergistic stimulation of (ω-1)-LAH and DIAH (20 times) while CA4H activity was strongly depressed. Cytochrome P450 content was increased to about 0.5

nmole/mg, one of the highest levels so far recorded in plants. The relative amounts of 11-, 10- and 9-hydroxylaurates formed remained unchanged under all conditions. Similarly, the (ω-1)-oleic acid hydroxylase activity was induced in treated seedlings to the same extent as (ω-1)-LAH, although these P450-dependent reactions were supported by distinct isoforms.

A wide range of chemicals has been found to induce the IC-LAH activity of tubers and bulbs from Jerusalem artichoke, tulip, and maize seedlings. Activity was induced above the untreated level by wounding slices of Jerusalem artichoke tubers in the presence of 25 mM $MnCl_2$ and 20 mM aminopyrine, but was even more enhanced when tissues were exposed to 8 mM phenobarbital.

The mechanism of cytochrome P450 induction in plant systems remains unknown but most of the P450 inducers active in mammals are also effective in induction of plant P450. Recently, experiments demonstrated that induction of cytochrome P450-dependent fatty acid hydroxylases from rodent liver by hypolipidemic drugs, such as clofibrate, and certain physiological conditions involves transcriptional activation of the genes which was mediated by receptors (peroxisome proliferator-activated receptors). The evidence suggested that perturbation of lipid metabolism is the common factor for fatty acid hydroxylase induction by peroxisomal proliferators.

Mechanism-based inhibitors (suicide substrates) containing a terminal acetylene are potent irreversible inhibitors of both plant and mammalian fatty acid omega-hydroxylases. Pre-incubation of microsomes from clofibrate-treated *V. sativa* seedlings with 11-dodecynoic acid (11-DDYA) and NADPH resulted in a pseudo-first-order loss of lauric acid omega-hydroxylation with K_i = 150 μM and a half-life of 2.4 min. The apparent rate constant for inactivation by 11-DDYA was 4.3-4.8 x 10^{-3}/sec.

Incubation of microsomes from *V. sativa* with (1-^{14}C)11-DDYA produces a major metabolite, 1,12-decanedioic acid, probably generated by addition of water to a ketene intermediate. This ketene may also interact with nucleophilic residues in the active site leading to a selective chemical labeling of two proteins bands (about 50 kDa). The labeling of microsomal proteins, which correlated well with diacid formation and inactivation of ω-LAHs, increased as a function of incubation time and concentration of (1-^{14}C)11-dodecynoic acid. Based on these results, two potential inhibitors targeted to inactivate the omega-hydroxylation of oleic acid were synthesized. Incubation of microsomes from *V. sativa* with terminal acetylenes, (Z)9-octadecen-17-ynoic acid (17-ODNYA) and the corresponding (Z)9,10-epoxyoctadecan-17-ynoic acid (17-EODNYA), resulted in a pseudo-first-order loss of oleic acid omega-hydroxylation with apparent K_i of 60 μM and 50 μM, respectively. The calculated half-lives of enzyme activity were 6 min and 8 min for saturating concentrations of 17-ODNYA and 17-EODNYA, respectively. Interestingly, these suicide substrates inhibit the omega hydroxylation of oleic acid, epoxide and diol derivatives, and also linoleic acid to a similar extent.

To purify and sequence plant cytochrome P450 proteins, a selective covalent binding of P450 apoproteins with labeled mechanism-based inhibitors would provide a useful means of following the labeled protein during purification steps.

The terminal olefin 11-dodecenoic acid inactivates a P450 from wheat which catalyzes mainly oxidation of the internal carbon (ω-l) of laurate. As proposed by Ortiz de Montellano and coworkers, P450 inactivation by a terminal olefin proceeds via an oxidative attack on the internal carbon (ω-1) of the double bond leaving a terminal methylene radical free to alkylate the heme unit. In contrast, the plant ω-LAH which

exclusively attacks the external position, catalyzed the formation of the 11-12 epoxide without any measurable loss of activity. Acetylenic derivatives of lauric acid are also potent inactivators of (ω-1)-LAH from wheat. Incubation of microsomes from etiolated wheat seedlings with 10-dodecynoic acid (10-DDYA) produced a dramatic inhibition of lauric acid hydroxylation. The inhibition was dependent upon time and concentration of inhibitor in a process characteristic of mechanism based inhibitors. A half-life of 3 min and an apparent inhibition constant K_i of 14 μM were determined from pseudo-first-order kinetic studies of (ω-1)-LAH inhibition. Similar results were obtained by incubating microsomes with a terminal acetylene, 11-dodecynoic acid (11-DDYA).

In addition, the oleic acid hydroxylase (ω-1)-OAH from wheat, oxidizing mainly the (ω-1) position, was irreversibly inhibited by a substrate analog displaying an acetylenic function at the (ω-1) position. The hydroxylation of oleic acid, but not of lauric acid, was inhibited when microsomes were incubated with cis-9-octadecen-16-ynoic acid (16-ODNYA). These results strongly suggest that at least two different P450 enzymes are involved in the oxidation of oleic and lauric acids.

Thus, an internal acetylene exerts a highly destructive effect on P450s catalyzing in-chain oxidation. The mechanism of inactivation remains unknown, but the chemical rearrangement of a putative unstable acetylene epoxide, already suspected in the formation of ketene from terminal acetylene, cannot be excluded.

Compared to studies of fatty acid hydroxylases in mammals, understanding of the catalytic mechanism and substrate specificity of plant fatty acid hydroxylases (Table 1) is limited, only two P450 forms catalyzing the dehydration of a fatty acid hydroperoxide have been isolated and cloned to date. Allene oxide synthase, by generating a precursor of jasmonic acid,

may be a key enzyme controlling various physiological steps in plant development. In this regard, it will also be of interest to understand the physiological role of the RPP from guayule rubber particles which apparently catalyzes a similar reaction. On the other hand, evidence suggests that long-chain fatty acid hydroxylases (omega and in-chain) play an important role in the biosynthesis of plant cuticles by generating terminal and internal hydroxy functions which appear essential to polymerization of cutin monomers.

At least three distinct roles for the plant fatty acid hydroxylases of the present invention are foreseeable: cutin and suberin synthesis, rapid catabolism of free fatty acids (i.e., detoxification), and synthesis of signaling molecules.

As discussed above, altering synthesis of cutin and suberin by controlling the activity of plant fatty acid hydroxylases is expected. Therefore, a plant with desirable characteristics (e.g., resistance to drought or chemical penetration) may result from the modification of cutin and suberin production. For example, we envision a null mutant or a hypomorph would be a slow growing plant relative to the wildtype plant.

Wounding or other types of stress lead to the activation of phospholipases, drastic liberation of fatty acids, and an oxidative burst (Low et al., 1996). There are several papers showing that elicitors activate phospholipases in plants (see Chandra et al., 1996) whose activation will lead to liberation of free fatty acids. By analogy with the role of P450 fatty acid hydroxylases in animals, one role could be the rapid catabolism of these free fatty acids. Recent data (Tijet et al., 1998) show that CYP94A1 is strongly induced after a few minutes, and up to 400 times after a few hours, in plant tissues exposed to clofibrate, a drug which provokes peroxisomal proliferation in animals and in plants (Palma et al., 1991).

Peroxisomal proliferation is strongly linked to oxidative burst.

The work of Schweizer et al. (1996ab) shows that omega-hydroxy C16:0 and C18:1 fatty acids are resistance elicitors. By analogy with the arachidonate cascade, other omega-hydroxylated fatty acids might be involved in stress signaling. In addition, increased omega and omega-1 hydroxylation may increase the production of pheromone-like molecules that could enhance insect attraction for pollination. This is based on the fact that many insect pheromones are (or are derived from) omega and (omega-1)-hydroxy fatty acids or alkanes (see Engels et al., 1997).

Furthermore, P450s involved in epoxidation of unsaturated fatty acids may also be involved in resistance to disease via the production of hydroxylated and epoxidated fatty acids which have been shown to inhibit the growth of pathogens (i.e., synthesis of signaling molecules). If the role of oxygenated fatty acids in fungal infections is considered, contradictory effects seem apparent, because reports indicate that certain monomers from cutin (i.e., dihydroxy fatty acids and 9,10,18-trihydoxystearic acid) are potent inducers of the cutinase of several pathogenic fungi. Schweizer et al. (1996ab) have shown that cutin monomers, and specifically the omega-hydroxy forms, induce resistance in barley against *Erysiphe graminis*. The highest effect is found for 9,10,18-trihydroxystearic acid. Pinot et al. (1993) showed that this compound is formed by the action of a 9,10 epoxygenase (not a P450), followed by hydroxylation at position 18 by P450 (CYP94A1, CYP94A4, CYP94A5 and CYP86A1 catalyze this reaction), followed by opening of the epoxide by an epoxide hydrolase. Blée et al. (1993) have shown that epoxygenase and epoxidase are extremely active, so that the limiting factor is the P450-catalyzed omega-hydroxylation. Overexpression of this gene, possibly under control of a pathogen-reactive promoter, may result in enhanced resistance.

The oil produced by altering the amount of plant fatty acid hydroxylase activity may exhibit different characteristics from oil produced by the wildtype plant that would be useful for the manufacture of lubricants, anti-slip agents, plasticizers, coating agents, detergents, and surfactants. For example, hydroxylated fatty acids of 10 to 14 carbon length (derived from capric, lauric, or myristic acid) may provide the basis for new detergents and plasticizers. Plastics that can be produced from hydroxylated fatty acids are polyurethanes and polyesters (Weber at al., 1994). It should be noted that cutin itself is a bioplastic constituted almost entirely of oxyfatty acids.

Omega hydroxylation is required for the chain-elongation reaction, and in-chain hydroxylation and/or epoxidation is required for reticulation. Plants can be engineered to produce C12 fatty acids by transforming them with the acyl-ACP thioesterase from *Umbellularia californica* which is specific for lauroyl-ACP. *Arabidopsis thaliana* transformed with this gene produce up to 25% laurate. If these plants would be further transformed with CYP94A1, they would produce high amounts of omega-hydroxylauric acid. Similarly, plants engineered to produce C14 FA and subsequently transformed with CYP94A2 would produce high amounts of omega-hydroxymyristic acid. To date, the properties and industrial uses of hydroxylated fatty acids have not been elucidated. Lack of commercial use of such fatty acids is due to the fact that these hydroxylated fatty acids do not accumulate in plants under normal conditions. The use of the plant fatty acid hydroxylases of the present invention will allow the mass production of such compounds. Chemical synthesis of omega-hydroxylated fatty acids is difficult and expensive, meaning the production of omega-hydroxylated fatty acids in plants would be of great economic significance.

Several ω- and in-chain fatty acid hydroxylases have been

characterized in higher plants. In microsomes from *Helianthus tuberosus* tuber the ω-2, ω-3 and ω-4 hydroxylation of lauric acid is catalyzed by one or a few closely related aminopyrine- and $MnCl_2$-inducible cytochrome P450(s). To isolate the cDNA and determine the sequences of the(se) enzyme(s), antibodies directed against a P450-enriched fraction purified from Mn^{++}-induced tissues were used. Screening of a cDNA expression library from aminopyrine-treated tubers led to the identification of a cDNA (*CYP81B1*) corresponding to a transcript induced by aminopyrine. CYP81B1 was expressed in yeast, and a systematic exploration of its function revealed that this enzyme specifically catalyzes the hydroxylation of medium chain saturated fatty acids, namely capric (C10:0), lauric (C12:0) and myristic (C14:0) acids. The same metabolites were obtained with transgenic yeast and plant microsomes: a mixture of ω-1 to ω-5 monohydroxylated products was observed. The three fatty acids were metabolized with high and similar efficiencies, the major position of attack depending on chain length. When lauric acid was the substrate, turnover was 30.7 ± 1.4 min^{-1} and $K_{m,app}$ 788 ± 400 nM. No metabolism of long chain fatty acids, aromatic molecules or herbicides was detected. This new fatty acid hydroxylase is typical from higher plants and differs from those already isolated from other living organisms.

Table 1: Summary of reactions with fatty acids catalyzed by a plant cytochrome P450.

Plant species	Substrate	Product(s) generated or carbon position oxidized
Vicia faba	palmitic (C16:0)	ω-OH
	16-hydroxy C16:0	8, 9 or 10-OH
Phaseolus aureus	lauric (C12:0)	ω-OH
Phaseolus vulgaris	lauric (C12:0)	ω-OH
Vicia sativa	C10:0-C14:0	ω-OH
	Cl2:1 Δ7-10	ω-OH
	Cl2:1 Δ11	11,12-epoxy
	C12:1 triple bonds 8-10	ω-OH
	Cl2:1 triple bond 11	1,12-dicarboxylic + inactivation
	Cl8:1 Δ9, C18:2 Δ9,12	ω-OH
	9,10-epoxy C18:0	ω-OH
	9,10-diOH C18:0	ω-OH
Pisum sativum	C10:0-C14:0	ω-OH
	12-oxo-C12:1 Δ9	12-OH-Cl2:1 Δ9
	C18:2 Δ9,12	9,10-epoxy-C18:1 Δ12
Glycine max	C10:0-C14:0	ω-OH
Triticum aestivum	C10:0-C14:0	(ω-3), (ω-2), (ω-1)-OH (mainly)
	Cl2:1 Δ9 or 10	9,10- or 10,11-epoxy
	Cl2:1 Δ11	11,12-epoxy + inactivation
	Cl8:1 Δ9	(ω-2), (ω-1) (mainly), ω-OH
	Diclofop	ring hydroxylation

TABLE 1 (continued)

Helianthus tuberosus	C10:0-C14:0	(ω-2), (ω-3) (mainly), (ω-4)-OH
	C12:1 Δ8 or 9	8,9- or 9,10-epoxy-C12:0
	C12:1 Δ7 or 10	Allylic hydroxy (9-OH)
Helianthus annuus	C 12:0	(ω-2), (ω-3) (mainly) or (ω-4)-OH
Zea mays	"	"
Tulipa fosteriana	"	"
Amaryllis belladonna	"	"
Spinacia oleracea	18-OH-C 18:1 -CoA	9,10-epoxy-18-OH-C18:0-CoA
Euphorbia lagascae	linoleyl-PC	12,13-epoxy-C18:1 Δ9
Parthenium argentantum	13-OOH-C18:2 Δ9,11	α and γ-ketol fatty acids
Linus usitanum	13-OOH-C18:2 Δ9, 11	α and γ-ketol, cyclopentenyl product

Since 1974, numerous reports have accumulated showing that plant P450 is induced by a great number of physical, physiological and chemical factors. However, in most cases, nothing is known about the identity of the induced isoforms and the actual mechanisms of induction. The results presented hereinbelow show the regulation of three distinct cloned P450 species, CYP73A1, CYP76B1 and CYP94A1, which have been functionally expressed and characterized.

CYP73A1 is the cinnamate 4-hydroxylase which catalyzes the first committed oxidation reaction in the general phenylpropanoid pathway, leading to lignins, flavonoids, defense molecules, anti-UV protectants etc. This enzyme was purified to homogeneity using Triton X114 phase partitioning, and cloned using a specific antibody raised against the pure protein (Teutsch *et al.*, 1993). Data suggest that the induction of cinnamate

4-hydroxylase activity primarily results from gene activation. Time-course experiments were performed after wounding and aminopyrine treatment. The timing of the induced changes in activity, protein and transcripts confirms that C4H induction results primarily from an increase in *CYP73A1* mRNA both in wounded and aminopyrine treated tissues. However, post-transcriptional mechanisms might also contribute to the regulation of C4H activity.

CYP76B1 is an alkoxycoumarin O-dealkylase (Batard *et al.*, 1995), whose true physiological function remains unknown. The protein was purified by the same method employed for CYP73A1, and the gene was cloned using cytochrome P450 primers deduced from the microsequenced peptide (Batard *et al.*, 1998). Determination of the steady-state level of *CYP76B1* transcripts after slicing tuber tissues and aging them in water, alone or in the presence of various chemicals, showed that the expression of this P450 was not responsive to mechanical stress, but was strongly induced by chemical treatments. Therefore, CYP76B1 appears to be a good potential marker of chemical stress and of environmental pollution.

The data illustrate that the existence of a 'plant P450 induction mechanism' is highly unlikely. The more than 60 P450 physiological activities which have been identified, and the several hundreds more expected to be characterized in the near future, are dispersed in the numerous pathways of plant secondary metabolites. As such, induction of plant P450 enzymes will probably be coordinate with that of other enzymes in the pathway. This is particularly true for all the enzymes engaged in the synthesis of defense compounds, which will be triggered by mechanical wounding, infection, and stress situations. The situation with the chemical inducers is more intriguing and very speculative. One can see the treatment of a plant by chemicals as 'painful', constituting a type of stress, that will

elicit a signal over one of the different stress signaling chains. On the other hand, some chemicals which induce P450 in animals also induce plant P450 with the same substrate and even regio-selectivity. In animals, plants and also *B. megaterium,* the fatty acid in-chain hydroxylases are selectively induced by phenobarbital, while in animals and plants the ω-hydroxylases are induced by clofibrate. The data suggest that in some instances, regulation mechanisms have been conserved along with the catalytic function during evolution. Finally, it should be stressed that some deviant (from a biochemical, phylogenetic, and probably structural point of view) forms of P450 like the allene oxide synthase (Song *et al.*, 1993) or the benzoate 2-hydroxylase (Leon *et al.*, 1995), catalyze key steps in the synthesis of jasmonate and salicylate, respectively.

Clofibrate stimulates efficiently (20-fold) the cytochrome P450-catalyzed activities of lauric and oleic acid omega-hydroxylation in microsomes of *Vicia sativa* seedlings. DEHP and 2,4-D have a similar stimulating effect on the lauric acid omega-hydroxylase in the same material. Recently, on the basis of an internal peptide sequence, the inventors isolated cDNAs coding for plant fatty acid omega-hydroxylases. After expression in yeast, the omega hydroxylase substrate specificities were characterized: CYP94A1 omega-hydroxylates fatty acids with different chain length (C10 to C18) and different degrees of unsaturation (C18:1, C18:2, C18:3). Northern blot analysis of RNAs from clofibrate-treated *Vicia sativa* seedlings revealed a very rapid (after 20 min) and large accumulation of the CYP94A1 transcripts, suggesting the involvement of a clofibrate receptor in the signal transduction. To assess the mechanism of regulation of CYP94A1 by clofibrate, and the possible involvement of PPARs in this regulation, a promoter sequence of CYP94A1 was isolated. A search for key regulator elements is currently in progress. In addition, a study of peroxisome

proliferation in *Vicia sativa* in response to clofibrate, at the level of Acyl CoA oxidase transcripts, has been initiated.

Furthermore, the inventors recently noted that treatment of etiolated *Vicia sativa* seedlings with the plant hormone methyl jasmonate (MetJA) led to an increase in cytochrome P450 content, suggesting this plant defense molecule may act via a CYP94A1 pathway (Pinot *et al.*, 1998). Treatment of the seedlings 48 hours in a 1mM solution of MetJA stimulated ω-hydroxylation of lauric acid 14 fold compared to control samples (153 pmol/min/mg protein versus 11 pmol/min/mg protein). Induction was dose-dependent. The increased activity (2.7 fold) was already detectable after three hours of treatment. Activity increased as a function of time and reached a steady level after 24 hours. Northern blot analysis revealed that the transcripts coding for the a fatty acid ω-hydroxylase CYP94A1 accumulated after one hour of exposure to MetJA, with maximal levels accruing between three and six hours. Under the same conditions, a study of the enzymatic hydrolysis of 9,10-epoxystearic acid showed that both microsomal and soluble epoxide hydrolase activities were not affected by MetJA treatment. Thus, regulation of microsomal ω-hydroxylation of fatty acids by methyl jasmonate may be a major event in the general mechanism of plant defense.

During the past years, the inventors have cloned and characterized at least three fatty acid ω-hydroxylases in *Vicia sativa*. These P450 enzymes are able to introduce an alcohol function on the terminal carbon of fatty acids with different chain lengths and desaturations. These novel enzymes are the first members of the CYP94 family.

Mammalian ω-hydroxylating enzymes have been extensively studied (Simpson 1997), and they have been classified in the CYP4A family. CYP4A are known to be involved in the metabolism of arachidonic acid

leading to the formation of physiologically important metabolites. They also participate in the catabolism of fatty acids (Gibson 1989).

In plants, fatty acid ω-hydroxylases are implicated in the biosynthesis of cuticle (Kolattukudy 1981). Moreover, ω-hydroxy fatty acids have been recently reported to play an important role in plant defense mechanisms (Schweizer 1996), and could also be involved in suberisation in elicitor-treated french bean cells (Bolwell 1997).

To characterize the physiological role of fatty acid ω-hydroxylases, the inventors developed a strategy based on the use of transgenic tobacco lines. To date, three new clones belonging to the CYP94 family (CYP94A4, CYP94A5 and CYP94A6) from a tobacco cDNA library have been isolated. To modulate *in vivo* the activity of these P450s, the two coding sequences were incorporated in sense and antisense orientations in a T-DNA vector and transformed tobacco leaf disks. Expression of a morphological phenotype in those transgenic lines is expected, but the quality and quantity of cuticle and the resistance to pathogens may be affected as well.

Arylphenoxy compounds such as the hypolipidemic drug clofibrate and the herbicide 2,4-D are members of a class of chemicals known to induce fatty acid hydroxylase activities and proliferation of peroxisomes. In plants, long-chain fatty acid omega-hydroxylases are believed to play a crucial role in synthesis of cuticles protecting plants from the outer environment. CYP94A1 which is the first P450-dependent fatty acid omega-hydroxylase cloned from a plant was isolated by tagging of the P450 apoprotein with a radiolabeled mechanism-based inhibitor. The functional expression of this novel P450 in *S. cerevisiae* shows that the methyl end of saturated (from C10 to C16) and unsaturated (C18:1, C18:2 and C18:3) fatty acids was mainly oxidized by CYP94A1. Similar to animal

omega-hydroxylases, this plant enzyme was strongly induced by clofibrate treatment. Rapid accumulation of CYP94A1 transcripts was detected less than 20 min after exposure of *Vicia sativa* seedlings to clofibrate. The rapid induction of CYP94A1 ensures that fatty acids (FAs) are effectively transformed into cutin monomers needed for repair and defense. The possible role of hydroxylated FAs as natural elicitors of plant defense mechanisms opens unexpected perspectives for investigating new regulatory routes of naturally occurring plant defense compounds under chemical or pathogenic stress.

All books, articles and patents cited in this specification are incorporated herein by reference in their entirety.

The following examples are meant to be illustrative of the present invention; however, the practice of the invention is not limited or restricted in any way by them.

EXAMPLES

Example I - Clone A (CYP94A1)

Clone A encodes the saturated and unsaturated fatty acid (FA) omega-hydroxylase, a microsomal cytochrome P450-dependent hydroxylase which catalyzes the transformation of capric (C10:0), lauric (C12:0), myristic (C14:0) palmitic (C16:0), oleic (C18:1), linoleic (C18:2 and enantiomers (9*E*,12*Z*); (9*Z*,12*E*)) and linolenic (C18:3) acids into their corresponding omega-hydroxy acids. In addition, two sulfur-containing lauric acid analogs, 10-methylsulfinyl-decanoic acid (10S-LAU) and 8-propylsulfinyl-octanoic acid (8S-LAU), are actively converted to their corresponding 10- and 8-sulfoxides respectively (Figure 3).

As previously demonstrated by incubating microsomes from *V. sativa*, a series of unsaturated lauric acid analogs containing a double or

a triple bond at carbon positions 8, 9 and 10 should be omega-hydroxylated. In addition, the terminal ethylenic lauric acid analog, 11-dodecenoic acid, should be converted to 11-epoxylauric acid by CYP94A1 (Clone A) (Weissbart et al., 1992; Pinot et al., 1992, 1993; Helvig et al., 1997).

Isolation of CYP94A1 (Clone A)

Specific peptide sequences of lauric acid omega-hydroxylase from clofibrate-treated *V. sativa* microsomes (Salaün et al., 1986) were obtained by employing a newly developed method based on the alkylation of the P450 apoprotein by radiolabeled (1-^{14}C)11-dodecynoic acid (11-DDYA) (Salaün et al., 1988; Helvig et al., 1997). A chemically labeled protein (about 53kDa) was isolated by successive SDS-PAGE analysis and subjected to «in-gel» V8 proteolysis. Resulting peptides were transferred to a nylon membrane (IMMOBILON™) and sequenced by the Edman degradation method. Four peptides were sequenced. Only two showed homology to P450s and were subsequently found in the deduced amino acid sequence of clone A (SEQ ID NO:4).

The first peptide contained the 18-20 amino acid hydrophobic domain which is typical of the membrane anchor found in all microsomal P450s. After isolation of the clone, it was confirmed that this peptide corresponds to the N-terminal amino acid sequence of the enzyme:
MFQFLLEVLLPYLLPLLLYILPF peptide microsequence
MFQFHLEVLLPYLLPLLLLILPT peptide deduced from the sequence of clone A (Residues 1-23 of SEQ ID NO:4).

The second peptide had the sequence LMNLYPPVPMMNAKEVVVXVLLXQ. A computer search with this peptide against all known cytochrome P450 enzymes showed partial homology of the beginning with a domain which is found at about 130 residues from the C-terminus in several P450s of family CYP4, the family

containing the mammalian fatty acid omega-hydroxylases: peptide p3, rat CYP4A1, rat CYP4B1 and rat CYP4A3. After cloning and sequencing, only eight out of the ten first amino acids, shown here in bold type, are found in the corresponding domain of CYP94A1 (SEQ ID NO:4, residues 370-394, SM**RLYPPVPM**DSKEAVNDDVLPDGW), which implies that peptide was contaminated with another protein. Examination of sequences from 428 cytochrome P450s indicated that two consecutive methionines are never found. The following PCR primer (peptide and nucleic acid sequences contained in SEQ ID NOS: 4 and 3, respectively) was deduced:

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      Tyr Pro Pro Val Pro Met
5' - TAY CCI CCI GTI CCI ATG - 3'
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This primer was used in association with an oligo (dT) primer to produce a probe of 661 bp by RT-PCR on total RNA from clofibrate-treated *V. sativa* seedlings. Conditions were the following: denaturation for 5 min at 93°C; followed by 30 cycles of denaturation for 1 min at 93°C, 2 min hybridization at 48°C, and 3 min elongation at 72°C; and terminated by 10 min elongation at 72°C.

A λZAP cDNA library, prepared from poly(A) RNA from 48 hour clofibrate-treated *V. sativa* seedlings, following the manufacturer's instructions (Stratagene), was screened at high stringency using the 650 bp probe. The probe was random labeled using (α-^{32}dCTP) and hybridized for 24 hours at 65°C in 5 x SSC, 0.5% SDS, 5 x Denhardt's solution, 100 μg/ml salmon sperm DNA, 2 mM EDTA, and 50 mM sodium phosphate, pH 6.0. After hybridization, the blot was washed twice with 2 x SSC, 0.1% SDS at room temperature for 15 min, and twice with 0.2 x SSC, 0.1% SDS at 55°C for 30 min. One clone (1862 bp) VAGH111 was isolated, sequenced, and found to encode a new cytochrome P450, CYP94A1 (Figure 1).

Heterologous Expression in Yeast

Complete sequencing of the genome of *Saccharomyces cerevisiae* has shown that this yeast has only four P450s, none of which is known to catalyze fatty acid hydroxylation. Furthermore, this yeast can be grown in conditions such that expression of endogenous P450s is minimal (i.e., P450 is spectrophotometrically undectable). Catalytic activity of CYP94A1 (Clone A) was assessed by functional expression in genetically engineered yeast. The system developed by Urban et al. (1990) was used for expression of heterologous P450 enzymes in *Saccharomyces cerevisiae*. All methods (e.g., subcloning of P450 cDNA, the pYeDP60 shuttle vector, transformation of yeast, and growth conditions allowing the expression of the cloned P450 gene) are described in Pompon et al. (1996). The coding sequence of Clone A (SEQ ID NO:3) was PCR cloned into expression vector pYeDP60 using the *Bam*HI and *Eco*RI restriction sites as follows.

Sense primer:

```
                    Met Phe Gln Phe His Leu Glu
5' - CGGC GGATCC ATG TTT CAA TTT CAT CTT GGA G - 3'
         BamHI
```

Antisense primer:

```
                         Ser Asp Arg Lys Gln Ile
5' - CGGC GAATTC TCA AGA ATC CCT CTT CTG AAT CG - 3'
               EcoRI  stop
```

Stratagene Pfu polymerase was used according to manufacturer's instructions and the amplified sequence was verified to avoid polymerase-generated errors. Yeast strain WAT11 (Urban et al., 1990) was transformed with the expression vector according to Schiestl and Gietz (1989).

Characterization of CYP94A1 (Clone A)

Preparation of Microsomes: Yeast strain WAT11 transformed with pYeDP60 harboring CYP94A1 (Clone A) was grown and induced according to Pompon et al. (1996). A culture was started from one isolated colony. After growth, cells were centrifuged 10 min at 7500g at 4°C. The pellet was washed with TEK (2 ml TEK/g cells), and centrifuged 10 min at 7500g at 4°C. The pellet was resuspended in 1ml TES and glass beads were added up to liquid surface level. Cells were broken by manual shaking for 5 min in the cold room using a 30 ml conical Falcon plastic tube with 0.5 mm diameter glass beads. Greater than 90% of cells were lysed. The homogenate and two 5ml TES washes of the beads were centrifuged 10 min at 7500g at 4°C; the supernatant was centrifuged 45 min at 100,000g at 4°C. The resultant pellet was resuspended in 2ml TEG with a loose Potter homogenizer to obtain a fraction designed as microsomes hereinafter. Microsomes can be stored at -20°C for several weeks without loss of activity. WAT11 cells transformed with pYeDP60 expression vector only were subjected to the same procedure for control experiments.

TEK: Tris-HCl 50mM pH 7.5; EDTA 1mM; KCl 100mM

TES: Tris-HCl 50mM pH 7.5; EDTA 1mM; sorbitol 600mM

TEG: Tris-HCl 50mM pH 7.5; EDTA 1mM; glycerol 20%

Measurement of P450: Microsomes were diluted 5-fold with TEG and P450 was measured (Figure 2) with the method of Omura and Sato (1964) using a mmolar absorbance coefficient of 91 $cm^{-1}mM^{-1}$.

Measurement of Activity: Enzymatic activities were measured as previously described (Weissbart et al., 1992; Pinot et al., 1992, 1993; Boucher et al., 1996) by following the rates of metabolite formation during incubation of transformed yeast microsomes with radiolabeled substrates. The standard assay contained in a final volume of 0.2 ml, 0.19-0.43 mg of

microsomal protein, 20 mM phosphate buffer (pH 7.4), and 100 μM radiolabeled substrate. Omega-hydroxylase activities were measured in the presence of 0.6 mM NADPH plus a regenerating system and 375 μM β-mercaptoethanol. Ethanol solutions containing radiolabeled substrate were evaporated under a stream of argon before addition of other fractions required for incubation. The reaction was initiated by adding NADPH at 27°C and stopped after 10 min incubation with 0.2 ml acetonitrile-acetic acid (99.8/0.2, v/v). After extraction with 2 x 600 μl diethyl ether, the organic phase was spotted on silica thin-layer plates and developed in a mixture of diethyl ether-light petroleum (b.p. 40-60°C)-formic acid (70/30/1, v/v/v for C10 to C16 substrates, and 50/50/1 for C18 substrates). Plates were scanned with a Berthold thin-layer scanner. For precise rate measurements, radioactive spots were scraped into counting vials and product formation was quantified by liquid scintillation. All the reactions products identified in these experiments have been identified by GC/MS spectroscopy. The activity of CYP94A1 (Clone A) with different fatty acid substrates are shown in Table 2.

Table 2: The activities were measured as described using purified radiolabeled substrates.

Substrates (100μM)	Vmax (mol/min/mol P450)	Km (μM)	Product formed (mol/min/mol P450)
Capric acid (C10:0)	4.9 ± 0.1	101 ± 7.0	-
Lauric acid (C12:0)	19.95 ± 1.2	14.7 ± 3.0	-
Myristic acid (C14:0)	24.6 ± 0.13	45.0 ± 5.5	-
Palmitic acid (C16:0)	7.16 ± 0.3	7.2 ± 0.8	-
Stearic acid (C18:0)	0	0	-
Oleic acid (C18:1)	14.3 ± 0.5	38.7 ± 4.3	-
Linoleic acid (C18:2)	9.1 ± 0.9	47.0 ± 1.9	-
Linolenic acid (C18:3)	24.6 ± 2.3	70.0 ± 3.5	-
9,10-epoxystearic acid (9R,10S)	18.5	1.3	
9.10-dihydroxystearic acid	8.1	25	
*C18:2 (9*E*,12*Z*)	-	-	52
*C18:2 (9*Z*,12*E*)	-	-	43
Linoleic (9*E*,12*E*)	-	-	25
*8-propylsulfinyloctanoic acid	-	-	40
*10-methylsulfinyldecanoic acid	-	-	133

*Values are means of triplicate measurements

The TLC radiochromatograms are shown in Figure 4 (capric, lauric, myristic, and palmitic acids), Figure 5 (oleic, linoleic, and linolenic acids), and Figure 6 (C18:2-9*E*,12*Z*; C18:2-9*Z*,12*E*; 8S-LAU and 10S-LAU

acids). For each substrate, two chromatograms are shown: with NADPH (A, hydroxylase is active) and without NADPH (B, hydroxylase is inactive).

Example II - Clone B (CYP94A2)

Clone B encodes ω-MAH (omega-myristic acid hydroxylase), a microsomal cytochrome P450-dependent hydroxylase which catalyzes the transformation of myristic (C14:0) acid into 14-hydroxytetradecanoic acid (i.e., the terminal methyl was hydroxylated). Low levels of transformation of lauric (C12:0) and palmitic (C16:0) acids into the corresponding omega-hydroxy fatty acids were also observed.

Isolation of VAGH811

The clone was obtained by screening a λZAP cDNA library prepared from clofibrate-treated *Vicia sativa* seedlings with Clone A, as follows. A λZAP cDNA library, prepared from poly(A) RNAs from 48 hour clofibrate-treated *V. sativa* seedlings following the manufacturer's instructions (Stratagene), was screened at low stringency using a 661 bp DNA fragment (starting at position 1201 in Figure 1) produced by RT-PCR during the isolation of Clone A. The fragment was ^{32}P-radiolabeled by random priming. Hybridization was at 55°C overnight in 5 x SSC, 0.5% SDS, 5 x Denhardt's solution, 100 μg/ml salmon sperm DNA, 2 mM EDTA, and 100 mM sodium phosphate, pH 6.0. After hybridization, blots were washed twice with 2 x SSC, 0.1% SDS at room temperature for 15 min, and twice with 0.2 x SSC, 0.1% SDS at 45°C for 30 min. One clone (1437 bp) that was isolated, VAGH811, was sequenced and found to encode a new cytochrome P450 (Figure 7). The clone was incomplete at the 5' end, but the sequence of the incomplete clone was used to synthesize a primer for 5'-RACE with poly(A) RNA from 96 hour clofibrate-treated

Vicia sativa seedlings to obtain the complete coding sequence of VAG811, named Clone B hereinafter (Figure 7).

Heterologous Expression in Yeast

Catalytic activity of CYP94A2 (Clone B) was assessed by functional expression in genetically engineered yeast, as described hereinabove for clone A. The coding sequence of CYP94A2 (Clone B, SEQ ID NO:5) was PCR cloned into expression vector pYeDP60 using the *Sma*I and *Sac*I restriction sites as follows (peptide sequences contained in SEQ ID NO:6).

Sense primer:

```
                          Met Glu Leu Glu Thr Leu
5' - GGAT CCCGGG GA ATG GAA CTC GAA ACA TTG - 3'
          SmaI
```

Antisense primer:

```
5' - AAG AGA AGC CCA CTT GTA TGA - 3'
     Lys Arg Ser Pro Leu Val stop
     3' - CT TCG GGT GAA CAT ACT CTCGAG CTCGCCTA -
5'
                                      SacI
```

Boehringer HiFi™ polymerase was used according to manufacturer's instructions and the amplified sequence was verified to avoid polymerase-generated errors. Yeast strain WAT11 (Urban et al., 1990) was transformed according to Schiestl and Gietz (1989).

Characterization of CYP94A2 (Clone B)

Preparation of microsomes: Yeast strain WAT11 transformed with the pYeDP60 expression vector harboring CYP94A2 (Clone B) was grown and induced according to the method described for Clone A.

Measurement of P450: Microsomes were diluted 5-fold with

TEG and P450 was measured (Figure 8) with the method of Omura and Sato (1964) using an absorbance coefficient of 91 $cm^{-1} mM^{-1}$.

Measurement of Activity: Enzymatic activities were measured as previously described for clone B. All the reaction products identified in these experiments have been identified in experiments with plant microsomes, by rechromatography with authentic compounds and by GC/MS spectroscopy. The activities of ω-MAH with different fatty acid substrates are shown in Table 3.

Table 3: The activities were measured as described using purified radiolabeled substrates.

Substrates (100 μM)	mole hydroxylated product/min/mole to ω-MAH
Capric acid (C10:0)	0
Lauric acid (C12:0)	3.8
Myristic acid (C14:0)	30.4
Palmitic acid (C16:0)	4.0
Stearic acid (C18:0)	0
Oleic acid (C18:1)	0

The actual TLC radiochromatograms are shown in Figure 9 (capric acid), Figure 10 (lauric acid), Figure 11 (myristic acid), Figure 12 (palmitic acid), Figure 13 (stearic acid), and Figure 14 (oleic acid). For each substrate, two chromatograms are shown: with NADPH (A, hydroxylase is active) and without NADPH (B, hydroxylase is inactive).

Kinetic Parameters and Specific Activity

In separate experiments, the apparent Km and Vmax of CYP94A2 (Clone B) for myristic acid were determined to be 3.8 μM and 80 moles 14-hydroxymyristic acid/min/mole ω-MAH.

Example III - Clone C (CYP94A3)

CYP94A3 encodes a cytochrome P450-dependent hydroxylase which catalyzes the methyl terminal oxidation of capric (C10:0), lauric (C12:0), and myristic (C14:0) acids and to a lesser extent the omega-hydroxylation of palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids.

Isolation of CYP94A3 (Clone C)

Clone C was obtained by screening a λZap cDNA library prepared from clofibrate-treated *Vicia sativa* seedlings with a 3' terminal cDNA fragment (300 bp) of CYP94A2 (Clone B) (from the sequence coding for the heme binding domain to the polyA tail) at high stringency. Hybridization was for 24 hours at 65°C in 5 x SSC, 0.5% SDS, 5 x Denhardt's solution, 100 μg/ml salmon sperm DNA, 2 mM EDTA, and 50 mM sodium phosphate, pH 6.0. After hybridization, the blot was washed twice with 2 x SSC, 0.1% SDS at room temperature for 15 min, and twice with 0.2 x SSC, 0.1%SDS at 55°C for 30 min. The fragment was ^{32}P-radiolabeled by random priming. A clone (1600 bp) was sequenced and found to encode a new cytochrome P450 (Figure 15). By sequence comparison with CYP94A2, CYP94A3 was missing nine nucleotides. For heterologous expression, the first nine nucleotides of CYP94A2 were added in front of the incomplete sequence of CYP94A3 (Clone C). The full-length cDNA was isolated since and found to be identical to the one used in these activity experiments.

Heterologous Expression in Yeast

Catalytic activity of CYP94A3 (Clone C) was assessed by functional expression in genetically engineered yeast as described hereinabove for the preceding clones. The coding sequence of CYP94A3 (Clone C, SEQ ID NO:7) was PCR cloned into expression vector pYeDP60 using the *Sma*I and *Sac*I restriction sites as follows (peptide sequences contained in SEQ ID NO:8).

Sense primer:

```
                    94A2          94A3
                     Met Glu Leu Glu Arg Leu Val Ala Trp
5' - TCC CCCGGG GT ATG GAA CTC GAA ACA TTG GTT GCA TGG - 3'
       SmaI
```

Antisense primer:

```
                              Glu Asp Thr His Ser
5' - ATC CGCTC GAGCTC TTA CTC ATC TGT GTG ACT - 3'
                   SacI  stop
```

Boehringer HiFi™polymerase was used according to manufacturer's instructions and the amplified sequence was verified for polymerase-generated errors. Yeast strain WAT11 (Urban et al., 1990) was transformed according to Schiestl and Gietz (1989).

Characterization of CYP94A3 (Clone C)

Preparation of Microsomes: Yeast strain WAT11 transformed with pYeDP60 harboring CYP94A3 (Clone C) was grown and induced as described for the preceding clones. Microsomes can be stored at -20°C for several weeks without loss of activity. WAT11 cells transformed with pYeDP60 expression vector only were subjected to the same procedure for control experiments.

Measurement of P450: Microsomes were diluted 5-fold with TEG and P450 was measured (Figure 2) with the method of Omura and Sato (1964) using a mmolar absorbance coefficient of 91 $cm^{-1}mM^{-1}$.

Measurement of Activity: Enzymatic activities were measured as previously described as described hereinabove for the preceding clones. The activity of CYP94A3 (Clone C) with different fatty acid substrates are shown in Table 4.

Table 4: The activities were measured as described using purified radiolabeled substrates.

Substrates (100 μM)	Mole ω-hydroxy-FA/min/mole P450
Capric acid (C10:0)	16.7
Lauric acid (C12:0)	10.8
Myristic acid (C14:0)	4.0
Palmitic acid (C16:0)	0.7
Oleic acid (C18:1)	0.7
Linoleic acid (C18:2)	1.6

Values are means of triplicate measurements

The TLC radiochromatograms are shown in Figures 17 (capric and lauric acids), 18 (myristic and palmitic acids), and 19 (oleic and linoleic acids). For each substrate, two chromatograms are shown: with NADPH (A, hydroxylase is active) and without NADPH (B, hydroxylase is inactive).

Example IV - Clone D (CYP81B1)

In-chain Hydroxylase (IC-LAH) of capric, lauric, and myristic acids

Clone D encodes a microsomal P450 from *Helianthus tuberosus* (Jerusalem artichoke), catalyzing the ω-2, ω-3 and ω-4 hydroxylation of capric (C10:0), lauric (C12:0), and myristic (C14:0) acids.

The major metabolite is the ω-3-hydroxylated compound.

Isolation of CYP81B1 (Clone D)

Purification of xenobiotic-inducible 7-ethoxycoumarin *O*-deethylase from *H. tuberosus* led to the isolation of a P450-enriched fraction containing a mixture of several P450 proteins (Batard et al., 1995). Polyclonal antibodies raised against this P450-enriched fraction were used to screen a λZAPII cDNA library prepared from *H. tuberosus* tuber tissues sliced and aged 24 hour in presence of 20 mM of aminopyrine. Positive clones (56) were isolated and tested for the presence of a P450 consensus sequence using the PCR technique previously described by Meijer et al. (1993). PCR fragments of expected size were obtained from 15 clones, labeled and hybridized with total RNA prepared from dormant, wounded or aminopyrine-treated tuber tissues. One of the 15 clones corresponded to a 2.2 kb transcript almost undetectable in dormant and wounded tuber, but induced by aminopyrine. Sequencing of its insert showed that it coded for a P450 missing about 150 nucleotides at the N-terminus; rescreening of the library led to the isolation of a longer cDNA missing only five nucleotides. The missing coding sequence was then obtained by 5'-RACE, using poly(A) RNA from 24 hour aminopyrine-treated tuber tissues. The full-length sequence was reconstituted and is named Clone D hereinafter (Figure 20).

Heterologous Expression in Yeast

Catalytic activity of CYP81B1 (Clone D) was assessed by functional expression in yeast. A genetically engineered yeast strain providing a suitable environment for plant P450 expression (membrane structures and presence of a plant P450 reductase) was used for this purpose. This strain WAT11, the expression vector, subcloning of the coding sequence, yeast growth, transformation, and preparation of yeast microsomes are described in Pompon et al. (1996). The CYP81B1 (Clone D) cDNA, trimmed of its non-coding sequences, was expressed in the pYeDP60 expression vector under the regulatory control of a galactose-inducible promoter (GAL10-CYC1). Subcloning of CYP81B1 (Clone D) for insertion into this vector was performed using Pfu DNA polymerase (Stratagene), and the modified cDNA was checked for PCR-generated errors.

P450 content and catalytic activities were measured in microsomes prepared from transformed and control yeast (control = yeast transformed with an empty plasmid). No P450 or fatty acid metabolism was detected in control microsomes. In microsomal membranes from CYP81B1 (Clone D) transformed yeast grown 16 hour in the presence of galactose, the P450 content measured by the method of Omura and Sato (1964) (Figure 21) was about 202 pmoles/mg protein (i.e., about 1% of the microsomal protein). Catalytic activity was tested with more than 20 potential radiolabeled substrates including aromatic compounds, sterols, herbicides, and fatty acids. Fatty acid metabolism was assayed as described by Salaün et al. (1981).

C10:0, C12:0 and C14:0 fatty acids were the only molecules metabolized by CYP81B1 (Clone D) (Table 5). Metabolism was dependent on the presence of NADPH.

Table 5: Substrate specificity of CYP81B1

Activities were measured with radiolabeled substrates. Metabolites (the sum of the three hydroxylated products) were quantified by radio-TLC. No activity was detected in control yeast (transformed with the empty expression vector), or in the absence of NADPH.

Substrate (100 μM)	Polar metabolites
	$pmoles.min^{-1}.mL^{-1}$
C10:0	51
C12:0	34
C14:0	16
C16:0	not detected
C18:0	not detected
C18:1	not detected
C18:2	not detected
C18:3	not detected

Kinetic Parameters and Specific Activity

Apparent Vmax and Km of the reaction were determined in the case of capric and lauric acids. In microsomes from yeast overexpressing *Arabidopsis* reductase (i.e., the WAT 11 strain), the reaction proceeds with an enzyme turnover of 41 ± 0.8 min^{-1} and Km of 903 ± 168 nM in the case of capric acid, and with an enzyme turnover of 30.7 ± 1.4 min^{-1} and Km of 788 ± 400 nM in the case of lauric acid.

Characterization of the Metabolites

Previous work performed by the inventors (Salaün et al., 1981) has shown that in *H. tuberosus* tuber microsomes, lauric acid is

converted into 8-, 9- and 10-hydroxylated metabolites (25:60:15, respectively). Products obtained with plant microsomes have been characterized by GC-MS. Lauric acid in-chain hydroxylase activitiy was also detected in maize and tulip microsomes, and was induced by aminopyrine, phenobarbital and other xenobiotics (Adelé et al., 1981; Salaün et al., 1982; Salaün et al., 1986; Fonne-Pfister et al., 1988).

TLC profiles in Figure 22 show that CYP81B1 (Clone D) codes for a P450 catalyzing formation of the same metabolites. The ω-2, ω-3, ω-4 hydroxylated metabolites are generated from the three fatty acid substrates (capric, lauric, and myristic acids) in the same proportions as in plant microsomes. An additional minor product is detected after incubation of lauric acid with the yeast-expressed enzyme; the structure of this metabolite is currently being investigated. In the case of lauric and myristic acids, the presence and proportions of the three metabolites were confirmed by HPLC (Figure 23).

Previous work by the inventors also indicates that the same enzyme very likely catalyzes allylic hydroxylation or epoxidation of unsaturated lauric acids (Salaün et al., 1989, 1992, 1993) and sulfoxidation of 9- and 11-thiadodecanoic acids (Bosch, 1992), some unsaturated analogs (in *Z* conformation) being metabolized with high stereoselectivity (Salaün et al., 1992).

Example V Clone E (CYP94A4)

CYP94A4 encodes a cytochrome P450-dependent hydroxylase which catalyzes the methyl terminal oxidation of capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, with very contrasted efficiencies. Highest activity is with C14:0 and C12:0.

Isolation of CYP94A4 (clone E)

Clone E was obtained by screening a lambda-Zap cDNA library prepared from TMV-infected tobacco leaves (Dr M. Legrand; IBMP Strasbourg) with CYP94A1, A2 and A3 as follows:

A λZAP cDNA library, prepared from poly(A^+) RNAs from tobacco (*Nicotiana tabacum* var. Samsun NN) leaves infected for 48h with TMV, was screened at low stringency using a mixture of the coding sequences of CYP94A1, A2 and A3 as probe. The probe was ^{32}P-radiolabeled by random priming. Fifteen clones greater than 1500 pb were isolated and sequenced. Ten of these clones were full-length and were found to encode for a new cytochrome P450 of the CYP94 family, which was named CYP94A4 (Figure 24).

Heterologous expression in yeast.

Catalytic activity of CYP94A4 (Clone E) was assessed by functional expression in a ad hoc engineered yeast, as described hereinabove for the preceding clones. The coding sequence of Clone E (SEQ ID NO:9) was PCR cloned into expression vector pYeDP60 using the BamHI restriction site as follows:

Sense primers (BamHI)

A4 M M I D L E L

5' CG **GGA TCC** ATG ATG ATA GAC TTG GAG CT 3'

Antisense primers (KpnI)

A4 GAA AGG AAC GGT ACG GAT ATT TGA

A4 E R N G T D I stop

3' CC TTG CCA TGC CTA TAA ACT **CCA TGG** GG 5'

Boehringer HIFI™ polymerase was used according to manufacturer's instructions and the amplified sequence was verified for polymerase errors. Yeast strain WAT11 (Urban et al. 1990) was transformed according to Schiestl and Gietz (1989).

Preparation of microsomes

Yeast (strain WAT11) transformed with pYeDP60 harboring clone E was grown and induced as previously described hereinabove. Untransformed WAT11 cells were subjected to the same procedure for control experiments.

P450 measurements

Microsomes were diluted 5-fold with TEG and P450 measured (Figure 2) with the method of Omura and Sato (1964) using a mmolar absorbance coefficient of 91.cm^{-1}.mM^{-1}.

Activity measurements

Enzymatic activities were measured as previously described hereinabove. All the reactions products identified in these experiments had been identified before in experiments with plant microsomes, by rechromatography with authentic compounds and by GC/MS spectroscopy. Complete kinetic studies were conducted with each substrate.

Table 6 : The activities for CYP94A4 were measured as described using radiolabeled substrates.

Substrates		Vmax	Km
Capric acid (C10:0)	3.5	2.6	1.3
Lauric acid (C12:0)	9.8	0.3	30.1
Myristic acid (C14:0)	14.6	2.2	6.6
Palmitic acid (C16:0)	3.1	46.5	0.067
Stearic acid (C18:0)	0	0	-
Oleic acid (C18:1)	1.8	24.8	0.072
Linoleic acid (C18:2)	4.5	47.9	0.094
Linolenic (C18:3)	4.0	95.5	0.042

Example VI Clone F (CYP94A5)

CYP94A5 encodes a cytochrome P450-dependent hydroxylase which catalyzes the methyl terminal oxidation of lauric (C12:0), myristic (C14:0), palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, with very contrasted efficiencies. Highest activity is with C14:0 and C18:2.

Isolation of CYP94A5 (Clone F)

Clone F was obtained by screening a lambda-Zap cDNA library prepared from TMV-infected tobacco leaves (Dr M. Legrand; IBMP Strasbourg) with CYP94A1, A2 and A3 as follows.

A λZAP cDNA library, prepared from poly(A^+) RNAs from tobacco (*Nicotiana tabacum* var. Samsun NN) leaves infected for 48h with TMV, was screened at low stringency using a mixture of the coding sequences for CYP94A1, A2 and A3 as probe. The probe was ^{32}P-radiolabeled by random priming. Fifteen clones greater than 1500 pb were isolated and sequenced. Two full-length clones were found to encode for a new cytochrome P450 of CYP94 family, which was termed CYP94A5 (Figure 25).

Heterologous expression in yeast.

Catalytic activity of CYP94A5 (Clone F) was assessed by functional expression in a ad hoc engineered yeast as described for the preceding clones.

Reformatting of Clone F

The coding sequence of Clone F (SEQ ID NO:11) was PCR cloned into vector pYeDP60 using the BamHI restriction site as follows (peptide sequences contained in SEQ ID NO:12).

Sense primers (BamHI)

A4 M M I D L E L

5′ CG **GGA TCC** ATG ATG ATA GAC TTG GAG CT 3′

Antisense primers (KpnI)

A4 GAA AGG AAC GGT ACG GAT ATT TGA

A4 E R N G T D I stop
3' CC TTG CCA TGC CTA TAA ACT **CCA TGG** GG 5'

Boehringer HIFI™ polymerase was used according to manufacturer's instructions and the amplified sequence was verified for polymerase errors. Yeast strain WAT11 (Urban et al. 1990) was transformed according to Schiestl and Gietz (1989).

Preparation of microsomes

Yeast (strain WAT11) transformed with pYeDP60 harboring clone F was grown and induced as described hereinabove for the preceding clones. Untransformed WAT11 cells were subjected to the same procedure for control experiments.

P450 measurements

Microsomes were diluted 5-fold with TEG and P450 measured (Figure 2) with the method of Omura and Sato (1964) using a mmolar absorbance coefficient of 91.cm^{-1}.mM^{-1}.

Activity measurements

Enzymatic activities were measured as previously described hereinabove. All the reactions products identified in these experiments had been identified before in experiments with plant microsomes, by rechromatography with authentic compounds and by GC/MS spectroscopy. Complete kinetic studies were conducted with each substrate.

Table 7 : The activities for CYP94A5 were measured as described using radiolabeled substrates

Substrates		Vmax	Km
Capric acid (C10:0)	0	0	-
Lauric acid (C12:0)	1.5	89.3	0.02
Myristic acid (C14:0)	5.1	50.7	0.1
Palmitic acid (C16:0)	0.8	53.5	0.015
Stearic acid (C18:0)	0	0	-
Oleic acid (C18:1)	1.7	36.5	0.046
Linoleic acid (C18:2)	4.3	36.5	0.12
Linolenic (C18:3)	2.2	17.1	0.13

Table 8 : The relative efficiency of 94A4 versus 94A5 was compared.

	CYP94A4			CYP94A5			
	Vm	Km	Vm/Km	Vm	Km	Vm/Km	Eff A4 / Eff A5
C10	3.5	2.6	1.3	0	0	-	-
C12	9.8	0.3	30.1	1.5	89.3	0.02	1505
C14	14.6	2.2	6.6	5.1	50.7	0.1	66
C16	3.1	46.5	0.067	0.8	53.5	0.015	4.5
C18:0	0	0	-	0	0	-	-
C18:1	1.8	24.8	0.072	1.7	36.5	0.046	1.5
C18:2	4.5	47.9	0.094	4.3	36.5	0.12	0.78
C18:3	4.0	95.5	0.042	2.2	17.1	0.13	0.32

Example VII Clone G (CYP94A6)

CYP94A6 encodes a cytochrome P450. The catalytic activity is presently being assessed. It is expected that it will show fatty acid hydroxylase activity since it displays the characteristic signature sequence for this class of enzymes.

Isolation of CYP94A6 (Clone G)

Clone G was obtained by screening a lambda-Zap cDNA library prepared from TMV-infected tobacco leaves (Dr M. Legrand; IBMP Strasbourg) with CYP94A1, A2 and A3 as follows.

A λZAP cDNA library, prepared from poly(A^+) RNAs from tobacco (*Nicotiana tabacum* var. Samsun NN) leaves infected for 48h with TMV, was screened at low stringency using a mixture of the coding sequences for CYP94A1, A2 and A3 as probe. The probe was ^{32}P-radiolabeled by random priming. Fifteen clones greater than 1500 pb were isolated and sequenced. One uncomplete clone was found to code for a new cytochrome P450 of the CYP94 family, which was named CYP94A6. The complete sequence for CYP94A6 was obtained by performing inverse-PCR on genomic tobacco (*Nicotiana tabacum* var. Samsun NN) DNA, using the NdeI restriction site ACATAT at position 594 (Figure 26) and sequence specific primers.

Heterologous expression in yeast.

CYP94A6 has been expressed in yeast as described hereinabove, and the protein produced has been detected by Western blotting. Catalytic activity of Clone G is being assessed by functional expression in a ad hoc engineered yeast.

Reformatting of Clone G

The coding sequence of Clone G (SEQ ID NO:13) was PCR cloned into vector pYeDP60 using the BamHI restriction site as follows (peptide sequences contained in SEQ ID NO:14).

Sense primer (BamHI)

A5 M A L L D L Q

5' CG **GGA TCC** ATG GCA CTA TTA GAC TTA CAA 3'

Anti sense primer (KpnI)

A5 GTT ACT ATT GAA GAA AGG ATA TAG

V T I E E R I stop

3' A TGA TAA CTT CTT TCC TAT ACT **CCA TGG** GG

Boehringer HIFI™ polymerase was used according to manufacturer's instructions and the amplified sequence was verified for polymerase errors. Yeast strain WAT11 (Urban et al. 1990) was transformed according to Schiestl and Gietz (1989).

Preparation of microsomes

Yeast (strain WAT11) transformed with pYeDP60 harboring clone G was grown and induced as described hereinabove. Untransformed WAT11 cells were subjected to the same procedure for control experiments.

P450 measurements

Microsomes were diluted 5-fold with TEG and P450 measured (Figure 2) with the method of Omura and Sato (1964) using a mmolar absorbance coefficient of 91.cm^{-1}.mM^{-1}.

Obtention of transgenic tobacco plants expressing CYP94A4, CYPA5, CYPA6

Tobacco (*Nicotiana tabacum* L. var Xanthi) was transformed with the open REPETITION open reading frames of clones E (CYP94A4), F (CYPA5) and G (CYPA6), in sense and antisense orientation. The coding sequences were cloned into pFB8, a custom built vector from our Institute (Atanassova et al. Plant J. 1995, 8, pp 465-477). The transformation was performed using tobacco leaf disks via Agrobacterium (strain LBA 4404) as described by Horsch (Science 1985, 227 pp 1227-1237).

Reformatting of CYP94A4, CYPA5, CYPA6 coding sequences

The coding sequences were PCR cloned into vector pFB8 using the BamHI and KpnI restriction sites indicated in bold type as follows (peptide and nucleic acid sequences contained in SEQ ID NOS:4 and 3, respectively, for 94A4, and SEQ ID NOS:13 and 12, respectively, for 94A5/94A6)

a) Sense orientation in pFB8:

Sense primers (KpnI)

```
94A4                    M   M   I   D   L   E   L
     5' CG CCA TGG ATG ATG ATA GAC TTG GAG CT 3'

94A5/94A6               M   A   L   L   D   L   Q
     5' CG CCA TGG ATG GCA CTA TTA GAC TTA CAA 3'
```

Antisense primers (BamHI)

```
94A4   GAA AGG AAC GGT ACG GAT ATT TGA
        E   R   N   G   T   D   I  stop
         3' CC TTG CCA TGC CTA TAA ACT GGA TCC GG 5'

94A5/94A6 GTT ACT ATT GAA GAA AGG ATA TAG
           V   T   I   E   E   R   I  stop
          3' A TGA TAA CTT CTT TCC TAT ACT GGA TCC GG
```

b) Antisense orientation in pFB8:

Sense primers (BamHI)

```
94A4                  M   M   I   D   L   E   L
     5' CG GGA TCC ATG ATG ATA GAC TTG GAG CT 3'

94A5/94A6             M   A   L   L   D   L   Q
     5' CG GGA TCC ATG GCA CTA TTA GAC TTA CAA 3'
```

Antisense primers (KpnI)

```
94A4   GAA AGG AAC GGT ACG GAT ATT TGA
94A4    E   R   N   G   T   D   I  stop
         3' CC TTG CCA TGC CTA TAA ACT CCA TGG GG 5'

94A5/94A6 GTT ACT ATT GAA GAA AGG ATA TAG
94A5/94A6  V   T   I   E   E   R   I  stop
          3' A TGA TAA CTT CTT TCC TAT ACT CCA TGG GG
```

Plants transformed in both sense and antisense directions are growing at present with seeds for T1 expected shortly.

Signature for fatty acid omega-hydroxylases

The inventors have identified a peptide sequence (SEQ ID

NO:2), marked by a double underline in Figures 1, 7, 15, 24, 25 and 26, which is a unique signature found in all plant fatty acid omega-hydroxylases characterized so far:

S(AVS)AL(TVS)WFFWL(LIV)

Wherein (AVS) means one of A, V or S; (TVS) means on eof T, V, or S; and (LIV) means one of L, I or V. This signature sequence is present in CYP86A1 (SEQ ID NO:1), CYP86A5, CYP94A1, CYP94A2, CYP94A3, CYP94A4, CYP94A5, and CYP94A6. All but CYP94A6 (characterization under way) have omega-hydroxylase activity. This signature sequence is not present in CYP81B1, the in-chain hydroxylase, as evidenced by sequence alignment.

A scan of all plant genes in Genbank for this signature sequence retrieved 12 sequences, all of which are cytochromes P450 isofoms. Some of the sequences are redundant because they originate from different laboratories recloning the same genes.

A scan of all sequences contained in the databases (Non-redundant GenBank+EMBL+DDBJ+PDB sequences = 364,804 sequences) confirms that this signature is not found in any other gene from any plant, animal or microbial origin. Therefore, any isolated gene presenting this signature is linked to the genes covered by the invention and presents the same type of catalytic activity.

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While the present invention has been described in connection with what is presently considered to be practical and preferred embodiments, it is understood that the present invention is not to be limited or restricted to the disclosed embodiments but, on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

Thus, it is to be understood that variations in the described invention will be obvious to those skilled in the art without departing from the novel aspects of the present invention and such variations are intended to come within the scope of the claims below.

What is claimed is:

1. An isolated nucleic acid encoding a plant fatty acid hydroxylase selected from the group consisting of omega hydroxylase, in-chain hydroxylase, and functional derivatives thereof.

2. An isolated nucleic acid encoding a plant fatty acid hydroxylase, wherein the plant fatty acid hydroxylase is an omega hydroxylase having a peptide sequence of SEQ ID NO:2 or a functional derivative thereof, which hydroxylates a fatty acid substrate at a terminal position.

3. The isolated nucleic acid of Claim 2 wherein the omega hydroxylase is selected from the group consisting of CYP94A1, CYP94A2, CYP94A3, CYP94A4, CYP94A5 and CYP94A6.

4. The isolated nucleic acid of Claim 1 wherein the plant fatty acid hydroxylase is an in-chain hydroxylase (CYP81) or a functional derivative thereof, which hydroxylates a fatty acid substrate at a subterminal position.

5. The isolated nucleic acid of Claim 4 wherein the in-chain hydroxylase is CYP81B1.

6. A recombinant nucleic acid comprising the isolated nucleic acid of any one of Claims 1-5.

7. The recombinant nucleic acid of Claim 6 further comprising a

regulatory region which is suitable for expression of the plant fatty acid hydroxylase in a host cell.

8. A host cell comprising the recombinant nucleic acid of Claim 6.

9. The host cell of Claim 8 wherein the host cell is selected from the group consisting of bacterial cell, fungal cell, and plant cell.

10. A transgenic plant comprising the recombinant nucleic acid of Claim 6.

11. A host cell comprising the recombinant nucleic acid of Claim 7.

12. The host cell of Claim 11 wherein the host cell is selected from the group consisting of bacterial cell, fungal cell, and plant cell.

13. A transgenic plant comprising the recombinant nucleic acid of Claim 7.

14. A plant fatty acid hydroxylase encoded by the isolated nucleic acid of any one of Claims 1-5.

15. A composition consisting essentially of the plant fatty acid hydroxlyase of Claim 14.

16. A polypeptide produced by the recombinant nucleic acid of

Claim 7.

17. A composition consisting essentially of the polypeptide of Claim 16.

18. A process of isolating additional fatty acid hydroxylase genes from a plant by using the isolated nucleic acid of any one of Claims 1-5.

19. The process of Claim 18 wherein the isolated nucleic acid is used as a labeled probe hybridized to a collection of nucleic acids from the plant to select the nucleic acid encoding the additional fatty acid hydroxylase gene.

20. The process of Claim 18 wherein a primer synthesized according to a conserved nucleotide sequence of the isolated nucleic acid amplifies a collection of nucleic acids from the plant to select the nucleic acid encoding the additional fatty acid hydroxylase gene.

21. An isolated nucleic acid selected by the process of Claim 18.

22. A process of altering fatty acid composition in a plant comprising:

introducing the isolated nucleic acid of any one of Claims 1-5 into a plant to produce a transgenic plant;

expressing the plant fatty acid hydroxylase in the transgenic plant; and

hydroxylating or epoxidating a fatty acid substrate in the transgenic plant.

23. The process of Claim 22 wherein the fatty acid substrate is a medium-chain fatty acid.

24. The process of Claim 23 wherein the medium-chain fatty acid is selected from the group consisting of capric fatty acid, lauric fatty acid, and myristic fatty acid.

25. The process of Claim 22 wherein the fatty acid substrate is a long-chain fatty acid.

26. The process of Claim 25 wherein the long-chain fatty acid is selected from the group consisting of palmitic fatty acid, oleic fatty acid, linoleic fatty acid, and linolenic fatty acid.

27. The process of Claim 25, wherein the fatty acid substrate is a member selected from the group consisting of fatty acids with odd carbon numbers, fatty acids with in-chain hydroxy groups, fatty acids with in-chain epoxy groups, thia-fatty acids, ether-fatty acids, modified fattyacids having ester linkages and modified fatty acids having amide linkages.

```
CYP94A1    cttaacttttcttctcaccaacaacatttgaatattcattttatctgaaaactctaaaca 60
PROTEIN

CYP94A1    gttagaacaATGTTTCAATTTCATCTTGAAGTCCTTCTTCCCTATCTCTTACCCCTTCTC 120
PROTEIN              M  F  Q  F  H  L  E  V  L  L  P  Y  L  L  P  L  L  17

CYP94A1    TTGTTAATCCTTCCCACAACAATCTTTTTCTTAACAAAACCAAACAACAAAGTATCTTCA 180
PROTEIN     L  L  I  L  P  T  T  I  F  F  L  T  K  P  N  N  K  V  S  S  37

CYP94A1    ACTTCAACCAACAACAACATCATCACTCTCCCAAAATCATACCCACTTATCGGCTCATAC 240
PROTEIN     T  S  T  N  N  N  I  I  T  L  P  K  S  Y  P  L  I  G  S  Y  57

CYP94A1    TTATCCTTCAGAAAAAACCTGCACCGCCGCATCCAATGGCTCTCCGACATAGTCCAAATC 300
PROTEIN     L  S  F  R  K  N  L  H  R  R  I  Q  W  L  S  D  I  V  Q  I  77

CYP94A1    TCCCCCTCCGCCACTTTCCAGCTCGACGGCACCTTAGGCAAACGCCAAATCATCACCGGA 360
PROTEIN     S  P  S  A  T  F  Q  L  D  G  T  L  G  K  R  Q  I  I  T  G  97

CYP94A1    AATCCATCCACAGTTCAACACATTCTCAAAAACCAATTCTCCAACTACCAGAAAGGCACA 420
PROTEIN     N  P  S  T  V  Q  H  I  L  K  N  Q  F  S  N  Y  Q  K  G  T  117

CYP94A1    ACCTTCACAAACACCCTCTCCGACTTCCTCGGCACCGGAATCTTCAACACCAACGGCCCA 480
PROTEIN     T  F  T  N  T  L  S  D  F  L  G  T  G  I  F  N  T  N  G  P  137

CYP94A1    AACTGGAAGTTCCAACGACAAGTCGCAAGCCACGAGTTCAACACGAAGTCCATCCGTAAC 540
PROTEIN     N  W  K  F  Q  R  Q  V  A  S  H  E  F  N  T  K  S  I  R  N  157

CYP94A1    TTCGTTGAACACATAGTCGACACTGAACTCACCAACCGGTTAATCCCAATCCTCACTTCA 600
PROTEIN     F  V  E  H  I  V  D  T  E  L  T  N  R  L  I  P  I  L  T  S  177

CYP94A1    TCAACCCAAACAAACAATATCCTCGACTTCCAAGACATTCTCCAACGTTTCACTTTCGAC 660
PROTEIN     S  T  Q  T  N  N  I  L  D  F  Q  D  I  L  Q  R  F  T  F  D  197

CYP94A1    AACATCTGCAACATTGCTTTTGGTTACGACCCAGAATACTTAACACCCTCAACCAACCGA 720

PROTEIN     N  I  C  N  I  A  F  G  Y  D  P  E  Y  L  T  P  S  T  N  R  217

CYP94A1    TCAAAATTCGCAGAAGCATATGAAGATGCAACTGAAATAAGCAGTAAACGTTTCCGTTTA 780
PROTEIN     S  K  F  A  E  A  Y  E  D  A  T  E  I  S  S  K  R  F  R  L  237

CYP94A1    CCGTTACCAATCATATGGAAAATCAAAAAATACTTCAACATAGGTTCAGAGAACGTGCTC 840
PROTEIN     P  L  P  I  I  W  K  I  K  K  Y  F  N  I  G  S  E  N  V  L  257

CYP94A1    AAGGAACGAGTAACAGAAGTACGAAGTTTCGCGAAAAAGCTAGTACGAGAGAAGAAACGA 900
PROTEIN     K  E  R  V  T  E  V  R  S  F  A  K  K  L  V  R  E  K  K  R  277

CYP94A1    GAACTAGAAGAGAAATCATCGCTTGAAACAGAAGATATGTTATCAAGGTTCTTGAGCTCG 960
PROTEIN     E  L  E  E  K  S  S  L  E  T  E  D  M  L  S  R  F  L  S  S  297

CYP94A1    GGTCATTCGGATGAAGATTTTGTTGCTGATATTGTAATAAGTTTTATTTTAGCGGGTAAA 1020
PROTEIN     G  H  S  D  E  D  F  V  A  D  I  V  I  S  F  I  L  A  G  K  317

CYP94A1    GATACAACTTCAGCTGCATTAACGTGGTTCTTCTGGCTGTTATGGAAGAATCCGCGTGTT 1080
PROTEIN     D  T  T  S  A  A  L  T  W  F  F  W  L  L  W  K  N  P  R  V  337

CYP94A1    GAGGAAGAGATTGTGAATGAATTAAGTAAAAAATCTGAGTTAATGGTTTATGATGAAGTG 1140
PROTEIN     E  E  E  I  V  N  E  L  S  K  K  S  E  L  M  V  Y  D  E  V  357
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FIG. 1A

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CYP94A1     AAGGAAATGGTTTATACTCACGCTGCTTTGAGCGAGAGTATGAGATTGTATCCACCTGTA 1200
PROTEIN      K  E  M  V  Y  T  H  A  A  L  S  E  S  M  R  L  Y  P  P  V  377

CYP94A1     CCGATGGATAGTAAGGAGGCTGTTAACGATGATGTTTTACCGGATGGATGGGTTGTGAAG 1260
PROTEIN      P  M  D  S  K  E  A  V  N  D  D  V  L  P  D  G  W  V  V  K  397

CYP94A1     AAAGGGACAATTGTGACTTATCATGTTTATGCAATGGGGAGGATGAAGAGTTTGTGGGGG 1320
PROTEIN      K  G  T  I  V  T  Y  H  V  Y  A  M  G  R  M  K  S  L  W  G  417

CYP94A1     GATGATTGGGCTGAGTTTAGGCCGGAGAGGTGGTTGGAGAAGGATGAGGTGAATGGGAAG 1380
PROTEIN      D  D  W  A  E  F  R  P  E  R  W  L  E  K  D  E  V  N  G  K  437

CYP94A1     TGGGTTTTTGTGGGGAGAGATTCGTATTCTTATCCGGTTTTTCAGGCCGGGCCGAGGGTT 1440
PROTEIN      W  V  F  V  G  R  D  S  Y  S  Y  P  V  F  Q  A  G  P  R  V  457

CYP94A1     TGTTTGGGGAAGGAAATGGCTTTTATGCAAATGAAGAGGATTGTTGCGGGGATTGTTGGA 1500
PROTEIN      C  L  G  K  E  M  A  F  M  Q  M  K  R  I  V  A  G  I  V  G  477

CYP94A1     AAGTTTAAGGTTGTTCCTGAGGCGCATTTGGCTCAAGAACCCGGTTTTATTTCTTTTTTG 1560
PROTEIN      K  F  K  V  V  P  E  A  H  L  A  Q  E  P  G  F  I  S  F  L  497

CYP94A1     AGTTCGCAGATGGAAGGTGGGTTTCCTGTCACGATTCAGAAGAGGGATTCTTGAttaatt 1620
PROTEIN      S  S  Q  M  E  G  G  F  P  V  T  I  Q  K  R  D  S  *        514

CYP94A1     catgagagcattcacattagttattaactcattactaattggttatatatatcgttttgt 1680
PROTEIN

CYP94A1     tttgtttgtgtgtctgtcgttgttgtagtcggatgttgcttaagtgatatgtatagtgta 1740
PROTEIN

CYP94A1     gtttcttatttagtagtatgttttatttacggttaacacttcagttgataaatacttgga 1800
PROTEIN

CYP94A1     ttgtattttgattgaatatgtatttataactttattatttattgtaaaaaaaaaaaaaaa 1860
PROTEIN

CYP94A1     aa                                                           1862
PROTEIN
```

FIG. 1B

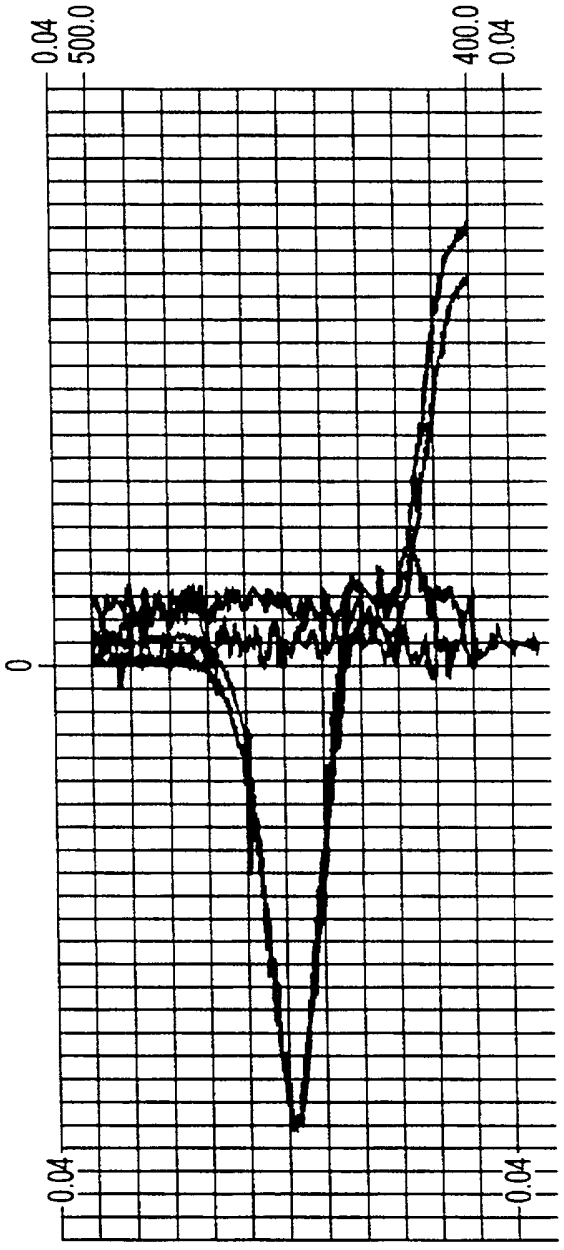


FIG. 2

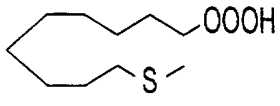
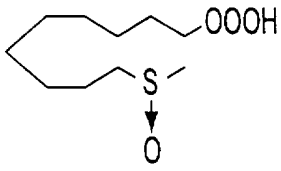
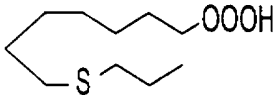
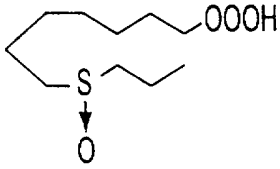
SUBSTRATES	METABOLITES
 10S-LAU	
 8S-LAU	

FIG. 3

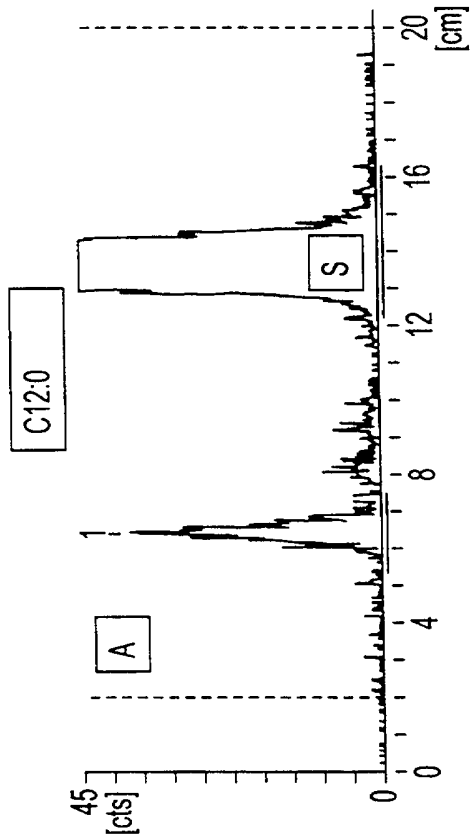


FIG. 4B

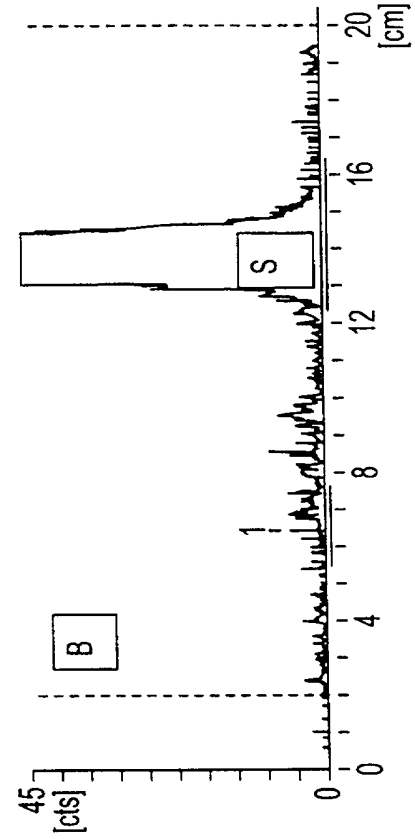


FIG. 4D

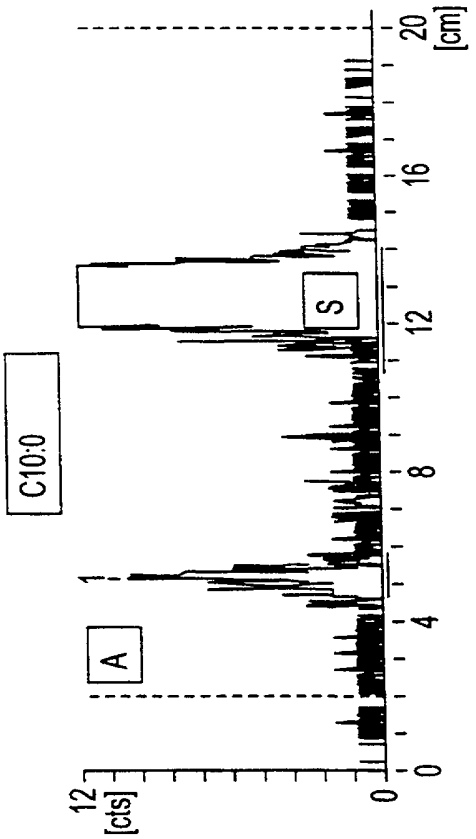


FIG. 4A

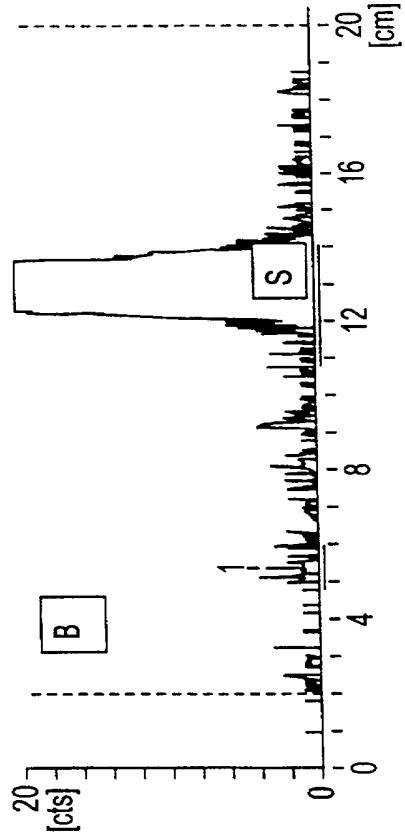


FIG. 4C

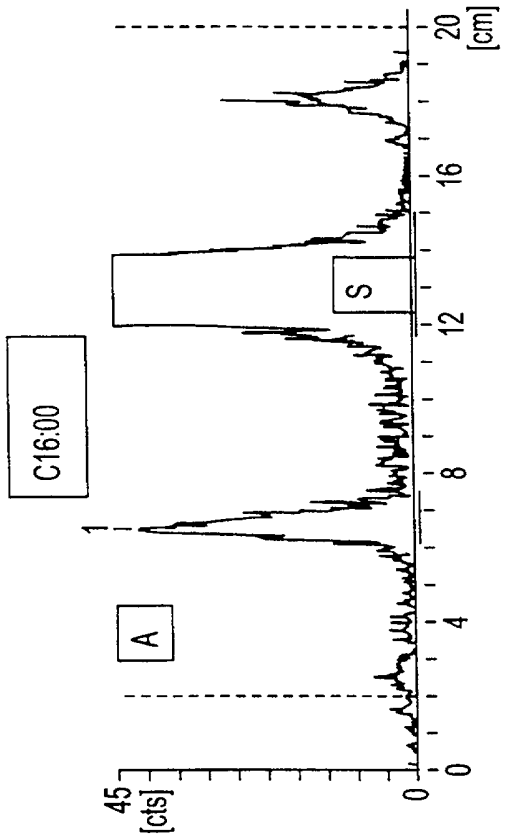


FIG. 4F

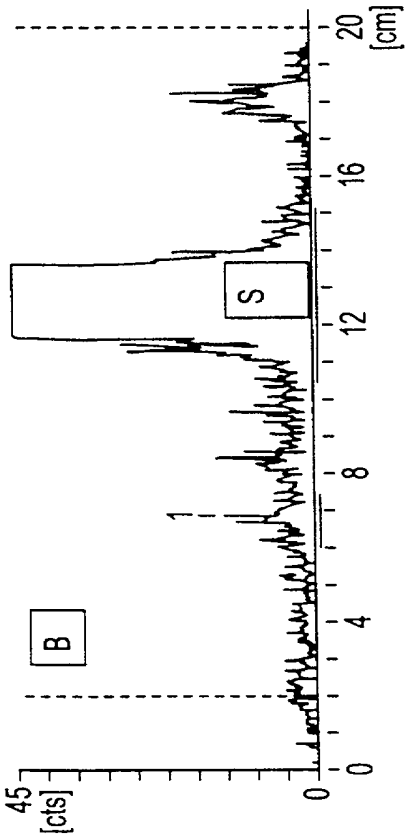


FIG. 4H

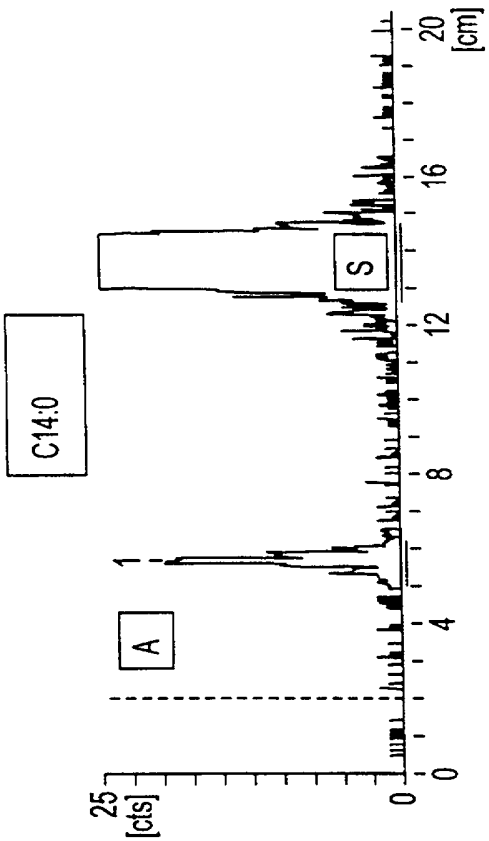


FIG. 4E

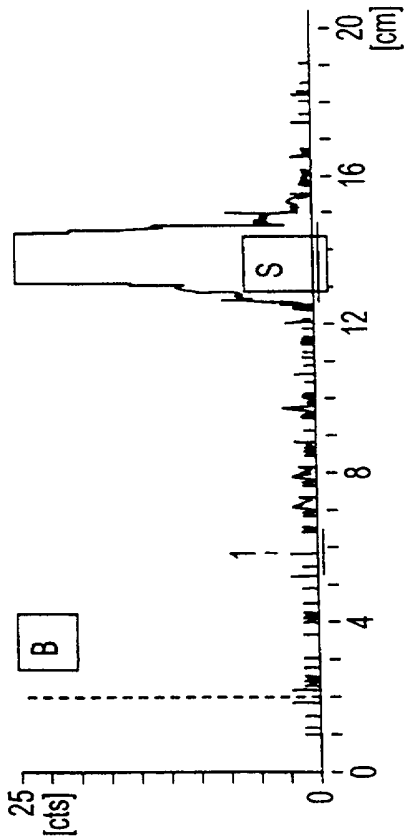


FIG. 4G

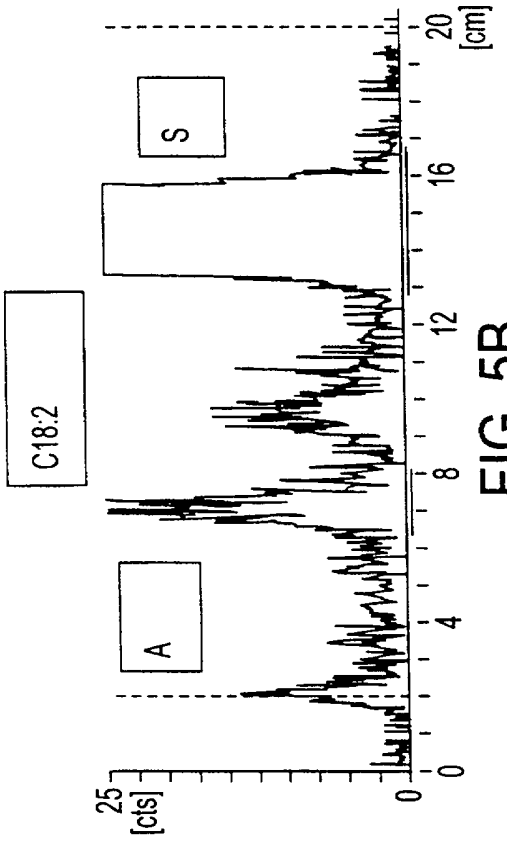


FIG. 5B

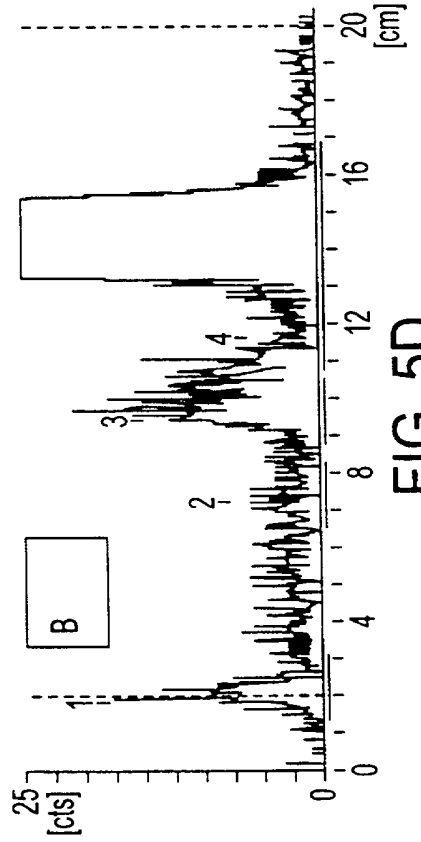


FIG. 5D

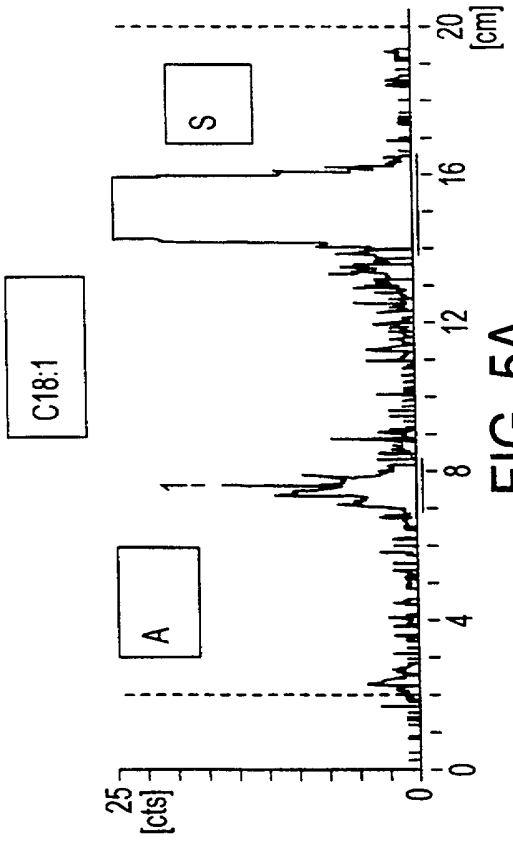


FIG. 5A

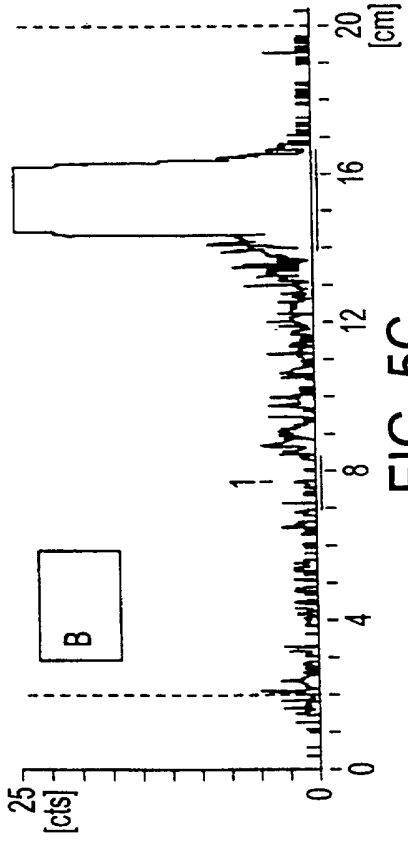


FIG. 5C

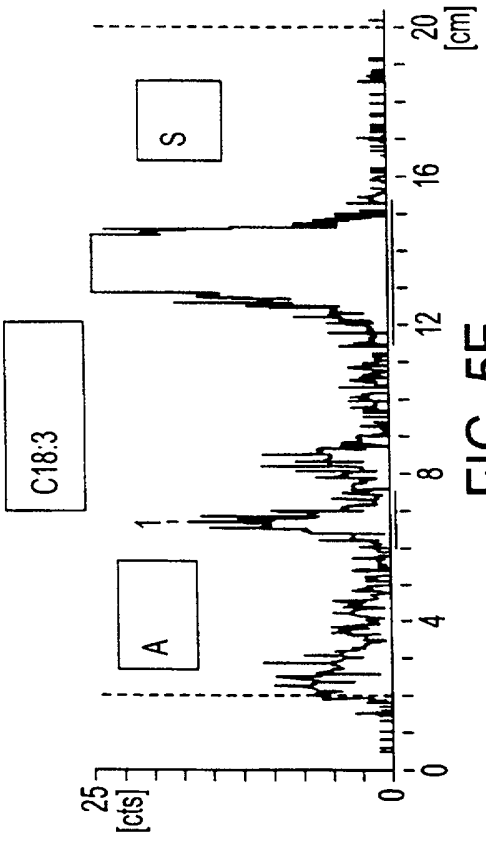


FIG. 5E

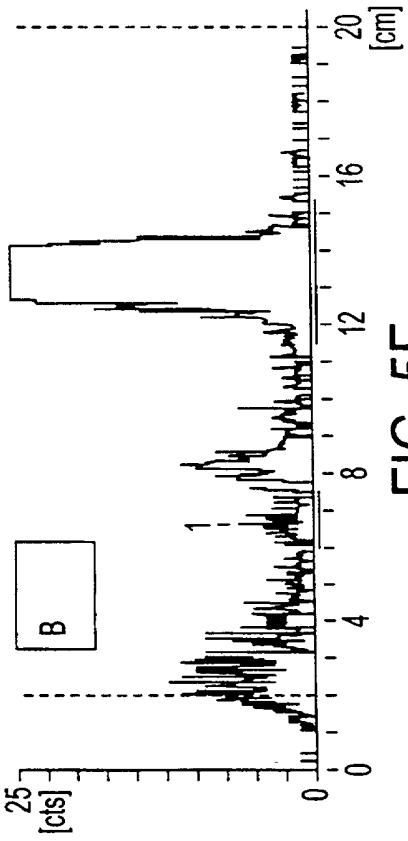


FIG. 5F

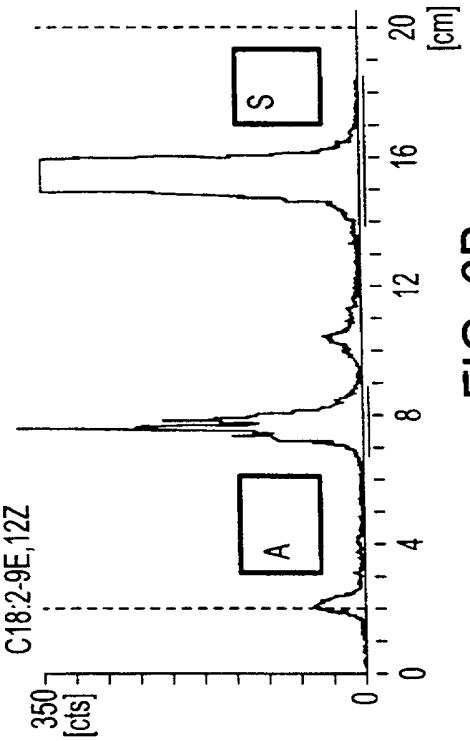


FIG. 6B

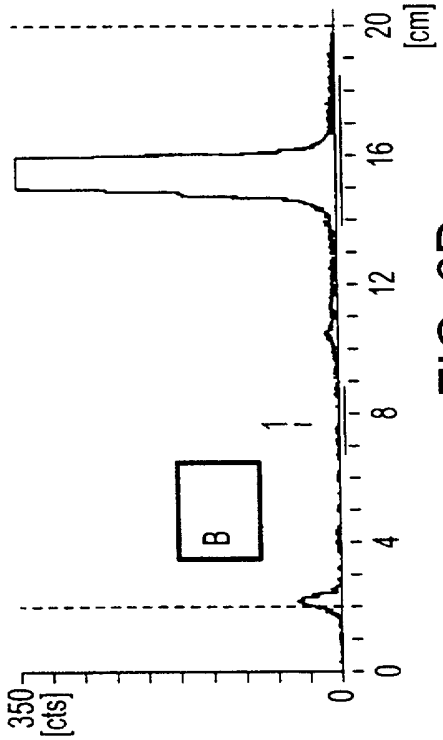


FIG. 6D

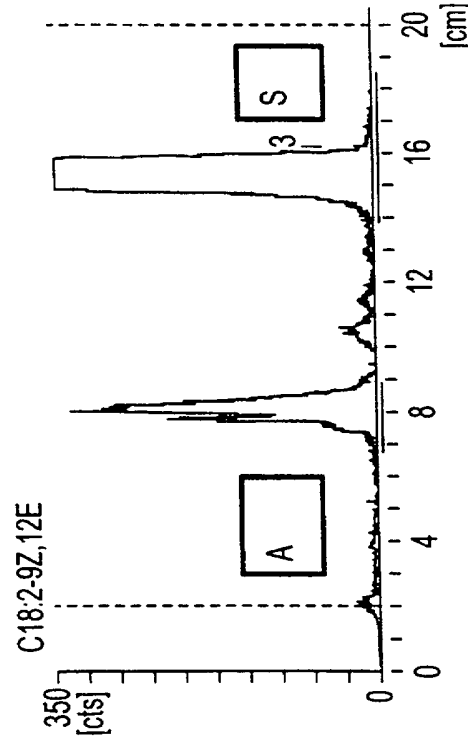


FIG. 6A

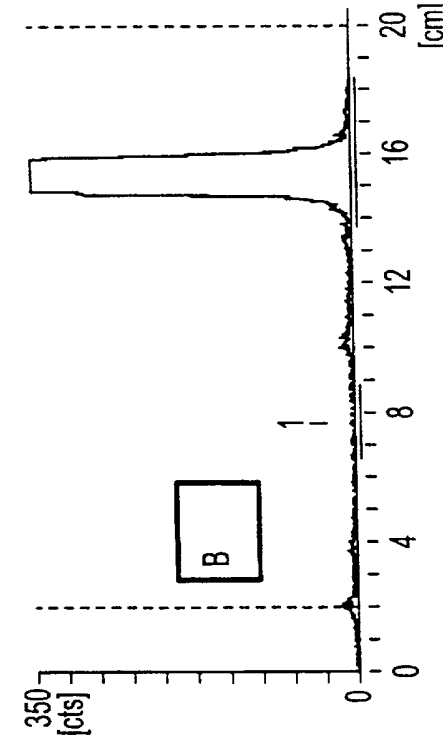


FIG. 6C

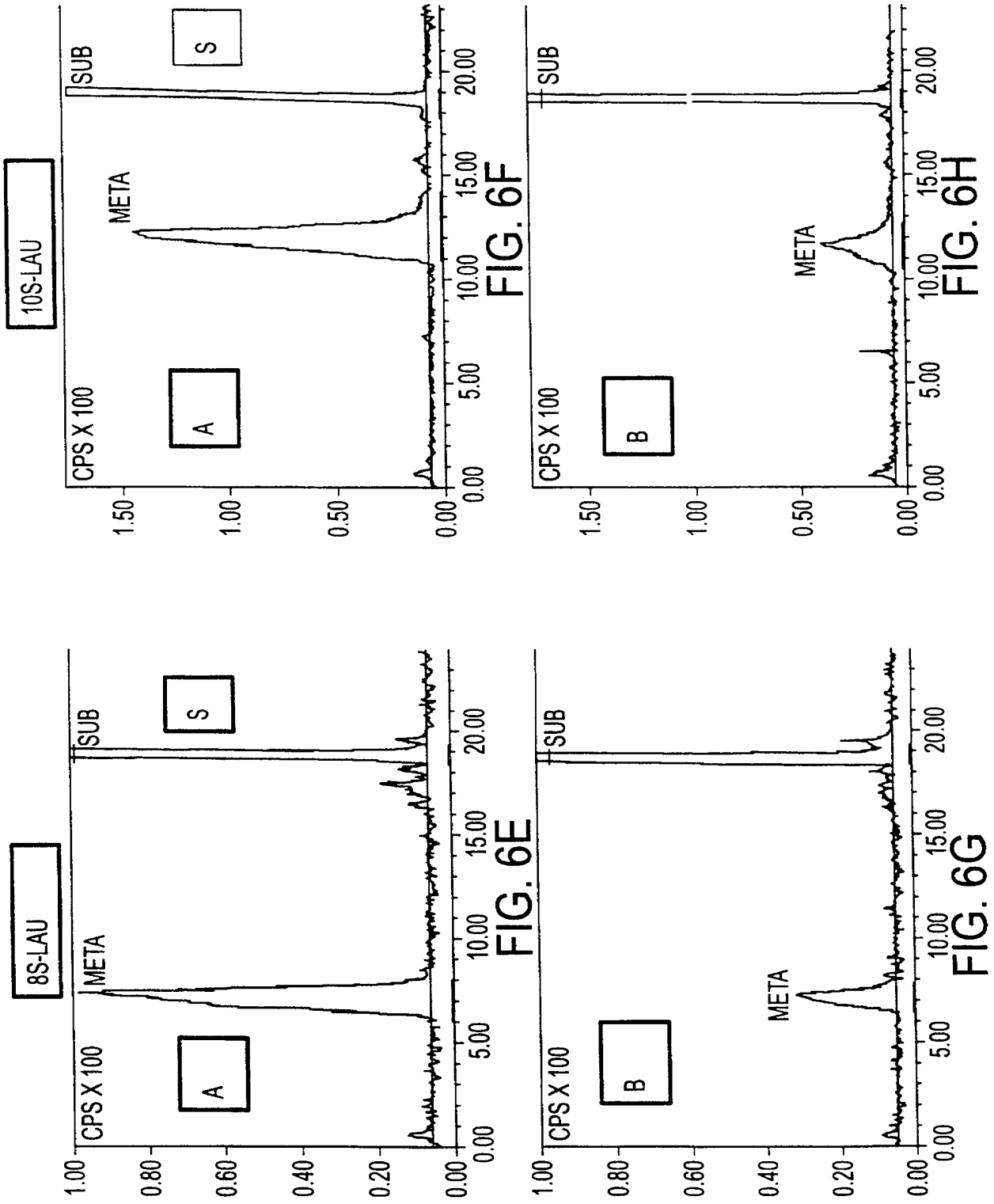


FIG. 6E

FIG. 6G

FIG. 6F

FIG. 6H

```
CYP94A2     agaataatccaagtgtaattacttttttagctctcactactcaatcataactatcaaact 60
PROTEIN

CYP94A2     tgaaaaATGGAACTCGAAACATTGATTTCTTGGTTACTTTTCTCTACAAGTTTATTTTGG 120
PROTEIN           M  E  L  E  T  L  I  S  W  L  L  F  S  T  S  L  F  W  18

CYP94A2     TTCTTATTCTTAGCCACAAAAACAAAATCCAAACCCCCAAAAACACCTTCCTCTACCACC 180
PROTEIN      F  L  F  L  A  T  K  T  K  S  K  P  P  K  T  P  S  S  T  T  38

CYP94A2     AACACCCCAATTCCTAAATCTTACCCCATTTTCGGTTCTGCCTTCTCTGTGCTAGCCAAC 240
PROTEIN      N  T  P  I  P  K  S  Y  P  I  F  G  S  A  F  S  V  L  A  N  58

CYP94A2     TTCCACCGACGCATACAATGGACCTCCGACATTCTCCAAACCATCCCTTCCTCCACCTTC 300
PROTEIN      F  H  R  R  I  Q  W  T  S  D  I  L  Q  T  I  P  S  S  T  F  78

CYP94A2     GTCCTCCACCGCCCTTTCGGCGCTCGCCAAGTCTTCACGGCTCAACCCGCCGTGGTGCAA 360
PROTEIN      V  L  H  R  P  F  G  A  R  Q  V  F  T  A  Q  P  A  V  V  Q  98

CYP94A2     CACATTCTCAGAACCAATTTCACTTGCTACGGCAAAGGTCTCACGTTTTACCAATCTATC 420
PROTEIN      H  I  L  R  T  N  F  T  C  Y  G  K  G  L  T  F  Y  Q  S  I  118

CYP94A2     AATGATTTTCTCGGCGACGGAATCTTCAATGCCGACGGTGAATCTTGGAAGTTCCAACGA 480
PROTEIN      N  D  F  L  G  D  G  I  F  N  A  D  G  E  S  W  K  F  Q  R  138

CYP94A2     CAAATCTCCAGCCACGAATTCAACACTAGATCCCTCCGGAAATTCGTTGAAACCGTAGTT 540
PROTEIN      Q  I  S  S  H  E  F  N  T  R  S  L  R  K  F  V  E  T  V  V  158

CYP94A2     GACGTTGAACTCTCCGATCGCCTAGTTCCTGTTCTCTCCCAAGCTTCTAACAGCCAAACC 600
PROTEIN      D  V  E  L  S  D  R  L  V  P  V  L  S  Q  A  S  N  S  Q  T  178

CYP94A2     ACTCTTGATTTCCAAGACATCCTCCAACGTTTAACTTTTGACAACATTTGCATGATTGCG 660
PROTEIN      T  L  D  F  Q  D  I  L  Q  R  L  T  F  D  N  I  C  M  I  A  198

CYP94A2     TTTGGATACGATCCAGAGTACCTCCTTCCTTCCCTTCCTGAAATACCATTTGCAAAAGCC 720
PROTEIN      F  G  Y  D  P  E  Y  L  L  P  S  L  P  E  I  P  F  A  K  A  218

CYP94A2     TTCGACGAAAGCTCGCAACTCAGTATCGAGAGGCTAAACGCGTTGATTCCATTACTATGG 780
PROTEIN      F  D  E  S  S  Q  L  S  I  E  R  L  N  A  L  I  P  L  L  W  238

CYP94A2     AAAGTGAAAAGATTCCTGAACATCGGAGTGGAGCGACAGCTGAAAGAAGCGGTTGCTGAA 840
PROTEIN      K  V  K  R  F  L  N  I  G  V  E  R  Q  L  K  E  A  V  A  E  258

CYP94A2     GTAAGAGGACTCGCCACTAAAATCGTTAAGAATAAGAAAAAAGAGCTTAAAGAAAAAGCA 900
PROTEIN      V  R  G  L  A  T  K  I  V  K  N  K  K  K  E  L  K  E  K  A  278

CYP94A2     CTACAGTCGGAATCCGAATCTGTTGATCTTTTATCGCGATTTTTAAGTTCTGGACATTCA 960
PROTEIN      L  Q  S  E  S  E  S  V  D  L  L  S  R  F  L  S  S  G  H  S  298

CYP94A2     GATGAATCTTTTGTTACTGATATGGTAATAAGTATTATTCTTGCTGGGAGAGATACGACT 1020
PROTEIN      D  E  S  F  V  T  D  M  V  I  S  I  I  L  A  G  R  D  T  T  318

CYP94A2     TCAGCTGCACTCACGTGGTTCTTTTGGTTACTCTCGAAGCATAGTCATGTGGAGAATGAG 1080
PROTEIN      S  A  A  L  T  W  F  F  W  L  L  S  K  H  S  H  V  E  N  E  338

CYP94A2     ATTCTCAAAGAGATAACTGGAAAATCGGAAACTGTTGGATACGATGAGGTGAAGGATATG 1140
PROTEIN      I  L  K  E  I  T  G  K  S  E  T  V  G  Y  D  E  V  K  D  M  358

CYP94A2     GTTTACACTCACGCGGCGCTTTGCGAGAGTATGAGGCTATATCCTCCGCTTCCGGTGGAT 1200
PROTEIN      V  Y  T  H  A  A  L  C  E  S  M  R  L  Y  P  P  L  P  V  D  378
```

FIG. 7A

```
CYP94A2     ACTAAAGTAGCCGTGCACGACGATGTTTTGCCGGATGGGACTTTAGTGAAGAAAGGATGG 1260
PROTEIN      T  K  V  A  V  H  D  D  V  L  P  D  G  T  L  V  K  K  G  W  398

CYP94A2     AGAGTGACGTATCATATATATGCTATGGGAAGATCTGAGAAGATATGGGGACCGGATTGG 1320
PROTEIN      R  V  T  Y  H  I  Y  A  M  G  R  S  E  K  I  W  G  P  D  W  418

CYP94A2     GCTGAATTTCGACCCGAGAGGTGGTTGAGTCGGGATGAGGTTGGGAAGTGGAGCTTTGTT 1380
PROTEIN      A  E  F  R  P  E  R  W  L  S  R  D  E  V  G  K  W  S  F  V  438

CYP94A2     GGGATTGATTATTATAGTTATCCGGTTTTCCAGGCTGGACCGAGGGTGTGTATAGGGAAG 1440
PROTEIN      G  I  D  Y  Y  S  Y  P  V  F  Q  A  G  P  R  V  C  I  G  K  458

CYP94A2     GAGATGGCATTTTTGCAGATGAAGAGGGTGGTTGCCGGGATTATGGGGCGGTTTAGGGTG 1500
PROTEIN      E  M  A  F  L  Q  M  K  R  V  V  A  G  I  M  G  R  F  R  V  478

CYP94A2     GTTCCGGCTATGGTTGAAGGGATTGAGCCGGAGTACACTGCCCACTTTACCTCAGTAATG 1560
PROTEIN      V  P  A  M  V  E  G  I  E  P  E  Y  T  A  H  F  T  S  V  M  498

CYP94A2     AAAGGTGGCTTCCCCGTGAAGATCGAAAAGAGAAGCCCACTTGTATGAataaaaaggaaa 1620
PROTEIN      K  G  G  F  P  V  K  I  E  K  R  S  P  L  V  *              513

CYP94A2     taatacaccatcaatttgaaatataaattacttttcttttaaaaa                1665
PROTEIN
```

FIG. 7B

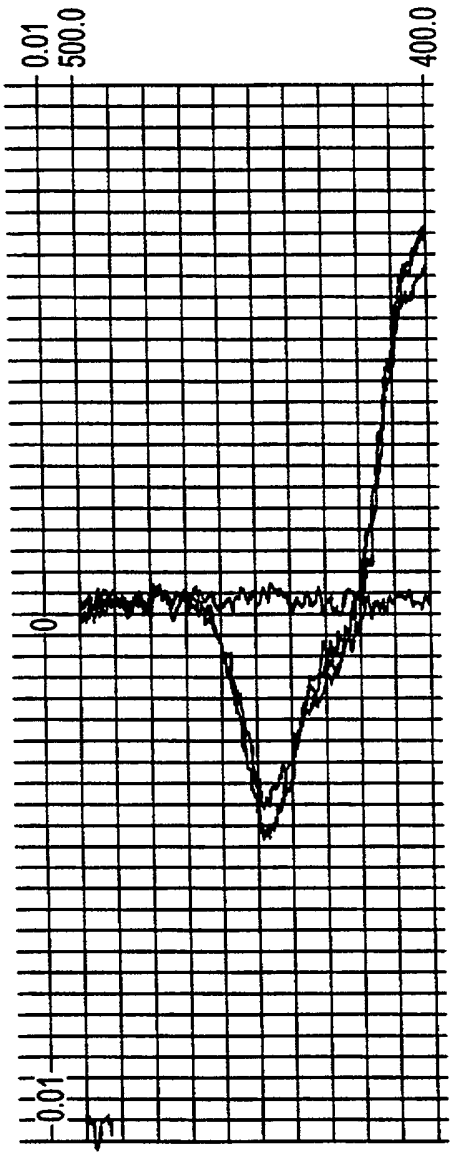


FIG. 8

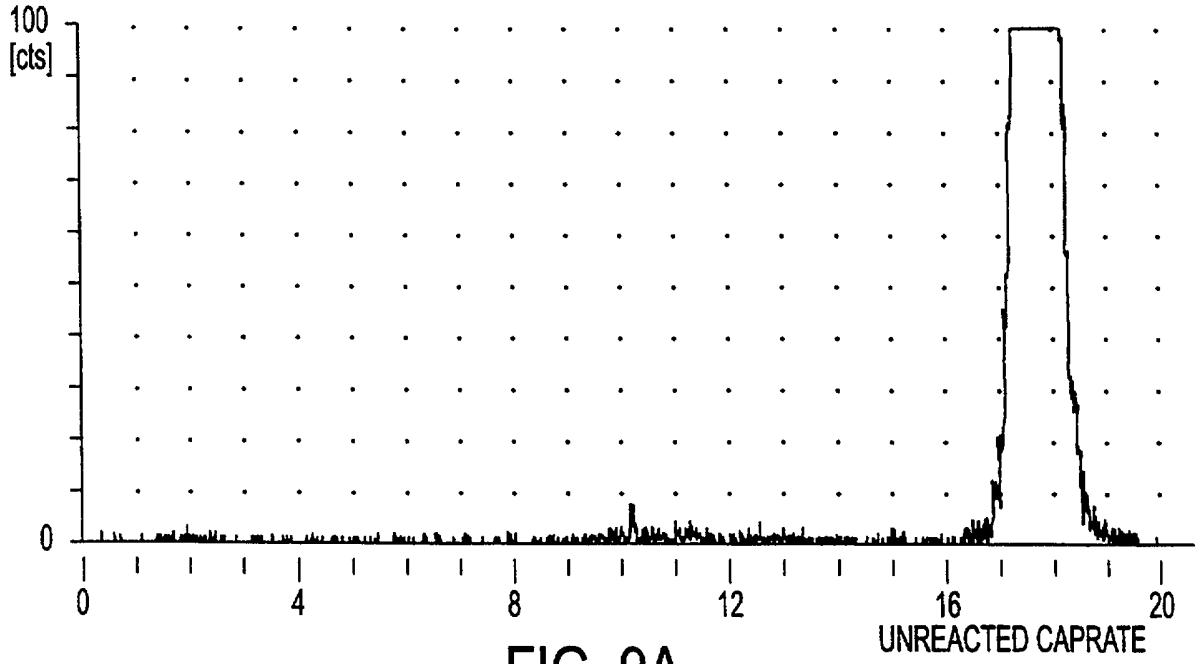


FIG. 9A

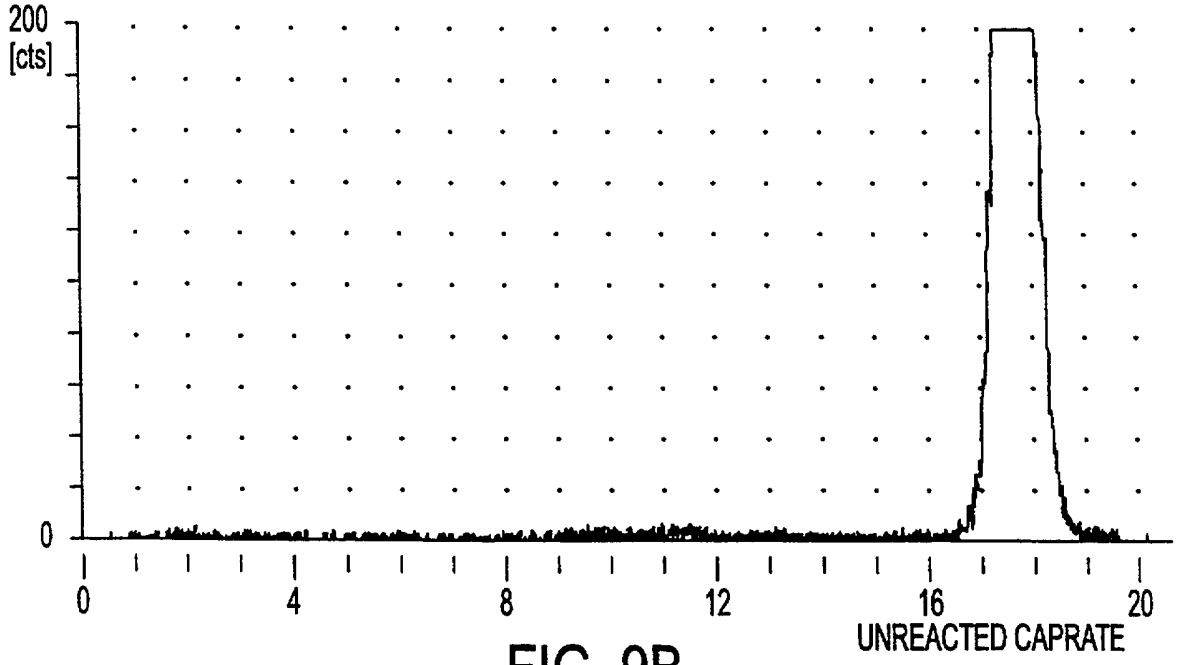


FIG. 9B

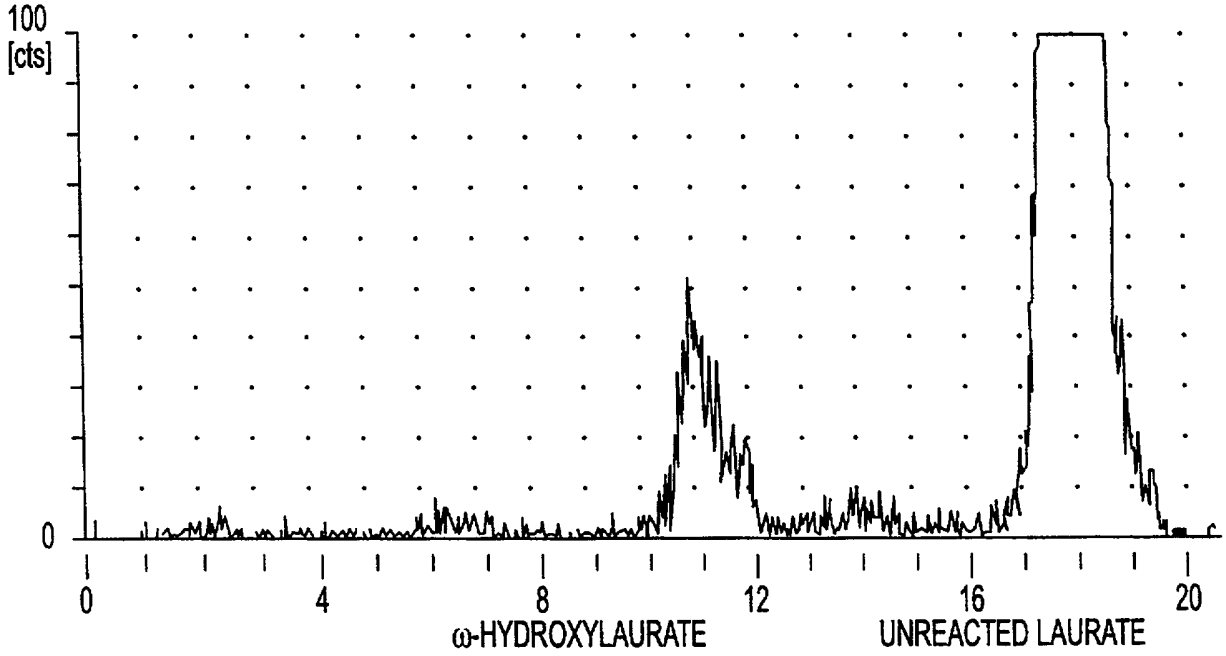


FIG. 10A

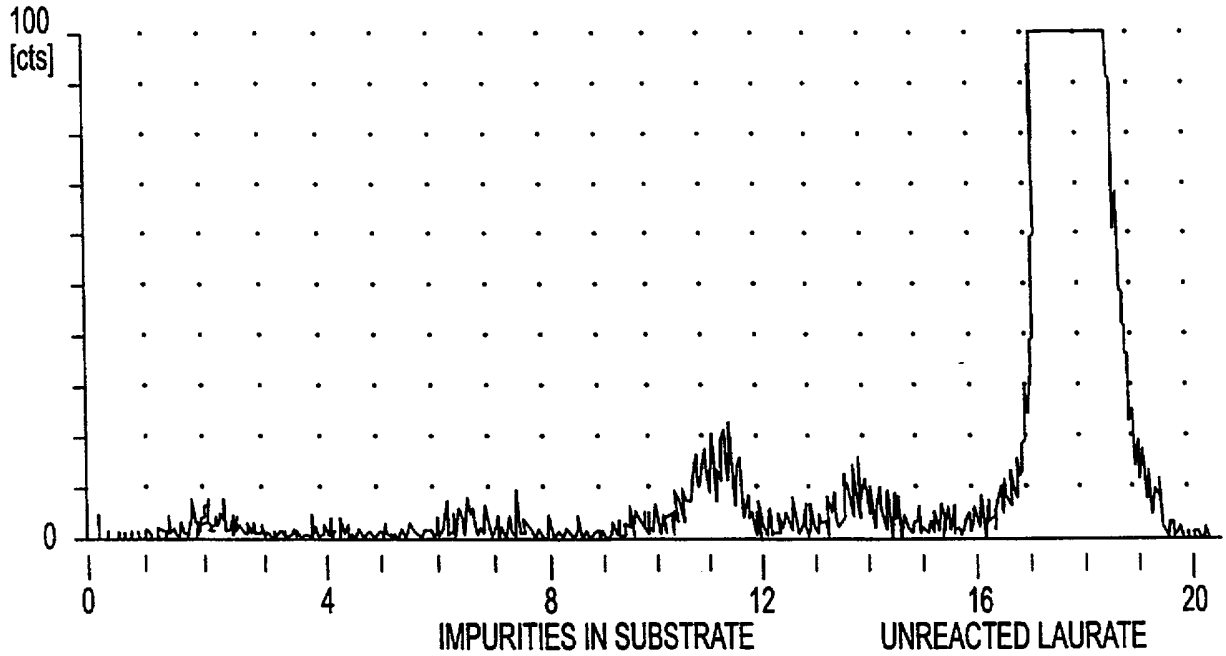


FIG. 10B

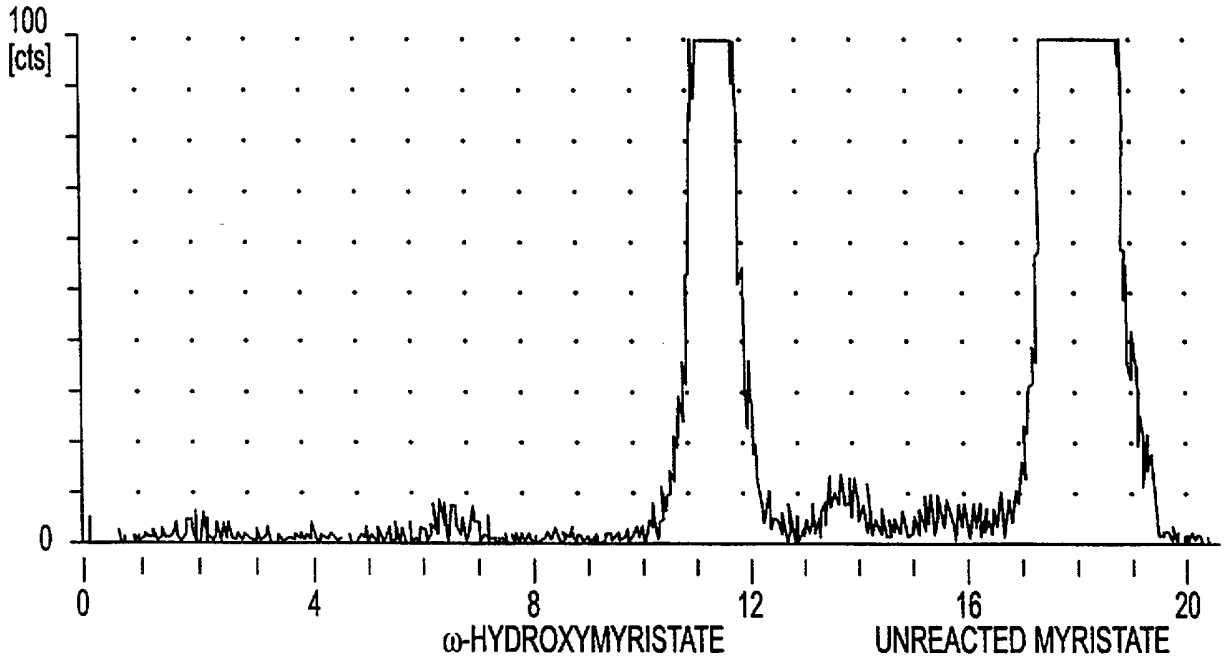


FIG. 11A

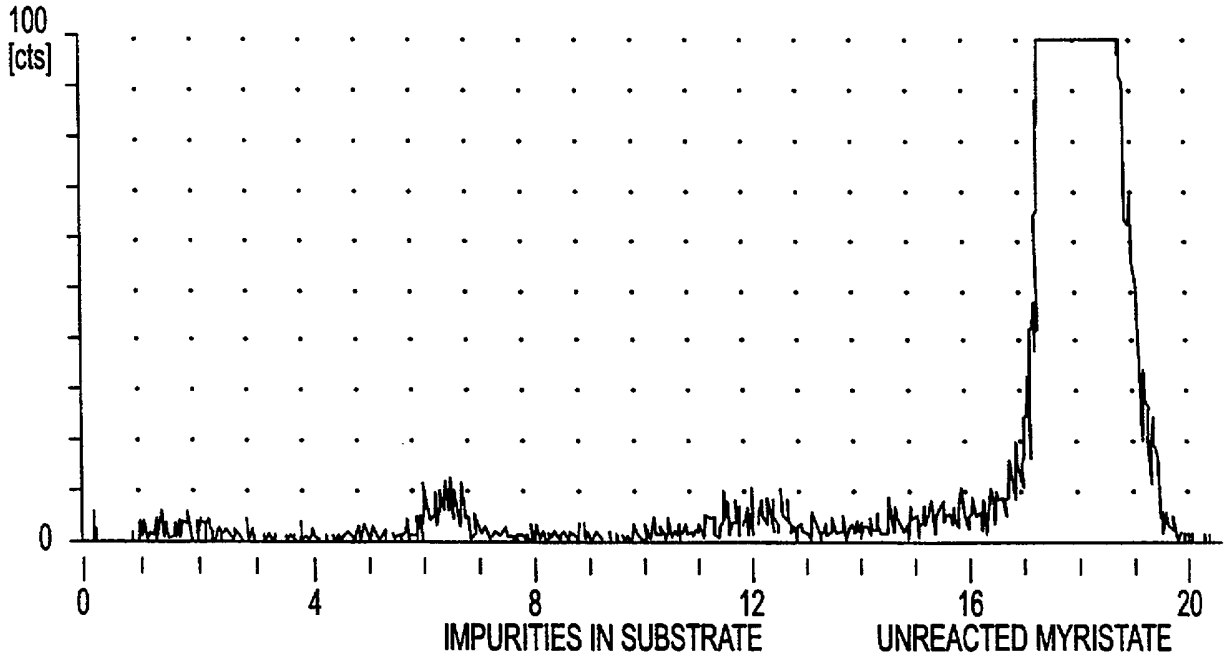


FIG. 11B

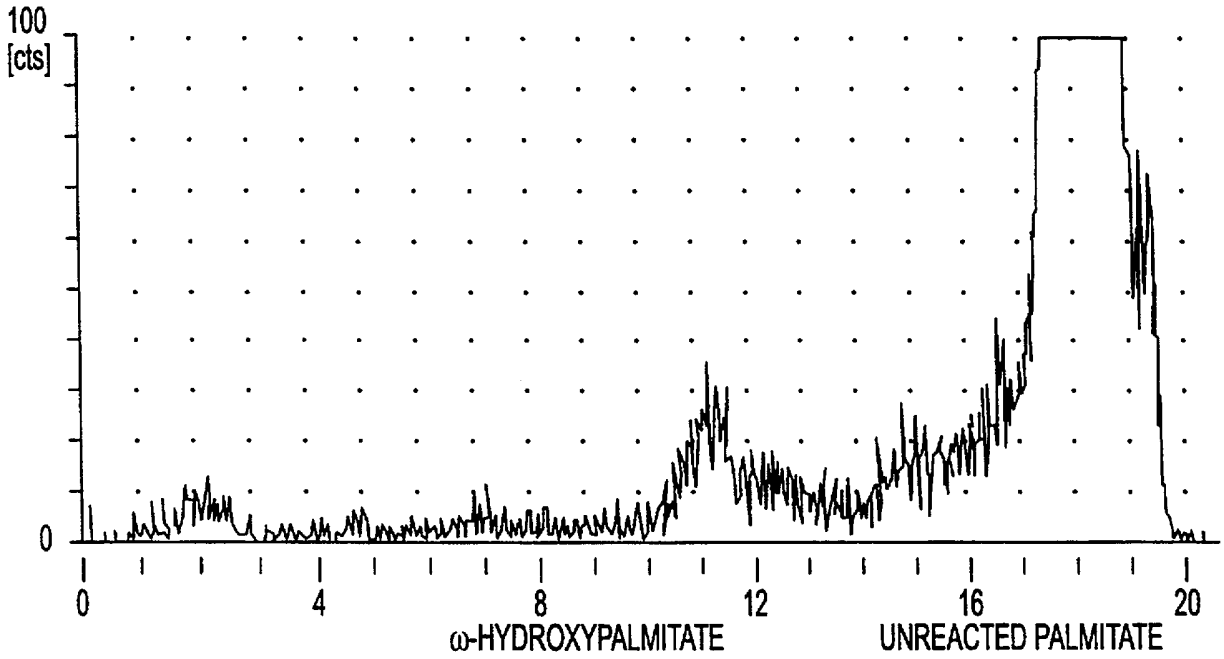


FIG. 12A

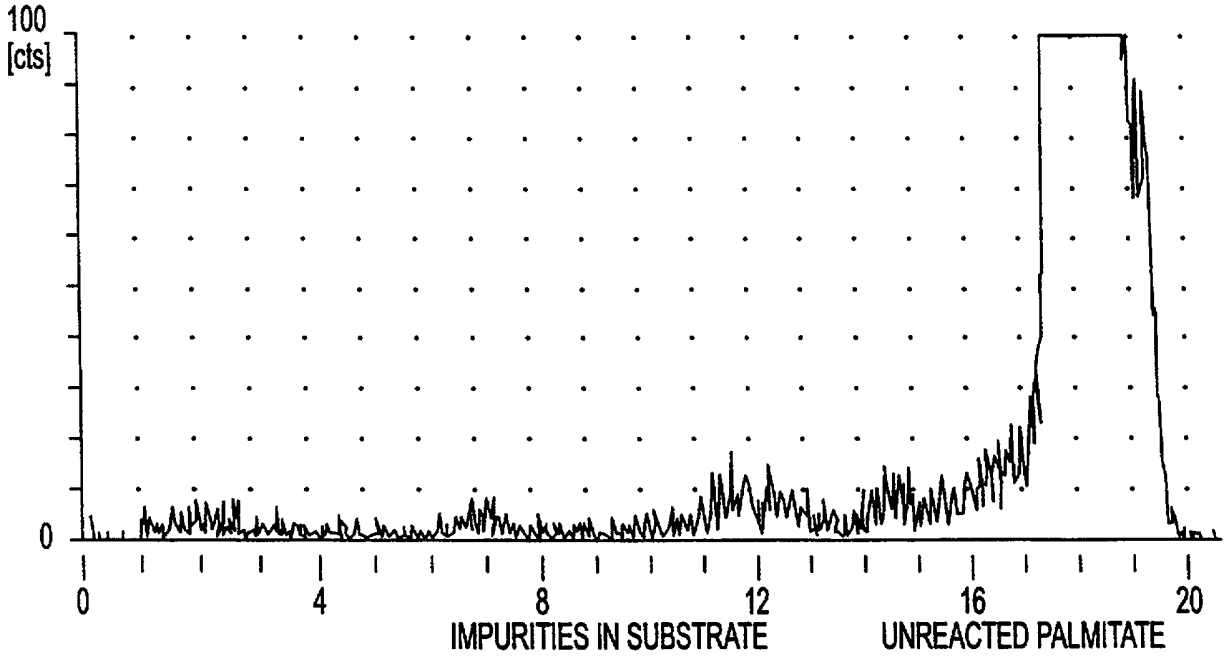


FIG. 12B

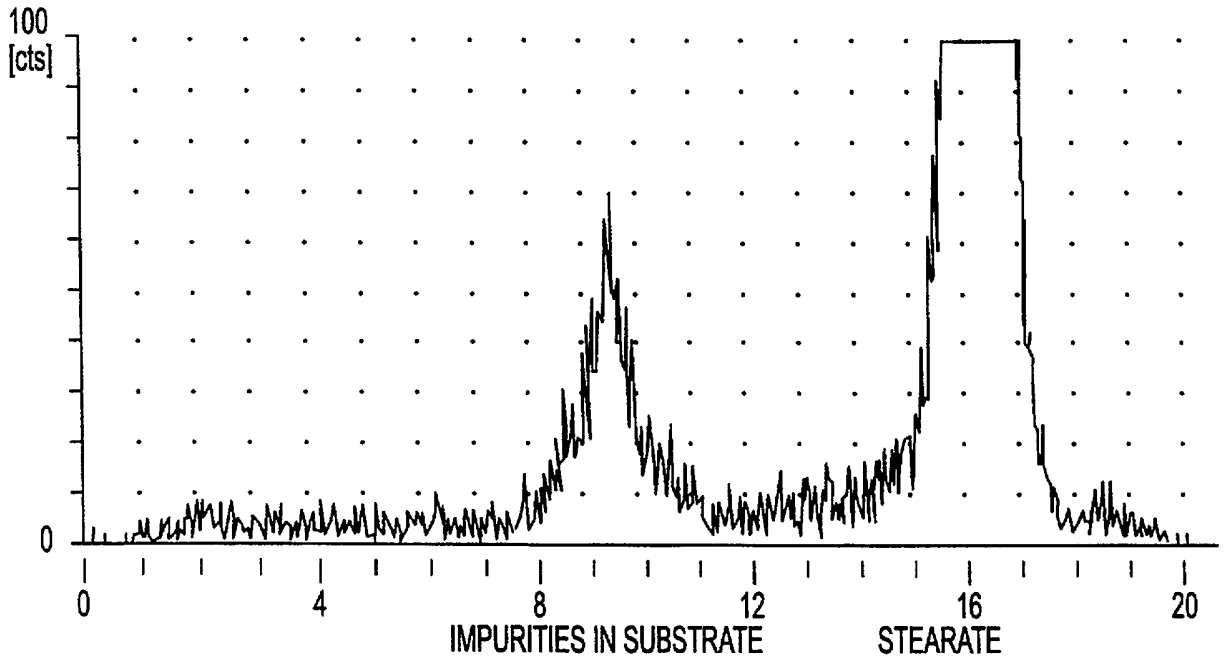


FIG. 13A

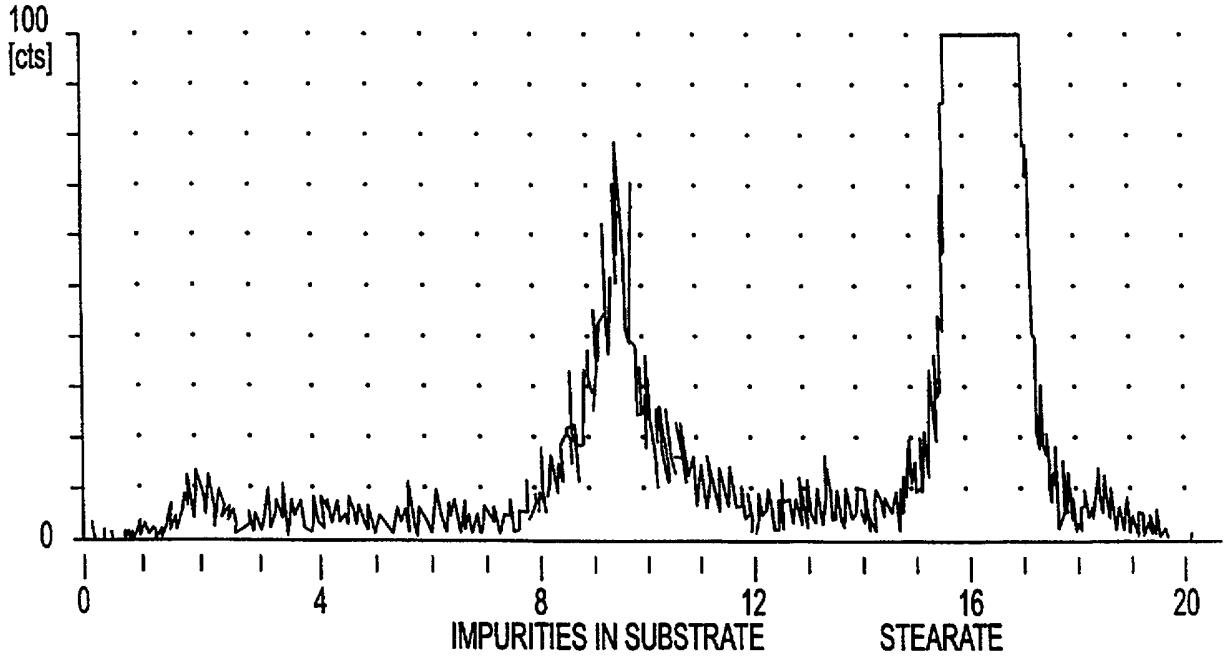


FIG. 13B

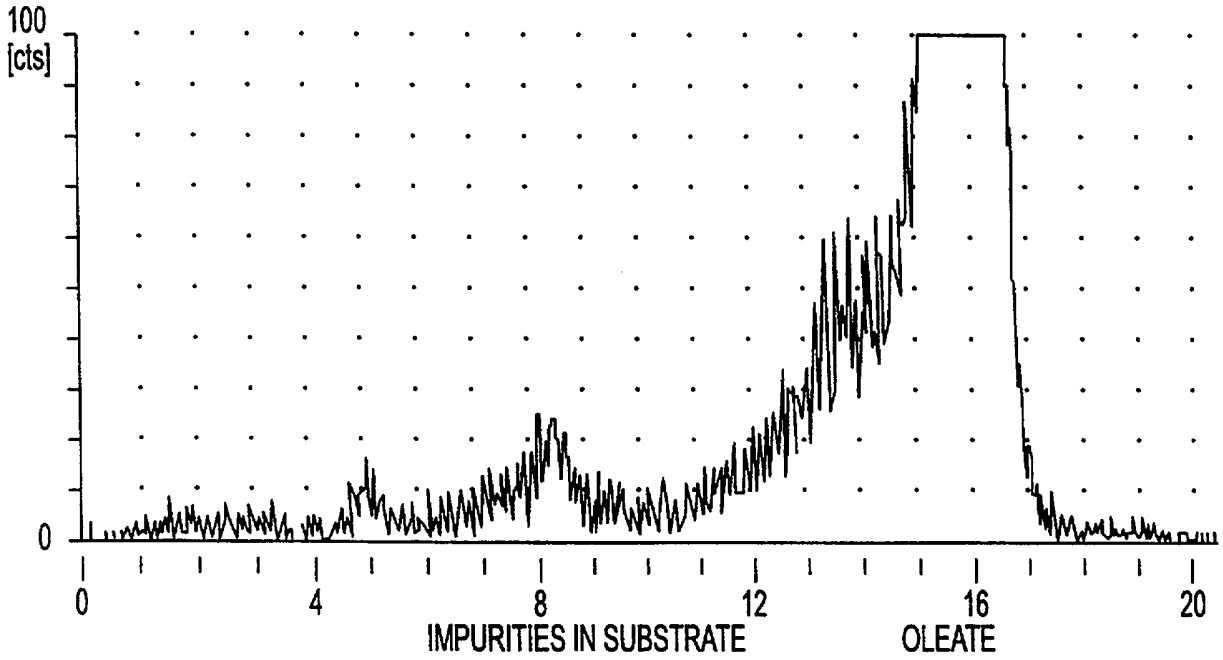


FIG. 14A

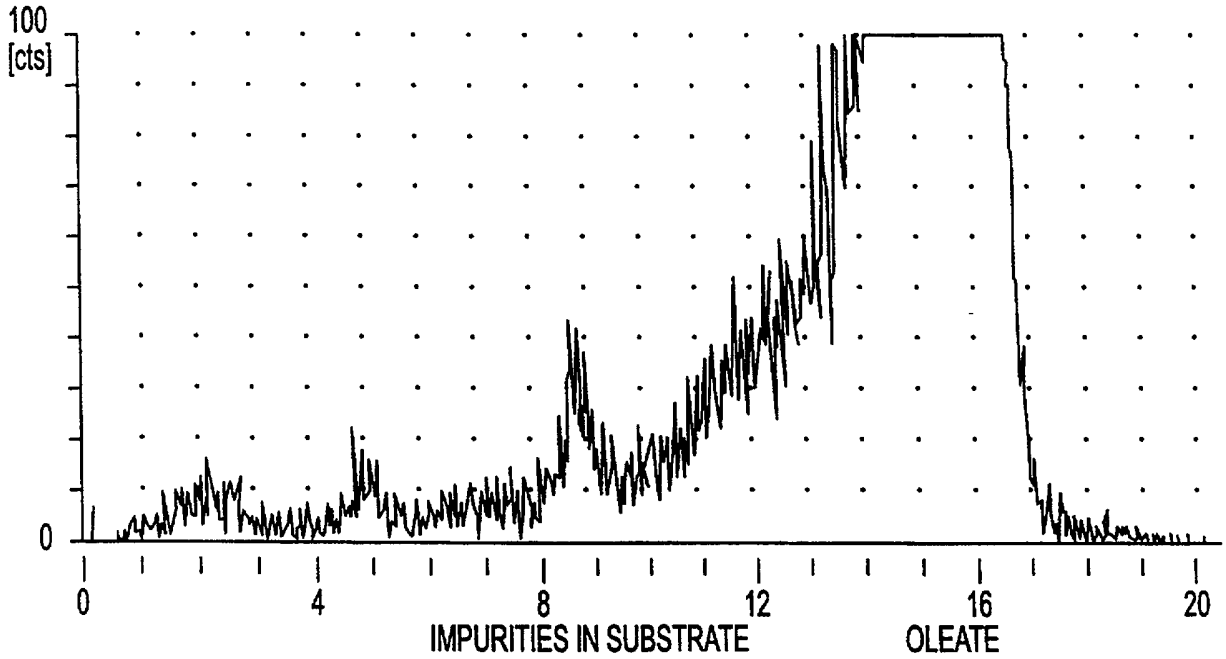


FIG. 14B

```
CYP94A3    tcattttgttatatataacaactgcgaagcaaagttaaactatgtcatgtaatcacaaca 60
PROTEIN

CYP94A3    ttggctctcatatcaactccaatctctctctcactaacgtgctaaaATGGAACTCGAAAC 120
PROTEIN                                                  M  E  L  E  T  5

CYP94A3    ATTGGTTGCATGGTTACTTTTCTCTGCAACTCTCCTTTGGCTCTTACTCTTAGCCACAAA 180
PROTEIN      L  V  A  W  L  L  F  S  A  T  L  L  W  L  L  L  L  A  T  K 25

CYP94A3    AACACAATCCAAATCCCTAAAATCACCCTCCTCAACCACCAACAGCACCATTCCCAAATC 240
PROTEIN      T  Q  S  K  S  L  K  S  P  S  S  T  T  N  S  T  I  P  K  S 45

CYP94A3    TTACCCCATCTTCGGTTCCATCTTTTCAATTGCAGCAAATTTTCACCGCCGCGTGCAATG 300
PROTEIN      Y  P  I  F  G  S  I  F  S  I  A  A  N  F  H  R  R  V  Q  W 65

CYP94A3    GATCTCCGACATCCTTCAAACCACCCCTTCCTCAACCTTCATCCTCCACCGCGCCTTCGG 360
PROTEIN      I  S  D  I  L  Q  T  T  P  S  S  T  F  I  L  H  R  A  F  G 85

CYP94A3    CTCCCGCCAAGTCTTCACAGCAAACCCCTTAGTAGTCCAACATATTCTCAAAACCAACTT 420
PROTEIN      S  R  Q  V  F  T  A  N  P  L  V  V  Q  H  I  L  K  T  N  F 105

CYP94A3    CCCTTGCTACCCTAAAGGTCTCACACTTAACCGTTCCCTCGGTGATTTCCTCGGTAACGG 480
PROTEIN      P  C  Y  P  K  G  L  T  L  N  R  S  L  G  D  F  L  G  N  G 125

CYP94A3    TATCTTCAACGCCGACGGTGAAACCTGGAAGCTCCAAAGACAAATCTCCAgCCATGAATT 540
PROTEIN      I  F  N  A  D  G  E  T  W  K  L  Q  R  Q  I  S  S  H  E  F 145

CYP94A3    CAACGCTAAATCTCTTCGGAAATTCGTTGAAACAGTAGTTGATGTAGAACTCTCCGGTCG 600
PROTEIN      N  A  K  S  L  R  K  F  V  E  T  V  V  D  V  E  L  S  G  R 165

CYP94A3    CCTCCTCCCTATTCTCTCTGAAGCTTCCAAAACTGAAAAAATCCTCCCTGATTTTCAAGA 660
PROTEIN      L  L  P  I  L  S  E  A  S  K  T  E  K  I  L  P  D  F  Q  D 185

CYP94A3    TATCCTTCAACGTTTTACATTCGATAACATCTGTATAATCGCCTTTGGATTCGATCCAGA 720
PROTEIN      I  L  Q  R  F  T  F  D  N  I  C  I  I  A  F  G  F  D  P  E 205

CYP94A3    GTATCTCCTCCCTTCTCTTCCCGAAACCGCCTTTGCAAAGGCCTTCGACTACGGCACCAG 780
PROTEIN      Y  L  L  P  S  L  P  E  T  A  F  A  K  A  F  D  Y  G  T  R 225

CYP94A3    AATAAGCAGCTTGAGATTCAACGCCGCAGTTCCATTAATATGGAAAGTCAAGAAAATCTT 840
PROTEIN      I  S  S  L  R  F  N  A  A  V  P  L  I  W  K  V  K  K  I  L 245

CYP94A3    AAACATCGGAACAGAACAGCGGTTAAAAGAAGCTGTTGCGGAAGTAAGAGGACTGGCTTC 900
PROTEIN      N  I  G  T  E  Q  R  L  K  E  A  V  A  E  V  R  G  L  A  S 265

CYP94A3    AAGAATTGTTAGAGAAAAGAAACAAGAGCTTTTAGAAAAATCAGCGTTGGAATCATTGGA 960
PROTEIN      R  I  V  R  E  K  K  Q  E  L  L  E  K  S  A  L  E  S  L  D 285

CYP94A3    TATTTTATCGCGATTTTTAAGTTCTGGTCATTCAGATGAATCATTTGTTATTGATATTGT 1020
PROTEIN      I  L  S  R  F  L  S  S  G  H  S  D  E  S  F  V  I  D  I  V 305

CYP94A3    AATAAGCTTTATTCTTGCTGGGAGAGATACAACTTCAGCTGCACTCACGTGGTTCTTTTG 1080
PROTEIN      I  S  F  I  L  A  G  R  D  T  T  S  A  A  L  T  W  F  F  W 325

CYP94A3    GTTACTCTCTAAGCATAGTCATGTGGAGACTGAGATTCTCAAAGAGGTTACTGCAAAATC 1140
PROTEIN      L  L  S  K  H  S  H  V  E  T  E  I  L  K  E  V  T  A  K  S 345

CYP94A3    GGAATCAGTTAGTTATGATGAAGTGAAGGACATGGTTTATACTCACGCGGCGCTGTGCGA 1200
```

FIG. 15A

PROTEIN	E S V S Y D E V K D M V Y T H A A L C E	365
CYP94A3	GAGTATGAGGCTGTATCCTCCTGTTCCAGTGGATACAAAAGAAGTAGCTTATGACGATGT	1260
PROTEIN	S M R L Y P P V P V D T K E V A Y D D V	385
CYP94A3	TTTACCAGATGGGACTTTTGTGAAGAAAGGGTGGAGAGTGGCGTATCATATATATGCTAT	1320
PROTEIN	L P D G T F V K K G W R V A Y H I Y A M	405
CYP94A3	GGGAAGGTCTGAGAAAATATGGGGATCTGACTGGGCTGAGTTTCGACCCGAGAGGTGGTT	1380
PROTEIN	G R S E K I W G S D W A E F R P E R W L	425
CYP94A3	GCGTCGGGATGAAGATGGGATGTGGAGCTTTGTTGGGATGGATCCTTATGCTTATCCAGT	1440
PROTEIN	R R D E D G M W S F V G M D P Y A Y P V	445
CYP94A3	TTTTCAAGCCGGGCCAAGGGTGTGTTTAGGGAAAGAAATGGCGTTcTTGCAAATGAAGAG	1500
PROTEIN	F Q A G P R V C L G K E M A F L Q M K R	465
CYP94A3	GGTGGCTGCCGGAGTTcTGAGAGAGTTTAGGGTGGTTCCGGCGATGGAAAATGGGATTGA	1560
PROTEIN	V A A G V L R E F R V V P A M E N G I E	485
CYP94A3	GCCGGAGTACAcTGCACACCTTACGGCTTTAATGAAAGGTGGTTTCCCTGTGAGGATTGA	1620
PROTEIN	P E Y T A H L T A L M K G G F P V R I E	505
CYP94A3	AAAGCGTAGTCACACAGATGAGTAAaaatagaaataattgttgtacaaaatattttccaa	1680
PROTEIN	K R S H T D E *	512
CYP94A3	aagttccattgttcatatttcgtttgttggaataatgtttaaattcgaatgtgatttgta	1740
PROTEIN		
CYP94A3	ctgtattagttattcagttagctagaactttctttttatgtgatacttgaataagtcctg	1800
PROTEIN		
CYP94A3	ttttttttttaaaaaaaaaaaaaaaaa	1828

FIG. 15B

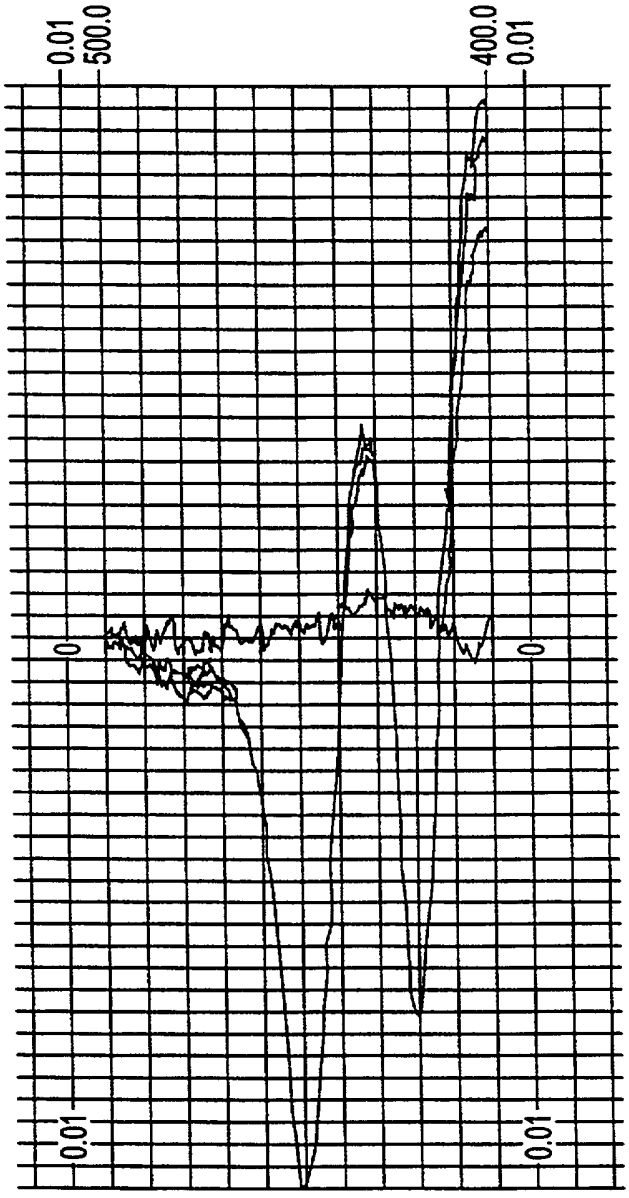


FIG. 16

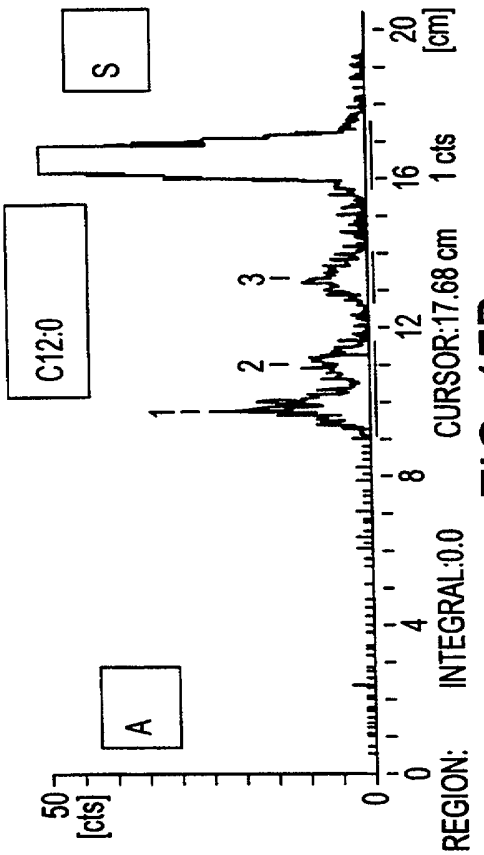


FIG. 17B

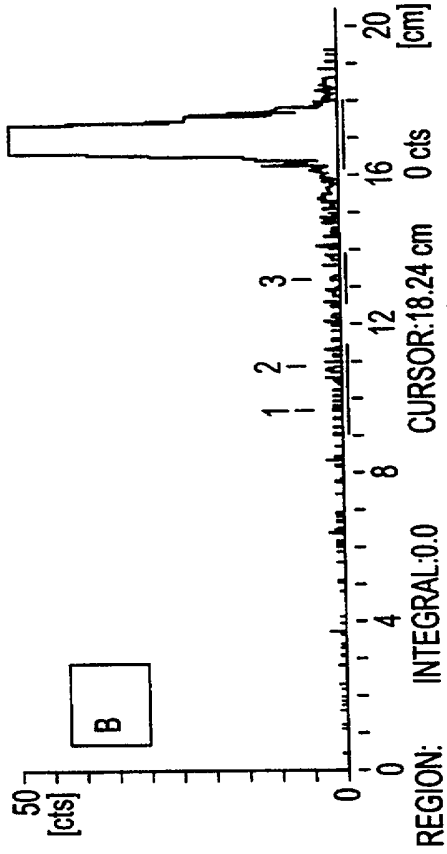


FIG. 17D

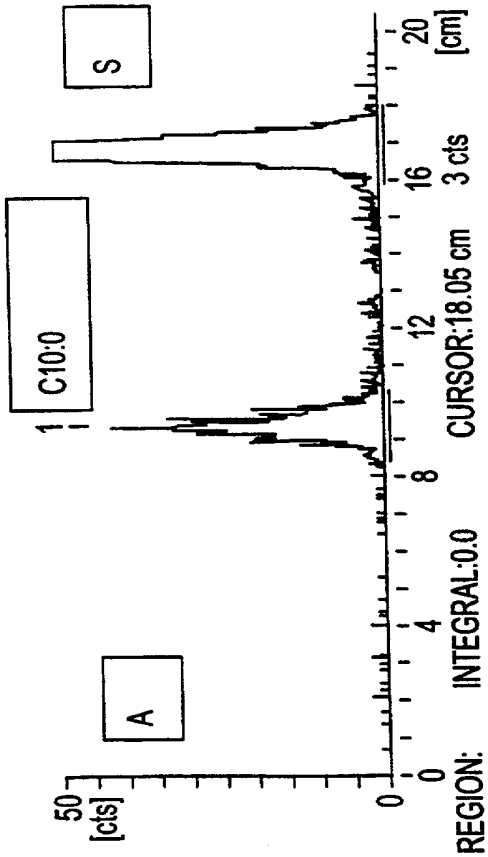


FIG. 17A

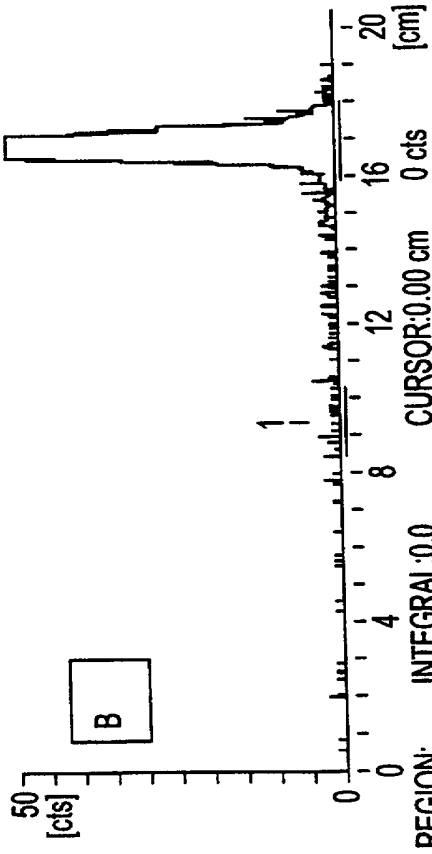


FIG. 17C

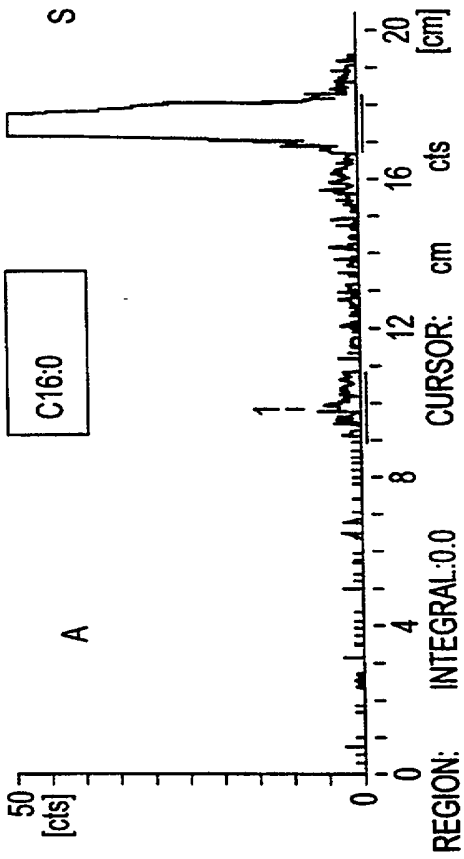


FIG. 18B

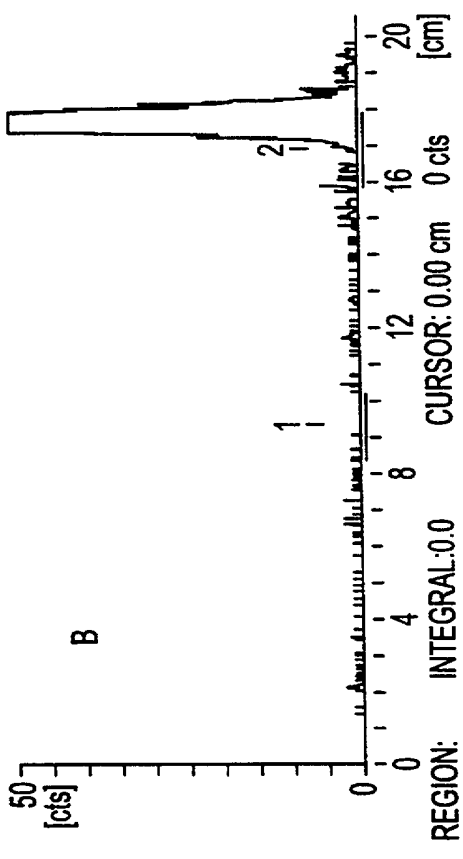


FIG. 18D

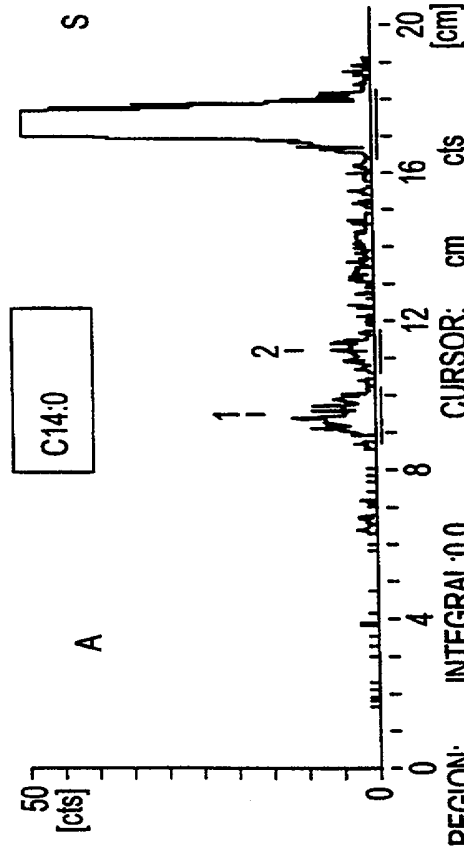


FIG. 18A

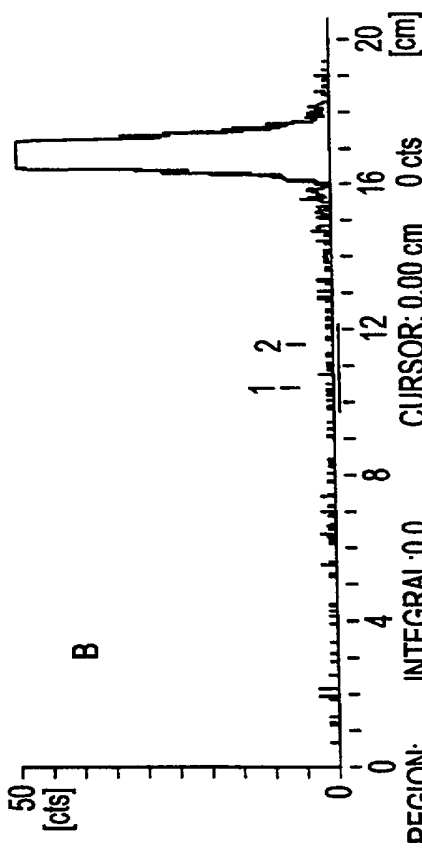


FIG. 18C

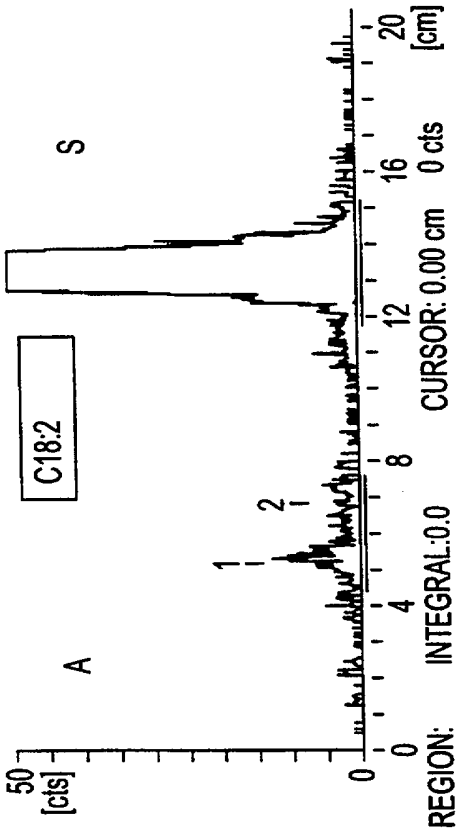


FIG. 19B

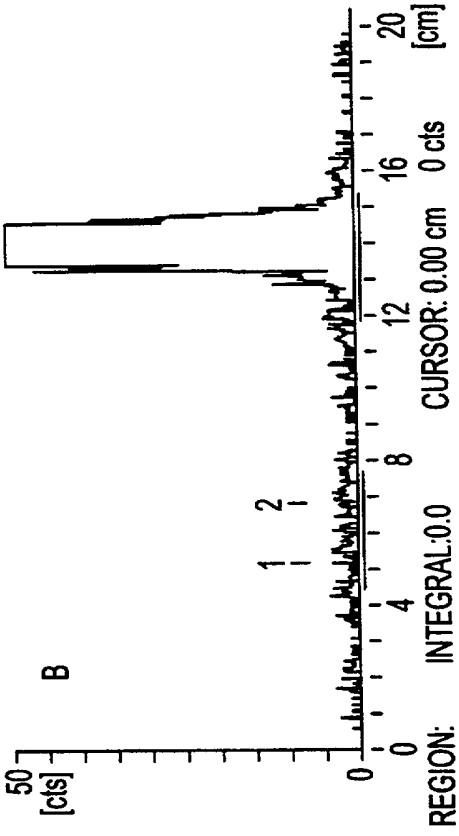


FIG. 19D

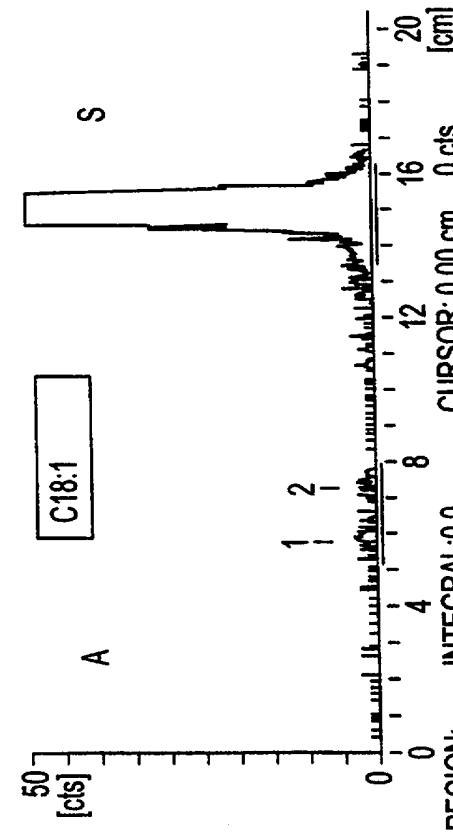


FIG. 19A

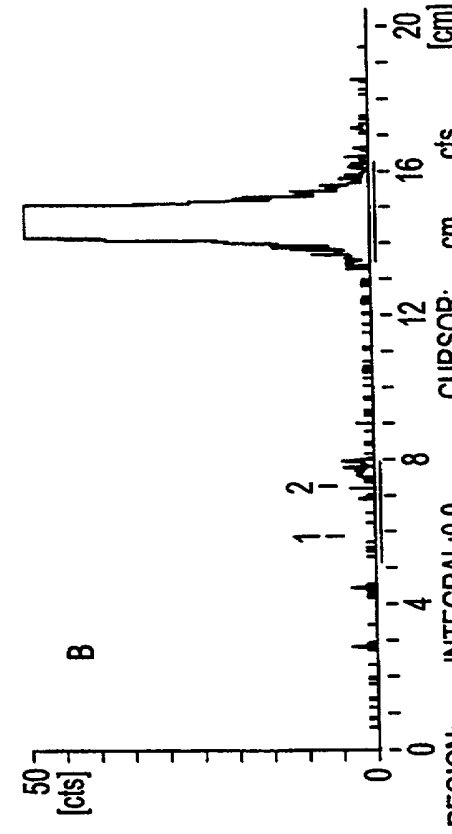


FIG. 19C

```
CYP81B1c    actcttcaacaatATGGAgATCCCATATCTACTCACCACCACCCTCCTCCTCCTCTTCAC 60
PROTEIN                  M  E  I  P  Y  L  L  T  T  T  L  L  L  F  T 16

CYP81B1c    CACCCTCTACCTCCTCCTCCGCCGCCGCTCCTCCACCCTCCCACCCACCATCTTCCCCTC 120
PROTEIN      T  L  Y  L  L  L  R  R  R  S  S  T  L  P  P  T  I  F  P  S 36

CYP81B1c    CCTCCCCATAATCGGCCACCTCTACCTCCTCAAACCACCACTCTACCGAACTCTAgCCAA 180
PROTEIN      L  P  I  I  G  H  L  Y  L  L  K  P  P  L  Y  R  T  L  A  K 56

CYP81B1c    ACTCTCCgCCAAACACGGCCAAATCCTCCGTCTCCAATTGGGTTTTCgACgTgTTCTTAT 240
PROTEIN      L  S  A  K  H  G  Q  I  L  R  L  Q  L  G  F  R  R  V  L  I 76

CYP81B1c    CgTCTCCTCCCCTTCGGCCGCTgAAGAgTgTTTTACTAAAAACgAcATCgTCTTTGCTAA 300
PROTEIN      V  S  S  P  S  A  A  E  E  C  F  T  K  N  D  I  V  F  A  N 96

CYP81B1c    CCgCCCCAAgATgTTgTTTGGGAAAATTATTGGTgTTAATTATACTAgCCTGGCGTgGTC 360
PROTEIN      R  P  K  M  L  F  G  K  I  I  G  V  N  Y  T  S  L  A  W  S 116

CYP81B1c    CCCGTATGGAGACAATTGGCGTAATTTACGCCGTATTGCCTCCATTGAGATCTTGTCCAT 420
PROTEIN      P  Y  G  D  N  W  R  N  L  R  R  I  A  S  I  E  I  L  S  I 136

CYP81B1c    TCATCGCCTTAACGAGTTCCATGATATTCGTGTTGAGGAAACCAGACTTCTAATCCAGAA 480
PROTEIN      H  R  L  N  E  F  H  D  I  R  V  E  E  T  R  L  L  I  Q  K 156

CYP81B1c    ACTGCTGTCCGCTTGCAACTCGGGTTCGTCTCAGGTGACAATGAAGTTTTCGTTTTACGA 540
PROTEIN      L  L  S  A  C  N  S  G  S  S  Q  V  T  M  K  F  S  F  Y  E 176

CYP81B1c    ACTAACATTGAATGTGATGATGAGGATGATCTCCGGTAAGAGGTACTTTGGGGGCGATAA 600
PROTEIN      L  T  L  N  V  M  M  R  M  I  S  G  K  R  Y  F  G  G  D  N 196

CYP81B1c    TCCGGAGTTGGAAGAGGAAGGGAAGCGGTTCCGGGATATGCTGGATGAGACGTTTGTGCT 660
PROTEIN      P  E  L  E  E  E  G  K  R  F  R  D  M  L  D  E  T  F  V  L 216

CYP81B1c    CGCAGGAGCTTCTAACGTCGGCGATTACTTGCCGGTGTTGAGTTGGTTGGGGGTGAAGGG 720
PROTEIN      A  G  A  S  N  V  G  D  Y  L  P  V  L  S  W  L  G  V  K  G 236

CYP81B1c    TTTGGAGAAGAAGTTGATTAAGTTGCAGGAAAAAAGAGATGTTTTCTTTCAGGGGTTAAT 780
PROTEIN      L  E  K  K  L  I  K  L  Q  E  K  R  D  V  F  F  Q  G  L  I 256

CYP81B1c    TGATCAACTTAGGAAATCTAAAGGGACTGAAGATGTAAATAAGAAAAAGACAATGATTGA 840
PROTEIN      D  Q  L  R  K  S  K  G  T  E  D  V  N  K  K  K  T  M  I  E 276

CYP81B1c    ACTGTTGTTATCGTTGCAAGAGACAGAACCGGAGTACTACACTGATGCGATGATTCGAAG 900
PROTEIN      L  L  L  S  L  Q  E  T  E  P  E  Y  Y  T  D  A  M  I  R  S 296

CYP81B1c    CTTTGTGCTGGTTTTATTAGCAGCAGGTAGTGATACATCGGCTGGAACCATGGAATGGGT 960
PROTEIN      F  V  L  V  L  L  A  A  G  S  D  T  S  A  G  T  M  E  W  V 316

CYP81B1c    TATGTCAcTTTTGCTAAACCACCCACAAGTTTTAAAAAAGGCACaAAACGAAATcGATAG 1020
PROTEIN      M  S  L  L  L  N  H  P  Q  V  L  K  K  A  Q  N  E  I  D  S 336

CYP81B1c    CGTTATTGGGaAAAATTGTcTAGTTGACGAGTcGGACATACCCAAcTTACCTTACCTTCG 1080
PROTEIN      V  I  G  K  N  C  L  V  D  E  S  D  I  P  N  L  P  Y  L  R 356

CYP81B1c    CTGTATCATAAACGAGACGTTAAGATTGTATCCTGCGgGCCCATTACTAgTTCCACACGA 1140
PROTEIN      C  I  I  N  E  T  L  R  L  Y  P  A  G  P  L  L  V  P  H  E 376

CYP81B1c    gGCGTCAAGTGATTGTGTTGTTGGCGGCTACAACGTCCCGCGTGGAACAATTTTGATTGT 1200
```

FIG. 20A

```
PROTEIN      A  S  S  D  C  V  V  G  G  Y  N  V  P  R  G  T  I  L  I  V 396

CYP81B1c     TAACCAATGGGCCATACATCATGACCCGAAAGTGTGGGATGAACCAGAAACGTTCAAACC 1260
PROTEIN        N  Q  W  A  I  H  H  D  P  K  V  W  D  E  P  E  T  F  K  P 416

CYP81B1c     AGAAAGGTTTGAAGGGTTAGAAGGGACACGGGATGGGTTTAAGTTATTGCCATTTGGGTC 1320
PROTEIN        E  R  F  E  G  L  E  G  T  R  D  G  F  K  L  L  P  F  G  S 436

CYP81B1c     TGGAAGGAGGAGTTGTCCTGGGGAAGGCTTGGCGGTTCGAATGCTTGGGATGACTTTAGG 1380
PROTEIN        G  R  R  S  C  P  G  E  G  L  A  V  R  M  L  G  M  T  L  G 456

CYP81B1c     GTCAATTATTCAATGCTTCGATTGGGAACGAACGAGTGAAGAGTTGGTTGATATGACTGA 1440
PROTEIN        S  I  I  Q  C  F  D  W  E  R  T  S  E  E  L  V  D  M  T  E 476

CYP81B1c     AGGTCCTGGGCTAACCATGCCTAAGGCTATACCATTGGTAGCTAAGTGCAAACCTCGGGT 1500
PROTEIN        G  P  G  L  T  M  P  K  A  I  P  L  V  A  K  C  K  P  R  V 496

CYP81B1c     TGAGATGACGAATCTACTGTCCGAACTGTGAgtcggttgctggttcctttgagataatgt 1560
PROTEIN        E  M  T  N  L  L  S  E  L  *                              505

CYP81B1c     ttggtcatatgatgggtctttctttttgctgtttctagccttgttctttggattttgaat 1620
PROTEIN

CYP81B1c     acaggtatttgtatgattatatagtattaattaaagttgaaatccttacgtagc        1674
PROTEIN
```

FIG. 20B

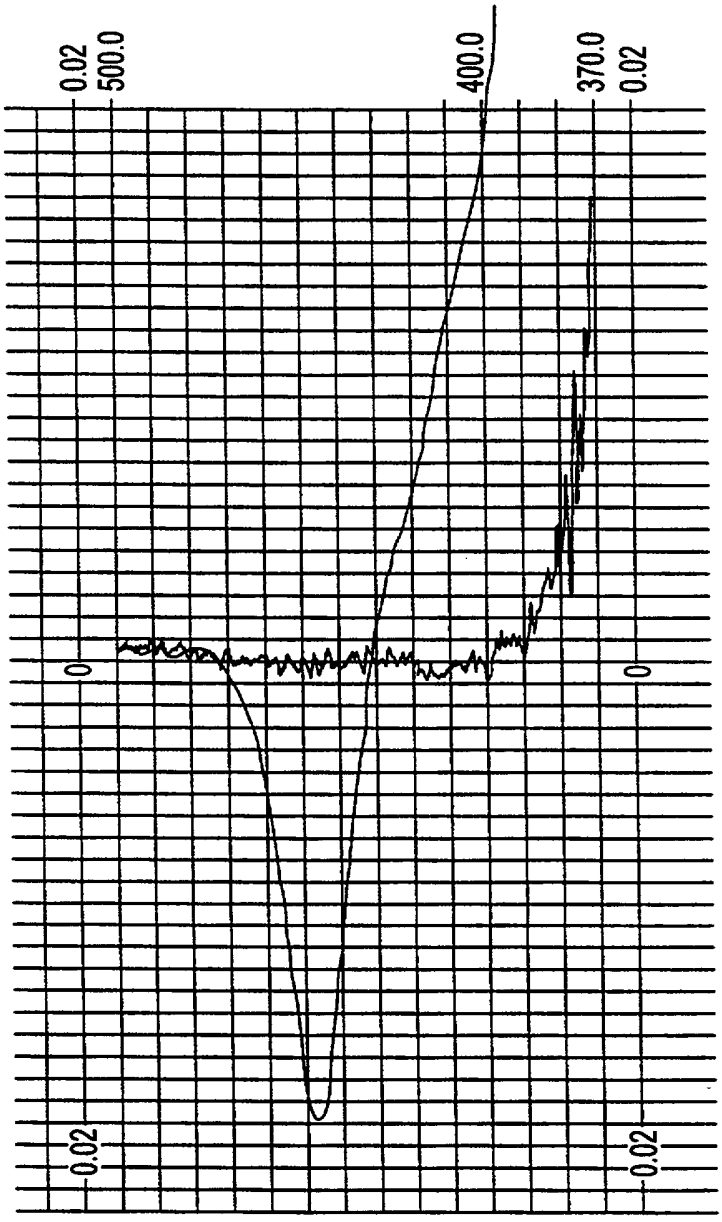


FIG. 21

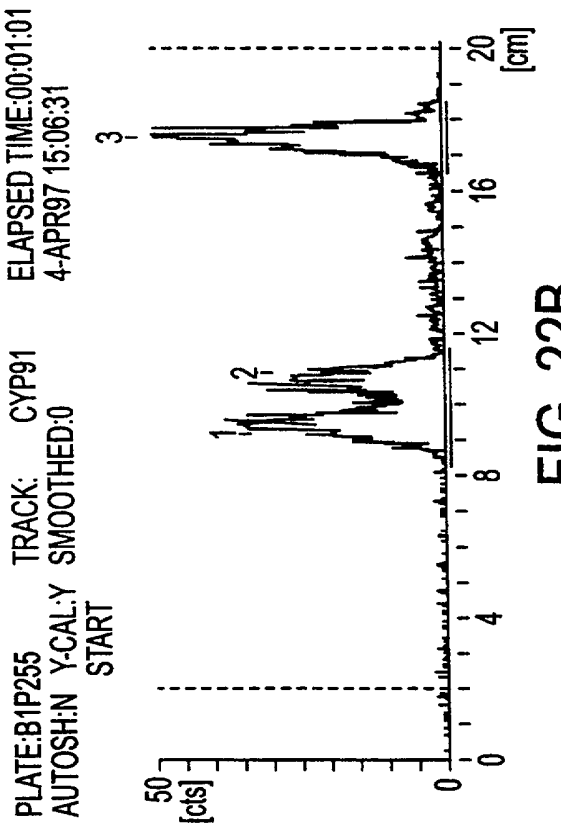


FIG. 22A

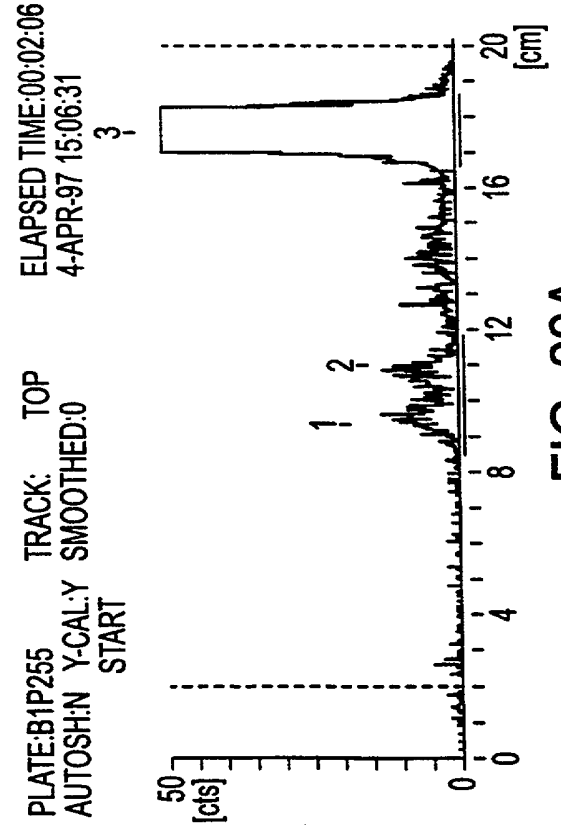


FIG. 22B

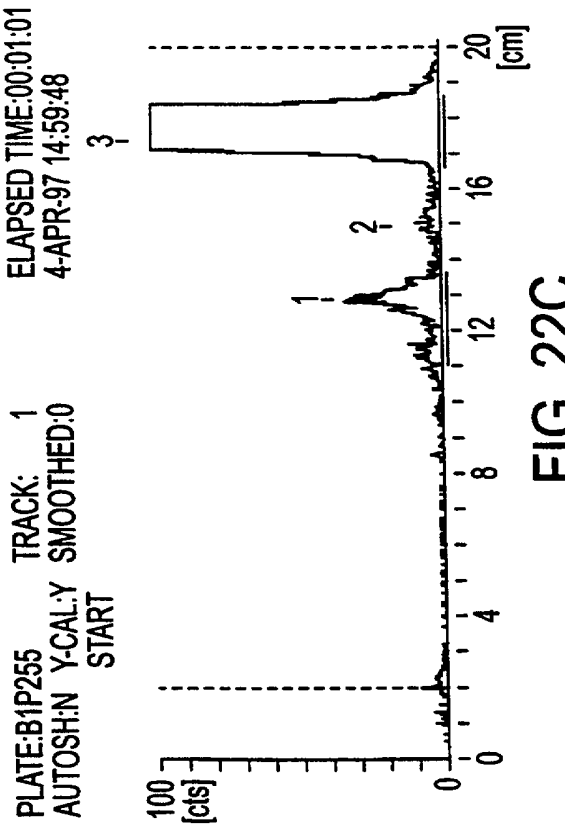


FIG. 22C

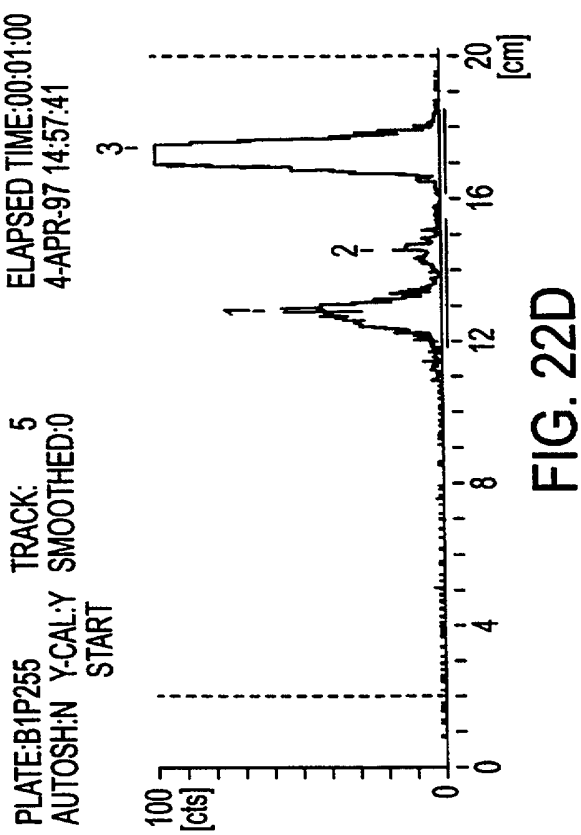


FIG. 22D

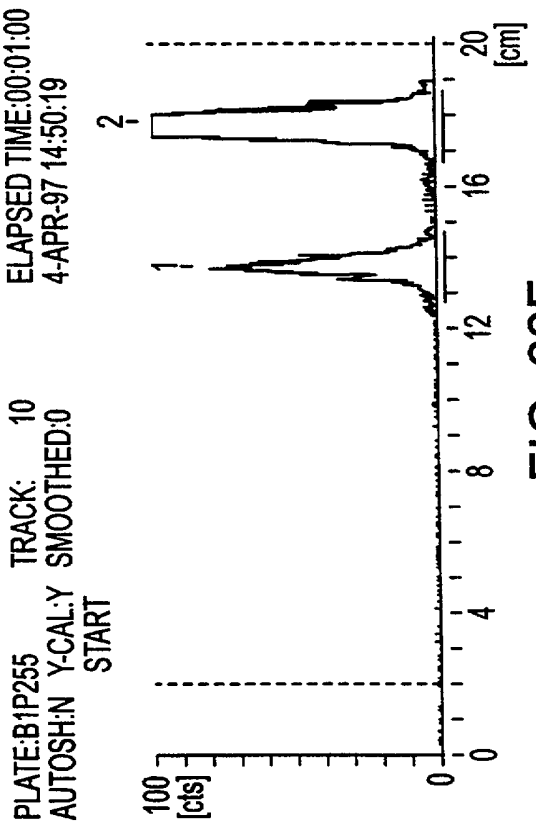


FIG. 22F

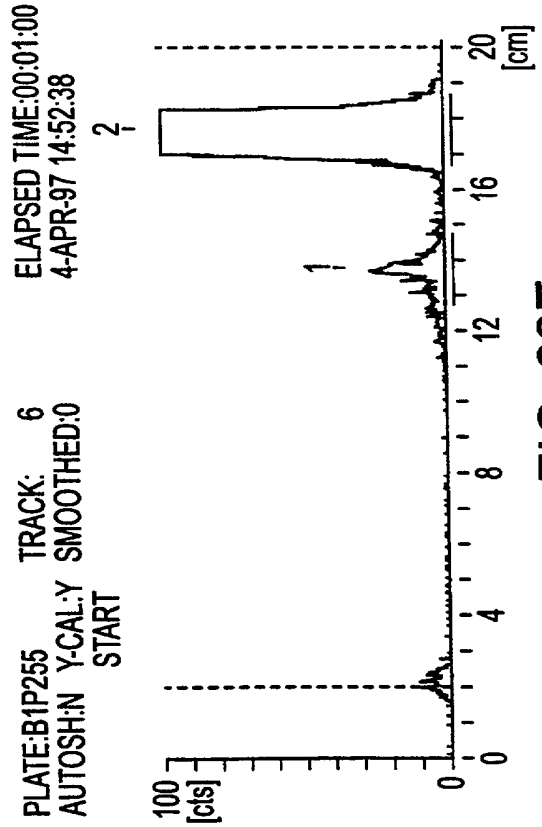


FIG. 22E

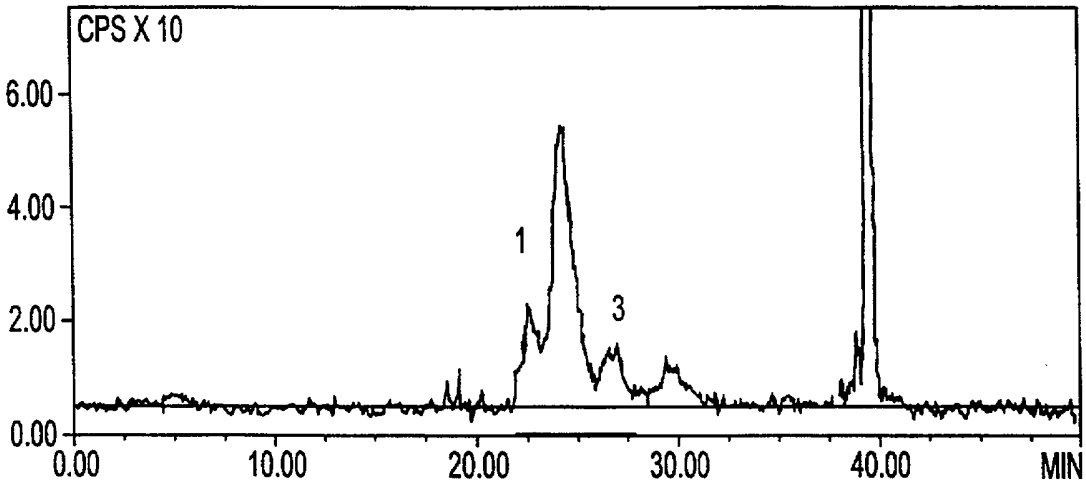


FIG. 23A

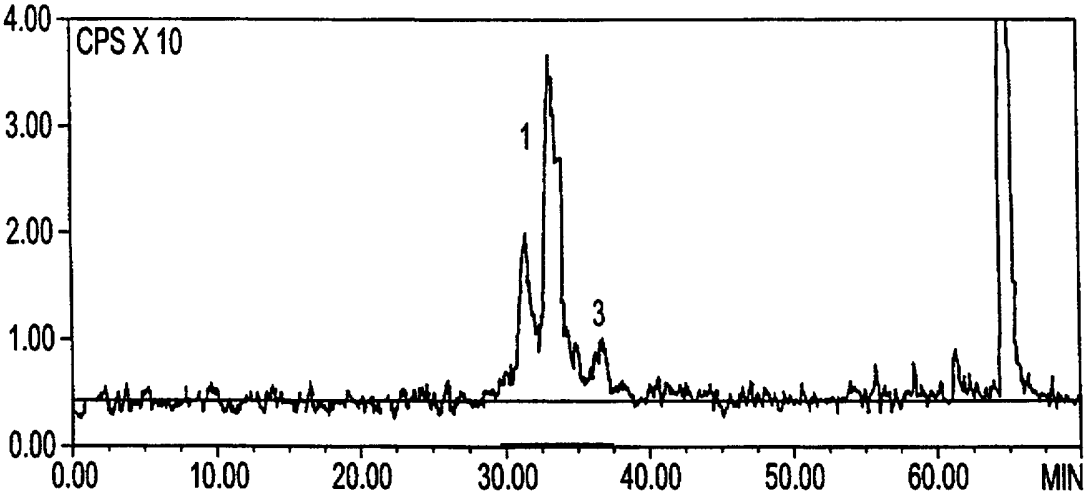


FIG. 23B

```
CYP94A4    gcggccgcaaaagaacaactaaaacctcaaaaagatatacgtaaATGATGATAGACTTGG 60
PROTEIN                                                M  M  I  D  L   5

CYP94A4    AGCTTTCAACCTCTTTACTCTTTTGCCTCATTCCTTTCCTCTTCCTCTTGTTCATCAAAA 120
PROTEIN    E  L  S  T  S  L  L  F  C  L  I  P  F  L  F  L  L  F  I  K   25

CYP94A4    CCACTTTAGCCAATCTTTTCTCATCCAATAAATCCACCAGCAAGATCCCAAAATCATATC 180
PROTEIN    T  T  L  A  N  L  F  S  S  N  K  S  T  S  K  I  P  K  S  Y   45

CYP94A4    CAATTATTGGTTCCTGTTTCTCGATCCTAGCAAACAAAGAACGTCGAATTCAATGGACTT 240
PROTEIN    P  I  I  G  S  C  F  S  I  L  A  N  K  E  R  R  I  Q  W  T   65

CYP94A4    CTGATATTATGCAAAACACTTCCAACTTAACTTTCACTCTCAATCGTCCTTTTGGTTTTC 300
PROTEIN    S  D  I  M  Q  N  T  S  N  L  T  F  T  L  N  R  P  F  G  F   85

CYP94A4    GCCAAATTTTCACAGCTAACCCCGCCAATGTCCAACACATGCTCAAAACCCAATTTCACA 360
PROTEIN    R  Q  I  F  T  A  N  P  A  N  V  Q  H  M  L  K  T  Q  F  H  105

CYP94A4    TTTACCAAAAAGGTGATGTTTTTAAAACAACTATGGCTGATTTTCTTGGTGATGGCATAT 420
PROTEIN    I  Y  Q  K  G  D  V  F  K  T  T  M  A  D  F  L  G  D  G  I  125

CYP94A4    TTAACGTGGACGGTGACATTTGGAAGTACCAAAGACAAGTTTCAAGCCATGAGTTTAACA 480
PROTEIN    F  N  V  D  G  D  I  W  K  Y  Q  R  Q  V  S  S  H  E  F  N  145

CYP94A4    CAAAATCTCTACGCAAGTTCGTTGAAACTGTTGTTGATACAGAACTCAACGAAAGGCTAA 540
PROTEIN    T  K  S  L  R  K  F  V  E  T  V  V  D  T  E  L  N  E  R  L  165

CYP94A4    TTCCAATTCTTGCTACTGCTGCTGTTGAAAAAACCGTTCTGGATTTTCAAGACATTTTAC 600
PROTEIN    I  P  I  L  A  T  A  A  V  E  K  T  V  L  D  F  Q  D  I  L  185

CYP94A4    AAAGGTTTGCTTTTGATAATATTTGTAAAATTGCTTTTGGCTATGATCCTGCTTATTTAT 660
PROTEIN    Q  R  F  A  F  D  N  I  C  K  I  A  F  G  Y  D  P  A  Y  L  205

CYP94A4    TACCATCTCTTCCTCAAGCAAAATTTGCTGTTGCTTTTGAAGAAGCTGTTAAGCTAAGTA 720
PROTEIN    L  P  S  L  P  Q  A  K  F  A  V  A  F  E  E  A  V  K  L  S  225

CYP94A4    GTGAAAGATTTAATGCTATTTTCCCTTTTGTATGGAAAATAAAACGAAATTTCAATATTG 780
PROTEIN    S  E  R  F  N  A  I  F  P  F  V  W  K  I  K  R  N  F  N  I  245

CYP94A4    GATCTGAGAAAAAAATCAGGGTAGCTGTGAATGAAGTTCGTCAATTTGCAAAAGAACTCG 840
PROTEIN    G  S  E  K  K  I  R  V  A  V  N  E  V  R  Q  F  A  K  E  L  265

CYP94A4    TGAAAGAAAAACAAAAAGAACTCAAAGAAAAATCATCACTCGATTCAGTGGATTTACTAT 900
PROTEIN    V  K  E  K  Q  K  E  L  K  E  K  S  S  L  D  S  V  D  L  L  285

CYP94A4    CAAGATTTTTAAGCAGTGGCCATTCGGATGAGGACTTTGTTACAGATATTGTTATAAGTT 960
PROTEIN    S  R  F  L  S  S  G  H  S  D  E  D  F  V  T  D  I  V  I  S  305

CYP94A4    TCATTTTGGCTGGTCGTGACACAACATCTGCTGCTTTAACATGGTTTTTTTGGTTAATTT 1020
PROTEIN    F  I  L  A  G  R  D  T  T  S  A  A  L  T  W  F  F  W  L  I  325

CYP94A4    TTGAACACCCAGAAACAGAAAACCAAATCTTAAAAGAGGCTAAAGCAAAATCCGAAAGTC 1080
PROTEIN    F  E  H  P  E  T  E  N  Q  I  L  K  E  A  K  A  K  S  E  S  345

CYP94A4    CAGTGTATGATGAAGTGAAGGACATGATTTACACACATGCTTCACTTTGTGAGAGCATGA 1140
PROTEIN    P  V  Y  D  E  V  K  D  M  I  Y  T  H  A  S  L  C  E  S  M  365

CYP94A4    GATTTTACCCACCAATTCCCATAGATACTAAAGCTGCTACAGAGGATAATATTTTGCCAG 1200
```

FIG. 24A

```
PROTEIN     R  F  Y  P  P  I  P  I  D  T  K  A  A  T  E  D  N  I  L  P   385

CYP94A4     ATGGTACTTTTGTGAAAAAGGGGACTAGAGTAAGTTATCATATCTATGCAATGGGGAGAG 1260
PROTEIN     D  G  T  F  V  K  K  G  T  R  V  S  Y  H  I  Y  A  M  G  R   405

CYP94A4     TGGAGAACTTATGGGGAAAAGATTGGGCAGAGTTTAGGCCAGAGAGGTGGTTAGATAAGG 1320
PROTEIN     V  E  N  L  W  G  K  D  W  A  E  F  R  P  E  R  W  L  D  K   425

CYP94A4     ATGAAGCGTCAGGTAATTGGACTTTTGTGGCTAGGGACACCTATACTTATCCTGTTTTTC 1380
PROTEIN     D  E  A  S  G  N  W  T  F  V  A  R  D  T  Y  T  Y  P  V  F   445

CYP94A4     AGGCGGGACCAAGAATTTGTTTAGGAAAAGAAATGGCATTTTTGCAGATGAAGAGGGTGG 1440
PROTEIN     Q  A  G  P  R  I  C  L  G  K  E  M  A  F  L  Q  M  K  R  V   465

CYP94A4     TGGCTGGTGTTTTACGGCGGTTCAAGGTGGTTCCGCTGGCAGAAAAAGGTGTTGAGCCGG 1500
PROTEIN     V  A  G  V  L  R  R  F  K  V  V  P  L  A  E  K  G  V  E  P   485

CYP94A4     TCTTTTTGTCTTACCTCACTGCGAAAATGAAAGGTGGTTTCCCTGTGACAATTGAGGAAA 1560
PROTEIN     V  F  L  S  Y  L  T  A  K  M  K  G  G  F  P  V  T  I  E  E   505

CYP94A4     GGAACGGTACGGATATTTGAtctttcaactatggccaaagagtacgagggtaaaggtttt 1620
PROTEIN     R  N  G  T  D  I  *                                           511

CYP94A4     tattgcttcttccacttaccttaaaagtgtttggattttgtgacatttatttatgtttgt 1680
PROTEIN

CYP94A4     ataaagctgctttataagaagtgagtattatttattaaaaaaaa                  1724
PROTEIN
```

FIG. 24B

```
CYP94A5    gcggccgccattaatcaaaaaccaaagtccaaaatccatcctcttgagagataaaaaaac 60
PROTEIN

CYP94A5    tttaggcaATGGCACTATTAGACTTACAACTCTCAACCTCATTACTCTTTTGCCTTGTTC 120
PROTEIN              M  A  L  L  D  L  Q  L  S  T  S  L  L  F  C  L  V  17

CYP94A5    CTTTGCTCTTCCTTTTTTTCGTCAAATTCAAGAAAACAATTACTAATACCCTTTTATCAT 180
PROTEIN    P  L  L  F  L  F  F  V  K  F  K  K  T  I  T  N  T  L  L  S  37

CYP94A5    CCAATAACTCTAGTAAGATACCAAGATCTTATCCTCTAATAGGTTCTTATTTTTCCATCT 240
PROTEIN    S  N  N  S  S  K  I  P  R  S  Y  P  L  I  G  S  Y  F  S  I  57

CYP94A5    TGGCAAATCACGACCGGCGGATAAAATGGATATCGGATATTATCCTAACCACCCCTAACC 300
PROTEIN    L  A  N  H  D  R  R  I  K  W  I  S  D  I  I  L  T  T  P  N  77

CYP94A5    TCACTTTTACTCTCATTCGCCCTCTCAATTTTCGCACAATTTTCACTGCAAACCCTTCCA 360
PROTEIN    L  T  F  T  L  I  R  P  L  N  F  R  T  I  F  T  A  N  P  S  97

CYP94A5    ATGTCCAACACGTGCTCAAAACAAATTTTCAAGTCTACCAAAAAGGTCATGGTTCGTACA 420
PROTEIN    N  V  Q  H  V  L  K  T  N  F  Q  V  Y  Q  K  G  H  G  S  Y  117

CYP94A5    GTACCCTCAAAGATTTTCTCAGTAATGGTATTTTTAATGTCGATGGTGATATATGGAAGT 480
PROTEIN    S  T  L  K  D  F  L  S  N  G  I  F  N  V  D  G  D  I  W  K  137

CYP94A5    ACCAAAGACAAGTTGCTAGCCATGAATTTAACACTAGGTCGTTACGTAAATTTGTTGAGA 540
PROTEIN    Y  Q  R  Q  V  A  S  H  E  F  N  T  R  S  L  R  K  F  V  E  157

CYP94A5    CAGTTGTTGATACTGAACTTTCTGAACGTTTGATACCAATTCTTGCCACTGCTGCTGCTA 600
PROTEIN    T  V  V  D  T  E  L  S  E  R  L  I  P  I  L  A  T  A  A  A  177

CYP94A5    ATAAAACTGTTCTTGATTTCCAAGACATATTACAAAGGTTTGCTTTTGACAACATTTGTA 660
PROTEIN    N  K  T  V  L  D  F  Q  D  I  L  Q  R  F  A  F  D  N  I  C  197

CYP94A5    AAATTGCTTTTGGATATGATCCTGGCTATTTGTTACCGTCACTTCCCGAGGCAGAATTTG 720
PROTEIN    K  I  A  F  G  Y  D  P  G  Y  L  L  P  S  L  P  E  A  E  F  217

CYP94A5    CTGTTGCTTTTGAAGATGCTGTTCGTCTCAGCACTGAAAGATTCATTGTTCCTTTCTCTC 780
PROTEIN    A  V  A  F  E  D  A  V  R  L  S  T  E  R  F  I  V  P  F  S  237

CYP94A5    TTATTTGGAAAATCAAACGAGCTTTAAACATTGGATCGGAGAAAAAACTAAGGGTTGCTG 840
PROTEIN    L  I  W  K  I  K  R  A  L  N  I  G  S  E  K  K  L  R  V  A  257

CYP94A5    TAGAACAAGTACGTGAATTTGCGAAAGAGATTGTTAGAGAAAAACAAAAGGAGCTAAACG 900
PROTEIN    V  E  Q  V  R  E  F  A  K  E  I  V  R  E  K  Q  K  E  L  N  277

CYP94A5    ATAAATCATCGCTCGATTCAGCTGATTTATTGTCAAGATTCTTGAGCACTGGACACTCCG 960
PROTEIN    D  K  S  S  L  D  S  A  D  L  L  S  R  F  L  S  T  G  H  S  297

CYP94A5    ATGAAGACTTCGTTACGGATATTGTGATCAGCTTTATATTGGCAGGACGTGACACAACTT 1020
PROTEIN    D  E  D  F  V  T  D  I  V  I  S  F  I  L  A  G  R  D  T  T  317

CYP94A5    CAGCAGCTTTAACATGGTTTTTTTGGCTAATTTCTAAACACCCTGAAGTAGAATCACAAA 1080
PROTEIN    S  A  A  L  T  W  F  F  W  L  I  S  K  H  P  E  V  E  S  Q  337

CYP94A5    TCATGAAAGAAGTTGGAGAGAAATCAGAATCTTTATTACTATATGATGAAGTGAAAAACA 1140
PROTEIN    I  M  K  E  V  G  E  K  S  E  S  L  L  L  Y  D  E  V  K  N  357

CYP94A5    TGATGTATACTCATGCATCTCTTTGTGAAAGCATGAGATTTTATCCGCCAGTTCCAATGG 1200
PROTEIN    M  M  Y  T  H  A  S  L  C  E  S  M  R  F  Y  P  P  V  P  M  377
```

FIG. 25A

```
CYP94A5     ATTCTAAAGAAGCAACAAAAGATGATATATTGCCAGATGGTACATTTGTGAAAAAGGGTA 1260
PROTEIN     D  S  K  E  A  T  K  D  D  I  L  P  D  G  T  F  V  K  K  G   397

CYP94A5     CGAGGGTAACTTATCATCCTTACGCAATGGGAAGAGTCGAGAAAGTATGGGGCGAAGATT 1320
PROTEIN     T  R  V  T  Y  H  P  Y  A  M  G  R  V  E  K  V  W  G  E  D   417

CYP94A5     GGGCAGAATTTAAGCCAGAAAGATGGTTGGATAAAGATGAAGTGACAGGGAATTGGACGT 1380
PROTEIN     W  A  E  F  K  P  E  R  W  L  D  K  D  E  V  T  G  N  W  T   437

CYP94A5     TTGTGCCAAAAGATGCATATACATATCCTGTGTTTCAAGCGGGGCCAAGAATTTGTTTAG 1440
PROTEIN     F  V  P  K  D  A  Y  T  Y  P  V  F  Q  A  G  P  R  I  C  L   457

CYP94A5     GGAAAGAAATGGCCTTTTTGCAAATGAAAAGAGTGGTGGCTGGTGTTTTACGGCGGTTTA 1500
PROTEIN     G  K  E  M  A  F  L  Q  M  K  R  V  V  A  G  V  L  R  R  F   477

CYP94A5     AGGTGGTTCCGGTGGTGGAACAAGGGGTGGAGCCAGTGTTCATATCGTATCTCACGGCCA 1560
PROTEIN     K  V  V  P  V  V  E  Q  G  V  E  P  V  F  I  S  Y  L  T  A   497

CYP94A5     AGATGAAAGGAGGTTTTCCTGTTACTATTGAAGAAAGGATATAGgaatatcctatggtca 1620
PROTEIN     K  M  K  G  G  F  P  V  T  I  E  E  R  I  *                  511

CYP94A5     attggtgaaaaaagtagttttgtttttatgtgttgctttaaatcttttgctttttcaac 1680
PROTEIN

CYP94A5     tgtgctactgtaattcattttgatattcataatttgtatttatattagttttaaaaaaa 1740
PROTEIN

CYP94A5     aaaaaaaaaa                                                  1750
PROTEIN
```

FIG. 25B

```
CYP94A6     cattttaatgtcgatggtgacatatggtaaaagtgtaggtgtagcagtctcaagaaaat 60
PROTEIN

CYP94A6     catgagacatcaaaacttctataaataacctttctagtagaccatagcttcaattcattg 120
PROTEIN

CYP94A6     tcaatcttagtcttccattaatcaaaaaccacaagtccaaaatccaccctcttgagaaaa 180
PROTEIN

CYP94A6     aaaacgactttaggcaATGGCACTATTAGACCTACAACCCTCAACCTCATTACTCTTTTG 240
PROTEIN                   M  A  L  L  D  L  Q  P  S  T  S  L  L  F  C  15

CYP94A6     CCTTGTTCCTTTGCTCTTTCTTTTCTTCATCAAATTCAACAAAACAATAACTAATACCCT 300
PROTEIN       L  V  P  L  L  F  L  F  F  I  K  F  N  K  T  I  T  N  T  L  35

CYP94A6     TTTGTCGTCCAACTCTAGTAAGATACCAAGATCTTATCCTCTAATAGGTTCCTATTTTTC 360
PROTEIN       L  S  S  N  S  S  K  I  P  R  S  Y  P  L  I  G  S  Y  F  S  55

CYP94A6     CATATTGGCAAATCACGACCAGCGAATAAAATGGATATCCGATATTATCCTAAGCACCCC 420
PROTEIN       I  L  A  N  H  D  Q  R  I  K  W  I  S  D  I  I  L  S  T  P  75

CYP94A6     TAACCTCACTTTTACTCTCATTCGCCCTCTCAATTTCCATACAATTTTCACCGCAAACCC 480
PROTEIN       N  L  T  F  T  L  I  R  P  L  N  F  H  T  I  F  T  A  N  P  95

CYP94A6     TTCCAATGTCCAGCACATGCTCAAAACAAATTTTCAAGTCTACCAAAAAGGCCACAATTC 540
PROTEIN       S  N  V  Q  H  M  L  K  T  N  F  Q  V  Y  Q  K  G  H  N  S  115

CYP94A6     GAACACTACTCTTAAAGACTTCCTTAGTAATGGCATTTTTAATGTCGATGGTGACATATG 600
PROTEIN       N  T  T  L  K  D  F  L  S  N  G  I  F  N  V  D  G  D  I  W  135

CYP94A6     GAAGTACCAAAGACAAGTTGCAAGCCATGAATTTAACACTAGGTCGTTACGTAAGTTTGT 660
PROTEIN       K  Y  Q  R  Q  V  A  S  H  E  F  N  T  R  S  L  R  K  F  V  155

CYP94A6     AGAGACAGTTGTTGATACTGAACTGTCCGAACGTTTGATACCTATTCTTGCCACTGCTGC 720
PROTEIN       E  T  V  V  D  T  E  L  S  E  R  L  I  P  I  L  A  T  A  A  175

CYP94A6     TGCTAACAAAACTGTTCTTGATTTCCAAGACATACTCCAAAGGTTTGCTTTTGACAACAT 780
PROTEIN       A  N  K  T  V  L  D  F  Q  D  I  L  Q  R  F  A  F  D  N  I  195

CYP94A6     TTGTAAAATTGCTTTTGGATATGATCCTGGTTATTTGTTACCATCACTTCCCGAGGCAGA 840
PROTEIN       C  K  I  A  F  G  Y  D  P  G  Y  L  L  P  S  L  P  E  A  E  215

CYP94A6     ATTTGCAGTTGCTTTTGAAGATGCTGTTCGTCTTAGCACTGAAAGGTTCATTCTTCCTTT 900
PROTEIN       F  A  V  A  F  E  D  A  V  R  L  S  T  E  R  F  I  L  P  F  235

CYP94A6     CCCTCTTATTTGGAAAATGAAACGAGCTTTAAACATCGGATCAGAGAAGAAACTAAGGTT 960
PROTEIN       P  L  I  W  K  M  K  R  A  L  N  I  G  S  E  K  K  L  R  F  255

CYP94A6     TGCTGTAGAACAAGTACGTGAATTTGCCAAGGAGATTGTTAGAGAAAAACAAAGGGAGCT 1020
PROTEIN       A  V  E  Q  V  R  E  F  A  K  E  I  V  R  E  K  Q  R  E  L  275

CYP94A6     AAAAGATAAATCATCGCTCGATTCAGCTGATTTATTGTCAAGATTCTTGAGTACAGGGCA 1080
PROTEIN       K  D  K  S  S  L  D  S  A  D  L  L  S  R  F  L  S  T  G  H  295

CYP94A6     TTCGGATGAAAACTTTGTTACTGATATTGTAATCAGCTTTATATTGGCAGGACGTGACAC 1140
PROTEIN       S  D  E  N  F  V  T  D  I  V  I  S  F  I  L  A  G  R  D  T  315
```

FIG. 26A

```
CYP94A6     AACTTCAGCAGCTTTAACATGGTTCTTTTGGCTAATTTCTAAACACCCTGAAGTGGAATC 1200
PROTEIN       T  S  A  A  L  T  W  F  F  W  L  I  S  K  H  P  E  V  E  S 335

CYP94A6     CCAAATCTTGAAAGAAATTGGAGAGAAATCAGAATCTTTATTACTCTATGATGAAGTAAA 1260
PROTEIN       Q  I  L  K  E  I  G  E  K  S  E  S  L  L  L  Y  D  E  V  K 355

CYP94A6     GAACATGATATATACTCATGCATCTCTTTGTGAAAGCATGAGATTTTATCCGCCCGTTCC 1320
PROTEIN       N  M  I  Y  T  H  A  S  L  C  E  S  M  R  F  Y  P  P  V  P 375

CYP94A6     AATGGACACTAAAGAAGCAACAAAAGATGATATATTGCCAGATGGTACATTTGTGAAAAA 1380
PROTEIN       M  D  T  K  E  A  T  K  D  D  I  L  P  D  G  T  F  V  K  K 395

CYP94A6     GGGCAATAGAGTAACTTATCATCCTTACGCAATGGGAAGAGTAGAGAAAGTGTGGGGCAA 1440
PROTEIN       G  N  R  V  T  Y  H  P  Y  A  M  G  R  V  E  K  V  W  G  K 415

CYP94A6     AGATTGGGCTGAATTTAGACCAGAAAGATGGTTGGATAAAGATGAAGTGACAGGGAATTG 1500
PROTEIN       D  W  A  E  F  R  P  E  R  W  L  D  K  D  E  V  T  G  N  W 435

CYP94A6     GACATTTGTGTCAAAAGATGCATATACATATCCTGTGTTTCAAGCGGGGCCAAGAGTTTG 1560
PROTEIN       T  F  V  S  K  D  A  Y  T  Y  P  V  F  Q  A  G  P  R  V  C 455

CYP94A6     TTTAGGGAAAGAAATGGCATTTTTGCAAATGAAAAGAGTGGTGGCTGGTGTTTTACGGCG 1620
PROTEIN       L  G  K  E  M  A  F  L  Q  M  K  R  V  V  A  G  V  L  R  R 475

CYP94A6     AATCAAGGTGGTTCCAGTGGTGGAACAAGGGGCGGAGCCAGTGTTCATATCGTATCTCAC 1680
PROTEIN       F  K  V  V  P  V  V  E  Q  G  A  E  P  V  F  I  S  Y  L  T 495

CYP94A6     GGCCAAGATGAAGGAGGTTTTCCCTGTTACTATTGAAGAAAGGATATAGgaatatcctat 1740
PROTEIN       A  K  M  K  E  V  F  P  V  T  I  E  E  R  I  *              510

CYP94A6     ggtcaaaaacgtcaacatgtcaaaaaagcagttttatttttctgtttaactgttttaag 1800
PROTEIN

CYP94A6     tcttctgcttttcaaatttgctactgtaattgattgtgatattcataatttgtatttat 1860
PROTEIN

CYP94A6     attagttttaaaattttctgataaaaaaa                                1890
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FIG. 26B

SEQUENCE LISTING

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<140> UNKNOWN

<141> 1998-10-06

<150> US 60/60960

<151> 1997-10-06

<160> 16

<170> PatentIn Ver. 2.0

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<223> Description of Artificial Sequence: Signature sequence characteristic of plant fatty acid omega-hydroxylases

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ccgttaccaa tcatatggaa aatcaaaaaa tacttcaaca taggttcaga gaacgtgctc 840

aaggaacgag taacagaagt acgaagtttc gcgaaaaagc tagtacgaga gaagaaacga 900

gaactagaag agaaatcatc gcttgaaaca gaagatatgt tatcaaggtt cttgagctcg 960

ggtcattcgg atgaagattt tgttgctgat attgtaataa gttttatttt agcgggtaaa 1020

gatacaactt cagctgcatt aacgtggttc ttctggctgt tatggaagaa tccgcgtgtt 1080

gaggaagaga ttgtgaatga attaagtaaa aaatctgagt taatggttta tgatgaagtg 1140

aaggaaatgg tttatactca cgctgctttg agcgagagta tgagattgta tccacctgta 1200

ccgatggata gtaaggaggc tgttaacgat gatgttttac cggatggatg ggttgtgaag 1260

aaagggacaa ttgtgactta tcatgtttat gcaatgggga ggatgaagag tttgtggggg 1320

gatgattggg ctgagtttag gccggagagg tggttggaga aggatgaggt gaatgggaag 1380

tgggtttttg tggggagaga ttcgtattct tatccggttt ttcaggccgg gccgagggtt 1440

tgtttgggga aggaaatggc ttttatgcaa atgaagagga ttgttgcggg gattgttgga 1500

aagtttaagg ttgttcctga ggcgcatttg gctcaagaac ccggttttat ttctttttg 1560

agttcgcaga tggaaggtgg gtttcctgtc acgattcaga agagggattc ttgattaatt 1620

catgagagca ttcacattag ttattaactc attactaatt ggttatatat atcgttttgt 1680

tttgtttgtg tgtctgtcgt tgttgtagtc ggatgttgct taagtgatat gtatagtgta 1740

gtttcttatt tagtagtatg ttttatttac ggttaacact tcagttgata aatacttgga 1800

ttgtattttg attgaatatg tatttataac tttattattt attgtaaaaa aaaaaaaaaa 1860

aa 1862

<210> 4

<211> 514

<212> PRT

<213> Vicia sativa

<220>

<221> PEPTIDE

<222> (1)..(514)

<223> DEDUCED SEQUENCE

<400> 4

Met Phe Gln Phe His Leu Glu Val Leu Leu Pro Tyr Leu Leu Pro Leu

1 5 10 15

Leu Leu Leu Ile Leu Pro Thr Thr Ile Phe Phe Leu Thr Lys Pro Asn

20 25 30

Asn Lys Val Ser Ser Thr Ser Thr Asn Asn Asn Ile Ile Thr Leu Pro

35 40 45

Lys Ser Tyr Pro Leu Ile Gly Ser Tyr Leu Ser Phe Arg Lys Asn Leu

50 55 60

His Arg Arg Ile Gln Trp Leu Ser Asp Ile Val Gln Ile Ser Pro Ser
65 70 75 80

Ala Thr Phe Gln Leu Asp Gly Thr Leu Gly Lys Arg Gln Ile Ile Thr
85 90 95

Gly Asn Pro Ser Thr Val Gln His Ile Leu Lys Asn Gln Phe Ser Asn
100 105 110

Tyr Gln Lys Gly Thr Thr Phe Thr Asn Thr Leu Ser Asp Phe Leu Gly
115 120 125

Thr Gly Ile Phe Asn Thr Asn Gly Pro Asn Trp Lys Phe Gln Arg Gln
130 135 140

Val Ala Ser His Glu Phe Asn Thr Lys Ser Ile Arg Asn Phe Val Glu
145 150 155 160

His Ile Val Asp Thr Glu Leu Thr Asn Arg Leu Ile Pro Ile Leu Thr
165 170 175

Ser Ser Thr Gln Thr Asn Asn Ile Leu Asp Phe Gln Asp Ile Leu Gln
180 185 190

Arg Phe Thr Phe Asp Asn Ile Cys Asn Ile Ala Phe Gly Tyr Asp Pro

195 200 205

Glu Tyr Leu Thr Pro Ser Thr Asn Arg Ser Lys Phe Ala Glu Ala Tyr
210 215 220

Glu Asp Ala Thr Glu Ile Ser Ser Lys Arg Phe Arg Leu Pro Leu Pro
225 230 235 240

Ile Ile Trp Lys Ile Lys Lys Tyr Phe Asn Ile Gly Ser Glu Lys Arg
245 250 255

Leu Lys Glu Ala Val Thr Glu Val Arg Ser Phe Ala Lys Lys Leu Val
260 265 270

Arg Glu Lys Lys Arg Glu Leu Glu Glu Lys Ser Ser Leu Glu Thr Glu
275 280 285

Asp Met Leu Ser Arg Phe Leu Ser Ser Gly His Ser Asp Glu Asp Phe
290 295 300

Val Ala Asp Ile Val Ile Ser Phe Ile Leu Ala Gly Lys Asp Thr Thr
305 310 315 320

Ser Ala Ala Leu Thr Trp Phe Phe Trp Leu Leu Trp Lys Asn Pro Arg
325 330 335

Val Glu Glu Glu Ile Val Asn Glu Leu Ser Lys Lys Ser Glu Leu Met
340 345 350

Val Tyr Asp Glu Val Lys Glu Met Val Tyr Thr His Ala Ala Leu Ser
355 360 365

Glu Ser Met Arg Leu Tyr Pro Pro Val Pro Met Asp Ser Lys Glu Ala
370 375 380

Val Asn Asp Asp Val Leu Pro Asp Gly Trp Val Val Lys Lys Gly Thr
385 390 395 400

Ile Val Thr Tyr His Val Tyr Ala Met Gly Arg Met Lys Ser Leu Trp
405 410 415

Gly Asp Asp Trp Ala Glu Phe Arg Pro Glu Arg Trp Leu Glu Lys Asp
420 425 430

Glu Val Asn Gly Lys Trp Val Phe Val Gly Arg Asp Ser Tyr Ser Tyr
435 440 445

Pro Val Phe Gln Ala Gly Pro Arg Val Cys Leu Gly Lys Glu Met Ala
450 455 460

Phe Met Gln Met Lys Arg Ile Val Ala Gly Ile Val Gly Lys Phe Lys
465 470 475 480

Val Val Pro Glu Ala His Leu Ala Gln Glu Pro Gly Phe Ile Ser Phe
485 490 495

Leu Ser Ser Gln Met Glu Gly Gly Phe Pro Val Thr Ile Gln Lys Arg
500 505 510

Asp Ser

<210> 5

<211> 1665

<212> DNA

<213> Vicia sativa

<400> 5

agaataatcc aagtgtaatt actttttag ctctcactac tcaatcataa ctatcaaact 60

tgaaaaatgg aactcgaaac attgatttct tggttacttt tctctacaag tttattttgg 120

ttcttattct tagccacaaa aacaaaatcc aaaccccaa aaacaccttc ctctaccacc 180

aacaccccaa ttcctaaatc ttaccccatt ttcggttctg ccttctctgt gctagccaac 240

ttccaccgac gcatacaatg gacctccgac attctccaaa ccatcccttc ctccaccttc 300

gtcctccacc gccctttcgg cgctcgccaa gtcttcacgg ctcaacccgc cgtggtgcaa 360

cacattctca gaaccaattt cacttgctac ggcaaaggtc tcacgtttta ccaatctatc 420

aatgattttc tcggcgacgg aatcttcaat gccgacggtg aatcttggaa gttccaacga 480

caaatctcca gccacgaatt caacactaga tccctccgga aattcgttga aaccgtagtt 540

gacgttgaac tctccgatcg cctagttcct gttctctccc aagcttctaa cagccaaacc 600

actcttgatt tccaagacat cctccaacgt ttaacttttg acaacatttg catgattgcg 660

tttggatacg atccagagta cctccttcct tcccttcctg aaataccatt tgcaaaagcc 720

ttcgacgaaa gctcgcaact cagtatcgag aggctaaacg cgttgattcc attactatgg 780

aaagtgaaaa gattcctgaa catcggagtg gagcgacagc tgaaagaagc ggttgctgaa 840

gtaagaggac tcgccactaa aatcgttaag aataagaaaa aagagcttaa agaaaaagca 900

ctacagtcgg aatccgaatc tgttgatctt ttatcgcgat ttttaagttc tggacattca 960

gatgaatctt ttgttactga tatggtaata agtattattc ttgctgggag agatacgact 1020

tcagctgcac tcacgtggtt cttttggtta ctctcgaagc atagtcatgt ggagaatgag 1080

attctcaaag agataactgg aaaatcggaa actgttggat acgatgaggt gaaggatatg 1140

gtttacactc acgcggcgct ttgcgagagt atgaggctat atcctccgct tccggtggat 1200

actaaagtag ccgtgcacga cgatgttttg ccggatggga ctttagtgaa gaaaggatgg 1260

agagtgacgt atcatatata tgctatggga agatctgaga agatatgggg accggattgg 1320

gctgaatttc gacccgagag gtggttgagt cgggatgagg ttgggaagtg gagctttgtt 1380

gggattgatt attatagtta tccggttttc caggctggac cgagggtgtg tatagggaag 1440

gagatggcat ttttgcagat gaagagggtg gttgccggga ttatggggcg gtttagggtg 1500

gttccggcta tggttgaagg gattgagccg gagtacactg cccactttac ctcagtaatg 1560

aaaggtggct tccccgtgaa gatcgaaaag agaagcccac ttgtatgaat aaaaaggaaa 1620

taatacacca tcaatttgaa atataaatta cttttctttt aaaaa 1665

<210> 6

<211> 513

<212> PRT

<213> Vicia sativa

<220>

<221> PEPTIDE

<222> (1)..(513)

<223> DEDUCED SEQUENCE

<400> 6

Met Glu Leu Glu Thr Leu Ile Ser Trp Leu Leu Phe Ser Thr Ser Leu
1 5 10 15

Phe Trp Phe Leu Phe Leu Ala Thr Lys Thr Lys Ser Lys Pro Pro Lys
20 25 30

Thr Pro Ser Ser Thr Thr Asn Thr Pro Ile Pro Lys Ser Tyr Pro Ile
35 40 45

Phe Gly Ser Ala Phe Ser Val Leu Ala Asn Phe His Arg Arg Ile Gln
50 55 60

Trp Thr Ser Asp Ile Leu Gln Thr Ile Pro Ser Ser Thr Phe Val Leu
65 70 75 80

His Arg Pro Phe Gly Ala Arg Gln Val Phe Thr Ala Gln Pro Ala Val
85 90 95

Val Gln His Ile Leu Arg Thr Asn Phe Thr Cys Tyr Gly Lys Gly Leu
100 105 110

Thr Phe Tyr Gln Ser Ile Asn Asp Phe Leu Gly Asp Gly Ile Phe Asn
115 120 125

Ala Asp Gly Glu Ser Trp Lys Phe Gln Arg Gln Ile Ser Ser His Glu
130 135 140

Phe Asn Thr Arg Ser Leu Arg Lys Phe Val Glu Thr Val Val Asp Val
145 150 155 160

Glu Leu Ser Asp Arg Leu Val Pro Val Leu Ser Gln Ala Ser Asn Ser
165 170 175

Gln Thr Thr Leu Asp Phe Gln Asp Ile Leu Gln Arg Leu Thr Phe Asp
180 185 190

Asn Ile Cys Met Ile Ala Phe Gly Tyr Asp Pro Glu Tyr Leu Leu Pro
195 200 205

Ser Leu Pro Glu Ile Pro Phe Ala Lys Ala Phe Asp Glu Ser Ser Gln
210 215 220

Leu Ser Ile Glu Arg Leu Asn Ala Leu Ile Pro Leu Leu Trp Lys Val
225 230 235 240

Lys Arg Phe Leu Asn Ile Gly Val Glu Arg Gln Leu Lys Glu Ala Val
245 250 255

Ala Glu Val Arg Gly Leu Ala Thr Lys Ile Val Lys Asn Lys Lys Lys
260 265 270

Glu Leu Lys Glu Lys Ala Leu Gln Ser Glu Ser Glu Ser Val Asp Leu
275 280 285

Leu Ser Arg Phe Leu Ser Ser Gly His Ser Asp Glu Ser Phe Val Thr
290 295 300

Asp Met Val Ile Ser Ile Ile Leu Ala Gly Arg Asp Thr Thr Ser Ala
305 310 315 320

Ala Leu Thr Trp Phe Phe Trp Leu Leu Ser Lys His Ser His Val Glu
325 330 335

Asn Glu Ile Leu Lys Glu Ile Thr Gly Lys Ser Glu Thr Val Gly Tyr
340 345 350

Asp Glu Val Lys Asp Met Val Tyr Thr His Ala Ala Leu Cys Glu Ser
355 360 365

Met Arg Leu Tyr Pro Pro Leu Pro Val Asp Thr Lys Val Ala Val His
370 375 380

Asp Asp Val Leu Pro Asp Gly Thr Leu Val Lys Lys Gly Trp Arg Val

385 390 395 400

Thr Tyr His Ile Tyr Ala Met Gly Arg Ser Glu Lys Ile Trp Gly Pro
405 410 415

Asp Trp Ala Glu Phe Arg Pro Glu Arg Trp Leu Ser Arg Asp Glu Val
420 425 430

Gly Lys Trp Ser Phe Val Gly Ile Asp Tyr Tyr Ser Tyr Pro Val Phe
435 440 445

Gln Ala Gly Pro Arg Val Cys Ile Gly Lys Glu Met Ala Phe Leu Gln
450 455 460

Met Lys Arg Val Val Ala Gly Ile Met Gly Arg Phe Arg Val Val Pro
465 470 475 480

Ala Met Val Glu Gly Ile Glu Pro Glu Tyr Thr Ala His Phe Thr Ser
485 490 495

Val Met Lys Gly Gly Phe Pro Val Lys Ile Glu Lys Arg Ser Pro Leu
500 505 510

Val

<210> 7

<211> 1828

<212> DNA

<213> Vicia sativa

<400> 7

tcattttgtt atatataaca actgcgaagc aaagttaaac tatgtcatgt aatcacaaca 60

ttggctctca tatcaactcc aatctctctc tcactaacgt gctaaaatgg aactcgaaac 120

attggttgca tggttacttt tctctgcaac tctcctttgg ctcttactct tagccacaaa 180

aacacaatcc aaatccctaa aatcaccctc ctcaaccacc aacagcacca ttcccaaatc 240

ttaccccatc ttcggttcca tcttttcaat tgcagcaaat tttcaccgcc gcgtgcaatg 300

gatctccgac atccttcaaa ccaccccttc ctcaaccttc atcctccacc gcgccttcgg 360

ctcccgccaa gtcttcacag caaacccctt agtagtccaa catattctca aaaccaactt 420

cccttgctac cctaaaggtc tcacacttaa ccgttccctc ggtgatttcc tcggtaacgg 480

tatcttcaac gccgacggtg aaacctggaa gctccaaaga caaatctcca gccatgaatt 540

caacgctaaa tctcttcgga aattcgttga aacagtagtt gatgtagaac tctccggtcg 600

cctcctccct attctctctg aagcttccaa aactgaaaaa atcctccctg attttcaaga 660

tatccttcaa cgttttacat tcgataacat ctgtataatc gcctttggat tcgatccaga 720

gtatctcctc ccttctcttc ccgaaaccgc ctttgcaaag gccttcgact acggcaccag 780

aataagcagc ttgagattca acgccgcagt tccattaata tggaaagtca agaaaatctt 840

aaacatcgga acagaacagc ggttaaaaga agctgttgcg gaagtaagag gactggcttc 900

aagaattgtt agagaaaaga aacaagagct tttagaaaaa tcagcgttgg aatcattgga 960

tattttatcg cgatttttaa gttctggtca ttcagatgaa tcatttgtta ttgatattgt 1020

aataagcttt attcttgctg ggagagatac aacttcagct gcactcacgt ggttcttttg 1080

gttactctct aagcatagtc atgtggagac tgagattctc aaagaggtta ctgcaaaatc 1140

ggaatcagtt agttatgatg aagtgaagga catggtttat actcacgcgg cgctgtgcga 1200

gagtatgagg ctgtatcctc ctgttccagt ggatacaaaa gaagtagctt atgacgatgt 1260

tttaccagat gggacttttg tgaagaaagg gtggagagtg gcgtatcata tatatgctat 1320

gggaaggtct gagaaaatat ggggatctga ctgggctgag tttcgacccg agaggtggtt 1380

gcgtcgggat gaagatggga tgtggagctt tgttgggatg gatccttatg cttatccagt 1440

ttttcaagcc gggccaaggg tgtgtttagg gaaagaaatg gcgttcttgc aaatgaagag 1500

ggtggctgcc ggagttctga gagagtttag ggtggttccg gcgatggaaa atgggattga 1560

gccggagtac actgcacacc ttacggcttt aatgaaaggt ggtttccctg tgaggattga 1620

aaagcgtagt cacacagatg agtaaaaata gaaataattg ttgtacaaaa tattttccaa 1680

aagttccatt gttcatattt cgtttgttgg aataatgttt aaattcgaat gtgatttgta 1740

ctgtattagt tattcagtta gctagaactt tctttttatg tgatacttga ataagtcctg 1800

ttttttttt aaaaaaaaaa aaaaaaaa 1828

<210> 8

<211> 512

<212> PRT

<213> Vicia sativa

<220>

<221> PEPTIDE

<222> (1)..(512)

<223> DEDUCED SEQUENCE

<400> 8

Met Glu Leu Glu Thr Leu Val Ala Trp Leu Leu Phe Ser Ala Thr Leu

1 5 10 15

Leu Trp Leu Leu Leu Leu Ala Thr Lys Thr Gln Ser Lys Ser Leu Lys
20 25 30

Ser Pro Ser Ser Thr Thr Asn Ser Thr Ile Pro Lys Ser Tyr Pro Ile
35 40 45

Phe Gly Ser Ile Phe Ser Ile Ala Ala Asn Phe His Arg Arg Val Gln
50 55 60

Trp Ile Ser Asp Ile Leu Gln Thr Thr Pro Ser Ser Thr Phe Ile Leu
65 70 75 80

His Arg Ala Phe Gly Ser Arg Gln Val Phe Thr Ala Asn Pro Leu Val
85 90 95

Val Gln His Ile Leu Lys Thr Asn Phe Pro Cys Tyr Pro Lys Gly Leu
100 105 110

Thr Leu Asn Arg Ser Leu Gly Asp Phe Leu Gly Asn Gly Ile Phe Asn
115 120 125

Ala Asp Gly Glu Thr Trp Lys Leu Gln Arg Gln Ile Ser Ser His Glu
130 135 140

Phe Asn Ala Lys Ser Leu Arg Lys Phe Val Glu Thr Val Val Asp Val

145 150 155 160

Glu Leu Ser Gly Arg Leu Leu Pro Ile Leu Ser Glu Ala Ser Lys Thr
165 170 175

Glu Lys Ile Leu Pro Asp Phe Gln Asp Ile Leu Gln Arg Phe Thr Phe
180 185 190

Asp Asn Ile Cys Ile Ile Ala Phe Gly Phe Asp Pro Glu Tyr Leu Leu
195 200 205

Pro Ser Leu Pro Glu Thr Ala Phe Ala Lys Ala Phe Asp Tyr Gly Thr
210 215 220

Arg Ile Ser Ser Leu Arg Phe Asn Ala Ala Val Pro Leu Ile Trp Lys
225 230 235 240

Val Lys Lys Ile Leu Asn Ile Gly Thr Glu Gln Arg Leu Lys Glu Ala
245 250 255

Val Ala Glu Val Arg Gly Leu Ala Ser Arg Ile Val Arg Glu Lys Lys
260 265 270

Gln Glu Leu Leu Glu Lys Ser Ala Leu Glu Ser Leu Asp Ile Leu Ser
275 280 285

Arg Phe Leu Ser Ser Gly His Ser Asp Glu Ser Phe Val Ile Asp Ile
290 295 300

Val Ile Ser Phe Ile Leu Ala Gly Arg Asp Thr Thr Ser Ala Ala Leu
305 310 315 320

Thr Trp Phe Phe Trp Leu Leu Ser Lys His Ser His Val Glu Thr Glu
325 330 335

Ile Leu Lys Glu Val Thr Ala Lys Ser Glu Ser Val Ser Tyr Asp Glu
340 345 350

Val Lys Asp Met Val Tyr Thr His Ala Ala Leu Cys Glu Ser Met Arg
355 360 365

Leu Tyr Pro Pro Val Pro Val Asp Thr Lys Glu Val Ala Tyr Asp Asp
370 375 380

Val Leu Pro Asp Gly Thr Phe Val Lys Lys Gly Trp Arg Val Ala Tyr
385 390 395 400

His Ile Tyr Ala Met Gly Arg Ser Glu Lys Ile Trp Gly Ser Asp Trp
405 410 415

Ala Glu Phe Arg Pro Glu Arg Trp Leu Arg Arg Asp Glu Asp Gly Met
420 425 430

Trp Ser Phe Val Gly Met Asp Pro Tyr Ala Tyr Pro Val Phe Gln Ala
435 440 445

Gly Pro Arg Val Cys Leu Gly Lys Glu Met Ala Phe Leu Gln Met Lys
450 455 460

Arg Val Ala Ala Gly Val Leu Arg Glu Phe Arg Val Val Pro Ala Met
465 470 475 480

Glu Asn Gly Ile Glu Pro Glu Tyr Thr Ala His Leu Thr Ala Leu Met
485 490 495

Lys Gly Gly Phe Pro Val Arg Ile Glu Lys Arg Ser His Thr Asp Glu
500 505 510

<210> 9

<211> 1724

<212> DNA

<213> Nicotiana tabacum

<400> 9

gcggccgcaa aagaacaact aaaacctcaa aaagatatac gtaaatgatg atagacttgg 60

agctttcaac ctctttactc ttttgcctca ttccttttcct cttcctcttg ttcatcaaaa 120

ccactttagc caatcttttc tcatccaata aatccaccag caagatccca aaatcatatc 180

caattattgg ttcctgtttc tcgatcctag caaacaaaga acgtcgaatt caatggactt 240

ctgatattat gcaaaacact tccaacttaa ctttcactct caatcgtcct tttggttttc 300

gccaaatttt cacagctaac cccgccaatg tccaacacat gctcaaaacc caatttcaca 360

tttaccaaaa aggtgatgtt tttaaaacaa ctatggctga ttttcttggt gatggcatat 420

ttaacgtgga cggtgacatt tggaagtacc aaagacaagt ttcaagccat gagtttaaca 480

caaaatctct acgcaagttc gttgaaactg ttgttgatac agaactcaac gaaaggctaa 540

ttccaattct tgctactgct gctgttgaaa aaaccgttct ggattttcaa gacattttac 600

aaaggtttgc ttttgataat atttgtaaaa ttgcttttgg ctatgatcct gcttatttat 660

taccatctct tcctcaagca aaatttgctg ttgcttttga agaagctgtt aagctaagta 720

gtgaaagatt taatgctatt ttccctttg tatggaaaat aaaacgaaat ttcaatattg 780

gatctgagaa aaaaatcagg gtagctgtga atgaagttcg tcaatttgca aaagaactcg 840

tgaaagaaaa acaaaaagaa ctcaaagaaa aatcatcact cgattcagtg gatttactat 900

caagattttt aagcagtggc cattcggatg aggactttgt tacagatatt gttataagtt 960

tcattttggc tggtcgtgac acaacatctg ctgctttaac atggtttttt tggttaattt 1020

ttgaacaccc agaaacagaa aaccaaatct taaaagaggc taaagcaaaa tccgaaagtc 1080

cagtgtatga tgaagtgaag gacatgattt acacacatgc ttcactttgt gagagcatga 1140

gattttaccc accaattccc atagatacta aagctgctac agaggataat attttgccag 1200

atggtacttt tgtgaaaaag gggactagag taagttatca tatctatgca atggggagag 1260

tggagaactt atggggaaaa gattgggcag agtttaggcc agagaggtgg ttagataagg 1320

atgaagcgtc aggtaattgg acttttgtgg ctagggacac ctatacttat cctgtttttc 1380

aggcgggacc aagaatttgt ttaggaaaag aaatggcatt tttgcagatg aagagggtgg 1440

tggctggtgt tttacggcgg ttcaaggtgg ttccgctggc agaaaaaggt gttgagccgg 1500

tctttttgtc ttacctcact gcgaaaatga aaggtggttt ccctgtgaca attgaggaaa 1560

ggaacggtac ggatatttga tctttcaact atggccaaag agtacgaggg taaaggtttt 1620

tattgcttct tccacttacc ttaaaagtgt ttggattttg tgacatttat ttatgtttgt 1680

ataaagctgc tttataagaa gtgagtatta tttattaaaa aaaa 1724

<210> 10

<211> 511

<212> PRT

<213> Nicotiana tabacum

<220>

<221> PEPTIDE

<222> (1)..(511)

<223> DEDUCED SEQUENCE

<400> 10

Met Met Ile Asp Leu Glu Leu Ser Thr Ser Leu Leu Phe Cys Leu Ile
1 5 10 15

Pro Phe Leu Phe Leu Leu Phe Ile Lys Thr Thr Leu Ala Asn Leu Phe
20 25 30

Ser Ser Asn Lys Ser Thr Ser Lys Ile Pro Lys Ser Tyr Pro Ile Ile
35 40 45

Gly Ser Cys Phe Ser Ile Leu Ala Asn Lys Glu Arg Arg Ile Gln Trp
50 55 60

Thr Ser Asp Ile Met Gln Asn Thr Ser Asn Leu Thr Phe Thr Leu Asn
65 70 75 80

Arg Pro Phe Gly Phe Arg Gln Ile Phe Thr Ala Asn Pro Ala Asn Val
85 90 95

Gln His Met Leu Lys Thr Gln Phe His Ile Tyr Gln Lys Gly Asp Val
100 105 110

Phe Lys Thr Thr Met Ala Asp Phe Leu Gly Asp Gly Ile Phe Asn Val
115 120 125

Asp Gly Asp Ile Trp Lys Tyr Gln Arg Gln Val Ser Ser His Glu Phe
130 135 140

Asn Thr Lys Ser Leu Arg Lys Phe Val Glu Thr Val Val Asp Thr Glu
145 150 155 160

Leu Asn Glu Arg Leu Ile Pro Ile Leu Ala Thr Ala Ala Val Glu Lys
165 170 175

Thr Val Leu Asp Phe Gln Asp Ile Leu Gln Arg Phe Ala Phe Asp Asn
180 185 190

Ile Cys Lys Ile Ala Phe Gly Tyr Asp Pro Ala Tyr Leu Leu Pro Ser

195 200 205

Leu Pro Gln Ala Lys Phe Ala Val Ala Phe Glu Glu Ala Val Lys Leu
210 215 220

Ser Ser Glu Arg Phe Asn Ala Ile Phe Pro Phe Val Trp Lys Ile Lys
225 230 235 240

Arg Asn Phe Asn Ile Gly Ser Glu Lys Lys Ile Arg Val Ala Val Asn
245 250 255

Glu Val Arg Gln Phe Ala Lys Glu Leu Val Lys Glu Lys Gln Lys Glu
260 265 270

Leu Lys Glu Lys Ser Ser Leu Asp Ser Val Asp Leu Leu Ser Arg Phe
275 280 285

Leu Ser Ser Gly His Ser Asp Glu Asp Phe Val Thr Asp Ile Val Ile
290 295 300

Ser Phe Ile Leu Ala Gly Arg Asp Thr Thr Ser Ala Ala Leu Thr Trp
305 310 315 320

Phe Phe Trp Leu Ile Phe Glu His Pro Glu Thr Glu Asn Gln Ile Leu
325 330 335

Lys Glu Ala Lys Ala Lys Ser Glu Ser Pro Val Tyr Asp Glu Val Lys
340 345 350

Asp Met Ile Tyr Thr His Ala Ser Leu Cys Glu Ser Met Arg Phe Tyr
355 360 365

Pro Pro Ile Pro Ile Asp Thr Lys Ala Ala Thr Glu Asp Asn Ile Leu
370 375 380

Pro Asp Gly Thr Phe Val Lys Lys Gly Thr Arg Val Ser Tyr His Ile
385 390 395 400

Tyr Ala Met Gly Arg Val Glu Asn Leu Trp Gly Lys Asp Trp Ala Glu
405 410 415

Phe Arg Pro Glu Arg Trp Leu Asp Lys Asp Glu Ala Ser Gly Asn Trp
420 425 430

Thr Phe Val Ala Arg Asp Thr Tyr Thr Tyr Pro Val Phe Gln Ala Gly
435 440 445

Pro Arg Ile Cys Leu Gly Lys Glu Met Ala Phe Leu Gln Met Lys Arg
450 455 460

Val Val Ala Gly Val Leu Arg Arg Phe Lys Val Val Pro Leu Ala Glu
465 470 475 480

Lys Gly Val Glu Pro Val Phe Leu Ser Tyr Leu Thr Ala Lys Met Lys
485 490 495

Gly Gly Phe Pro Val Thr Ile Glu Glu Arg Asn Gly Thr Asp Ile
500 505 510

<210> 11

<211> 1750

<212> DNA

<213> Nicotiana tabacum

<400> 11

gcggccgcca ttaatcaaaa accaaagtcc aaaatccatc ctcttgagag ataaaaaaac 60

tttaggcaat ggcactatta gacttacaac tctcaacctc attactcttt tgccttgttc 120

ctttgctctt cctttttttc gtcaaattca agaaaacaat tactaatacc cttttatcat 180

ccaataactc tagtaagata ccaagatctt atcctctaat aggttcttat ttttccatct 240

tggcaaatca cgaccggcgg ataaaatgga tatcggatat tatcctaacc acccctaacc 300

tcacttttac tctcattcgc cctctcaatt ttcgcacaat tttcactgca aacccttcca 360

atgtccaaca cgtgctcaaa acaaattttc aagtctacca aaaaggtcat ggttcgtaca 420

gtaccctcaa agattttctc agtaatggta tttttaatgt cgatggtgat atatggaagt 480

accaaagaca agttgctagc catgaattta acactaggtc gttacgtaaa tttgttgaga 540

aaaaaaaaaa cagttgttga tactgaactt tctgaacgtt tgataccaat tcttgccact 600

gctgctgcta ataaaactgt tcttgatttc caagacatat tacaaaggtt tgcttttgac 660

aacatttgta aaattgcttt tggatatgat cctggctatt tgttaccgtc acttcccgag 720

gcagaatttg ctgttgcttt tgaagatgct gttcgtctca gcactgaaag attcattgtt 780

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<212> PRT

<213> Nicotiana tabacum

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<223> DEDUCED SEQUENCE

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Asn Thr Leu Leu Ser Ser Asn Asn Ser Ser Lys Ile Pro Arg Ser Tyr
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Pro Leu Ile Gly Ser Tyr Phe Ser Ile Leu Ala Asn His Asp Arg Arg
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Ile Lys Trp Ile Ser Asp Ile Ile Leu Thr Thr Pro Asn Leu Thr Phe
65 70 75 80

Thr Leu Ile Arg Pro Leu Asn Phe Arg Thr Ile Phe Thr Ala Asn Pro
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Ser Asn Val Gln His Val Leu Lys Thr Asn Phe Gln Val Tyr Gln Lys
100 105 110

Gly His Gly Ser Tyr Ser Thr Leu Lys Asp Phe Leu Ser Asn Gly Ile

115 120 125

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130 135 140

His Glu Phe Asn Thr Arg Ser Leu Arg Lys Phe Val Glu Thr Val Val
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165 170 175

Ala Asn Lys Thr Val Leu Asp Phe Gln Asp Ile Leu Gln Arg Phe Ala
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Phe Asp Asn Ile Cys Lys Ile Ala Phe Gly Tyr Asp Pro Gly Tyr Leu
195 200 205

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210 215 220

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225 230 235 240

Lys Ile Lys Arg Ala Leu Asn Ile Gly Ser Glu Lys Lys Leu Arg Val
245 250 255

Ala Val Glu Gln Val Arg Glu Phe Ala Lys Glu Ile Val Arg Glu Lys
260 265 270

Gln Lys Glu Leu Asn Asp Lys Ser Ser Leu Asp Ser Ala Asp Leu Leu
275 280 285

Ser Arg Phe Leu Ser Thr Gly His Ser Asp Glu Asp Phe Val Thr Asp
290 295 300

Ile Val Ile Ser Phe Ile Leu Ala Gly Arg Asp Thr Thr Ser Ala Ala
305 310 315 320

Leu Thr Trp Phe Phe Trp Leu Ile Ser Lys His Pro Glu Val Glu Ser
325 330 335

Gln Ile Met Lys Glu Val Gly Glu Lys Ser Glu Ser Leu Leu Leu Tyr
340 345 350

Asp Glu Val Lys Asn Met Met Tyr Thr His Ala Ser Leu Cys Glu Ser
355 360 365

Met Arg Phe Tyr Pro Pro Val Pro Met Asp Ser Lys Glu Ala Thr Lys
370 375 380

Asp Asp Ile Leu Pro Asp Gly Thr Phe Val Lys Lys Gly Thr Arg Val
385 390 395 400

Thr Tyr His Pro Tyr Ala Met Gly Arg Val Glu Lys Val Trp Gly Glu
405 410 415

Asp Trp Ala Glu Phe Lys Pro Glu Arg Trp Leu Asp Lys Asp Glu Val
420 425 430

Thr Gly Asn Trp Thr Phe Val Pro Lys Asp Ala Tyr Thr Tyr Pro Val
435 440 445

Phe Gln Ala Gly Pro Arg Ile Cys Leu Gly Lys Glu Met Ala Phe Leu
450 455 460

Gln Met Lys Arg Val Val Ala Gly Val Leu Arg Arg Phe Lys Val Val
465 470 475 480

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<212> DNA

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<223> DEDUCED SEQUENCE

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Asn Thr Leu Leu Ser Ser Asn Ser Ser Lys Ile Pro Arg Ser Tyr Pro
35 40 45

Leu Ile Gly Ser Tyr Phe Ser Ile Leu Ala Asn His Asp Gln Arg Ile
50 55 60

Lys Trp Ile Ser Asp Ile Ile Leu Ser Thr Pro Asn Leu Thr Phe Thr
65 70 75 80

Leu Ile Arg Pro Leu Asn Phe His Thr Ile Phe Thr Ala Asn Pro Ser
85 90 95

Asn Val Gln His Met Leu Lys Thr Asn Phe Gln Val Tyr Gln Lys Gly
100 105 110

His Asn Ser Asn Thr Thr Leu Lys Asp Phe Leu Ser Asn Gly Ile Phe
115 120 125

Asn Val Asp Gly Asp Ile Trp Lys Tyr Gln Arg Gln Val Ala Ser His
130 135 140

Glu Phe Asn Thr Arg Ser Leu Arg Lys Phe Val Glu Thr Val Val Asp
145 150 155 160

Thr Glu Leu Ser Glu Arg Leu Ile Pro Ile Leu Ala Thr Ala Ala Ala

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165 170 175

Asn Lys Thr Val Leu Asp Phe Gln Asp Ile Leu Gln Arg Phe Ala Phe
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Asp Asn Ile Cys Lys Ile Ala Phe Gly Tyr Asp Pro Gly Tyr Leu Leu
195 200 205

Pro Ser Leu Pro Glu Ala Glu Phe Ala Val Ala Phe Glu Asp Ala Val
210 215 220

Arg Leu Ser Thr Glu Arg Phe Ile Leu Pro Phe Pro Leu Ile Trp Lys
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Met Lys Arg Ala Leu Asn Ile Gly Ser Glu Lys Lys Leu Arg Phe Ala
245 250 255

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Glu Val Lys Asn Met Ile Tyr Thr His Ala Ser Leu Cys Glu Ser Met
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Arg Phe Tyr Pro Pro Val Pro Met Asp Thr Lys Glu Ala Thr Lys Asp
370 375 380

Asp Ile Leu Pro Asp Gly Thr Phe Val Lys Lys Gly Asn Arg Val Thr
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Trp Ala Glu Phe Arg Pro Glu Arg Trp Leu Asp Lys Asp Glu Val Thr
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<211> 1674

<212> DNA

<213> Helianthus tuberosus

<400> 15

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<221> PEPTIDE

<222> (1)..(505)

<223> DEDUCED SEQUENCE

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Ile Phe Pro Ser Leu Pro Ile Ile Gly His Leu Tyr Leu Leu Lys Pro
35 40 45

Pro Leu Tyr Arg Thr Leu Ala Lys Leu Ser Ala Lys His Gly Gln Ile
50 55 60

Leu Arg Leu Gln Leu Gly Phe Arg Arg Val Leu Ile Val Ser Ser Pro
65 70 75 80

Ser Ala Ala Glu Glu Cys Phe Thr Lys Asn Asp Ile Val Phe Ala Asn
85 90 95

Arg Pro Lys Met Leu Phe Gly Lys Ile Ile Gly Val Asn Tyr Thr Ser
100 105 110

Leu Ala Trp Ser Pro Tyr Gly Asp Asn Trp Arg Asn Leu Arg Arg Ile
115 120 125

Ala Ser Ile Glu Ile Leu Ser Ile His Arg Leu Asn Glu Phe His Asp
130 135 140

Ile Arg Val Glu Glu Thr Arg Leu Leu Ile Gln Lys Leu Leu Ser Ala
145 150 155 160

Cys Asn Ser Gly Ser Ser Gln Val Thr Met Lys Phe Ser Phe Tyr Glu
165 170 175

Leu Thr Leu Asn Val Met Met Arg Met Ile Ser Gly Lys Arg Tyr Phe
180 185 190

Gly Gly Asp Asn Pro Glu Leu Glu Glu Glu Gly Lys Arg Phe Arg Asp
195 200 205

Met Leu Asp Glu Thr Phe Val Leu Ala Gly Ala Ser Asn Val Gly Asp
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Tyr Leu Pro Val Leu Ser Trp Leu Gly Val Lys Gly Leu Glu Lys Lys
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Leu Ile Lys Leu Gln Glu Lys Arg Asp Val Phe Phe Gln Gly Leu Ile
245 250 255

Asp Gln Leu Arg Lys Ser Lys Gly Thr Glu Asp Val Asn Lys Lys Lys
260 265 270

Thr Met Ile Glu Leu Leu Leu Ser Leu Gln Glu Thr Glu Pro Glu Tyr
275 280 285

Tyr Thr Asp Ala Met Ile Arg Ser Phe Val Leu Val Leu Leu Ala Ala
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Gly Ser Asp Thr Ser Ala Gly Thr Met Glu Trp Val Met Ser Leu Leu
305 310 315 320

Leu Asn His Pro Gln Val Leu Lys Lys Ala Gln Asn Glu Ile Asp Ser
325 330 335

Val Ile Gly Lys Asn Cys Leu Val Asp Glu Ser Asp Ile Pro Asn Leu
340 345 350

Pro Tyr Leu Arg Cys Ile Ile Asn Glu Thr Leu Arg Leu Tyr Pro Ala
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Gly Pro Leu Leu Val Pro His Glu Ala Ser Ser Asp Cys Val Val Gly

370 375 380

Gly Tyr Asn Val Pro Arg Gly Thr Ile Leu Ile Val Asn Gln Trp Ala
385 390 395 400

Ile His His Asp Pro Lys Val Trp Asp Glu Pro Glu Thr Phe Lys Pro
405 410 415

Glu Arg Phe Glu Gly Leu Glu Gly Thr Arg Asp Gly Phe Lys Leu Leu
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Pro Phe Gly Ser Gly Arg Arg Ser Cys Pro Gly Glu Gly Leu Ala Val
435 440 445

Arg Met Leu Gly Met Thr Leu Gly Ser Ile Ile Gln Cys Phe Asp Trp
450 455 460

Glu Arg Thr Ser Glu Glu Leu Val Asp Met Thr Glu Gly Pro Gly Leu
465 470 475 480

Thr Met Pro Lys Ala Ile Pro Leu Val Ala Lys Cys Lys Pro Arg Val
485 490 495

Glu Met Thr Asn Leu Leu Ser Glu Leu
500 505

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/IB 98/01716

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N9/02 C12N5/10 C12Q1/68 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and. where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HELVIG,C., ET AL. : "suicide inactivation of cytochrome P450 by midchain and terminal acetylenes" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 1, 3 January 1997, pages 414-421, XP002091704 cited in the application page 420, last paragraph ---	1-3,14, 15,17
X	FRANK, M.R., ET AL. : "cloning of wound-induced cytochrome P450 monooxygenases expressed in pea" PLANT PHYSIOLOGY, vol. 110, 1996, pages 1035-1046, XP002091705 abstract, page 1040; Fig.5; Fig. 3B; page 1042, right column, first paragraph; page 1045 --- -/--	1,2,6-8, 11

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1999	16/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/IB 98/01716

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BENVENISTE, I. AND DURST, F.: "cloning, sequencing and expression of CYP86, a new Cyt. P450 from Arabidopsis thaliana (Accession No. X90458), in Plant Physiology,1995,Vol.109,page 722)" EMBL SEQUENCE DATA LIBRARY,9 August 1995, XP002091706 heidelberg, germany accession no. X90458 ---	1,2
X	DEWAR, K., ET AL. : "untitled" EMBL SEQUENCE DATA LIBRARY,1 July 1997, XP002091707 heidelberg, germany accession no. o04948 ---	1,2
P,X	TIJET, N., ET AL. : "functional expression in yeast and charcterization of a clofibrate-inducible plant cytochrome P-450 (CYP94A1) involved in cutin monomers synthesis" BIOCHEMICAL JOURNAL, vol. 332, June 1998, pages 583-589, XP002091708 cited in the application see the whole document ---	1-3,6-9, 11,12, 14-17
P,X	CABELLO-HURTADO,F., ET AL. : "cloning, expression in yeast, and functional characterization of CYP81B1, a plant cytochrome P450 that catalyzes in-chain hydroxylation of fatty acids" THE JOURNAL OF BIOLOGICAL CHEMISTRY , vol. 273, no. 13, pages 7260-7267, XP002091709 cited in the application see the whole document ---	1,2,4-9, 11,12, 14-17
P,X	BENVENISTE, I., ET AL. : "CYP86A1 from Arabidopsis thaliana encodes a cytochrome P450-dependent fatty acid omega-hydroxylase" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 243, 24 February 1998, pages 688-693, XP002091710 cited in the application see the whole document -----	1,2,6

1

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB 98/01716

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: claims 2,3 completely, 1, 6-27 partially

   Isolated nucleic acids encoding plant fatty acid omega-hydroxylases, comprising the clones CYP94A1, CYP94A2, CYP94A3, CYP94A4, CYP94A5, CYP94A6; recombinant expression of the enzyme in host cells; furthermore processes for isolating related fatty acid hydroxylases and for altering the fatty acid composition utilizing transgenic plants.

2. Claims: claims 4-5 completely, 1,6-27 partially

   Isolated nucleic acid encoding plant fatty acid in-chain hydroxylase, comprising the clone CYP81B1; recombinant expression of the enzyme in host cells; furthermore processes for isolating related fatty acid hydroxylases and for altering the fatty acid composition utilizing transgenic plants.