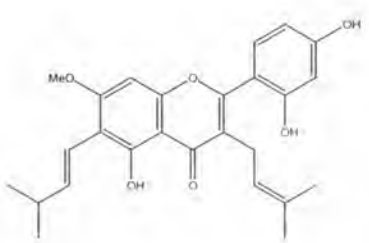
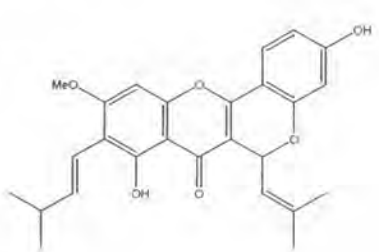
CHAPTER II

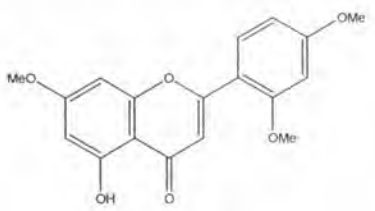
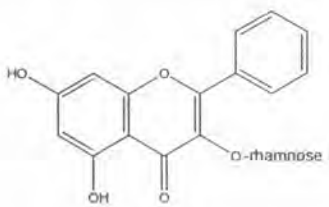
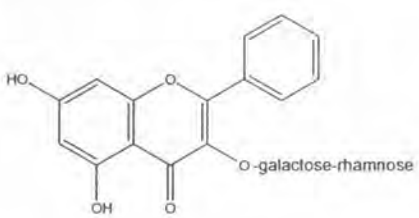
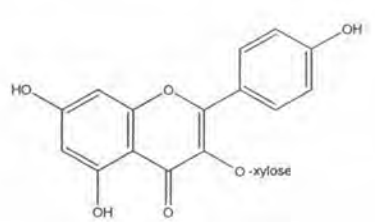
HISTORICAL

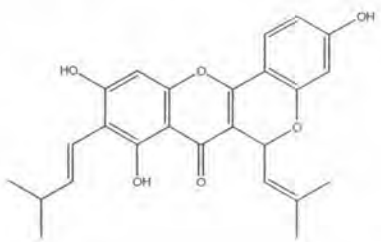
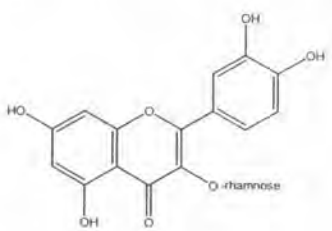
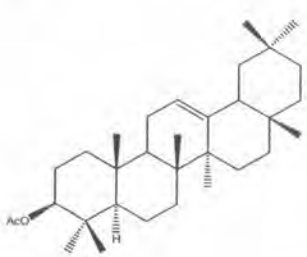
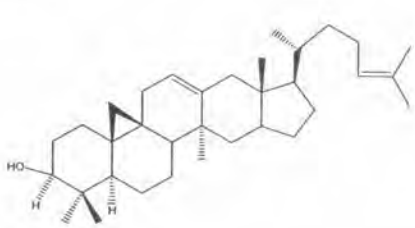
1. Chemical constituents of *Artocarpus lakoocha*

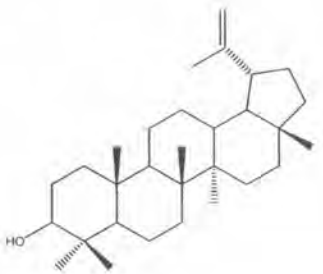
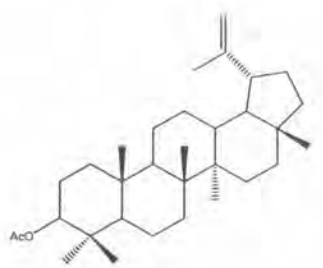
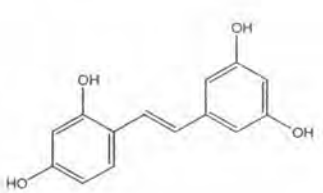
Flavonoids, stilbenoids, steroids and triterpenoids have been reported from the stem and root parts whereas lectins and related compounds have been found in the seed. These are summarized in Table 1.

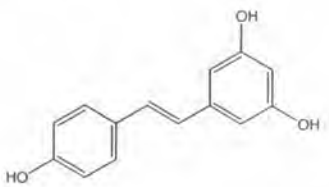
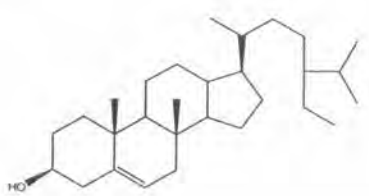
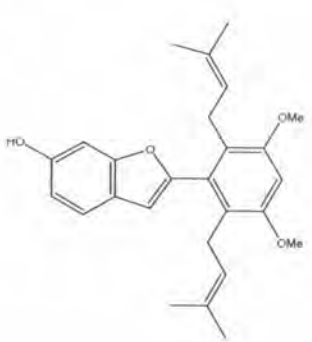
Table 1: Chemical investigations of *A. lakoocha*

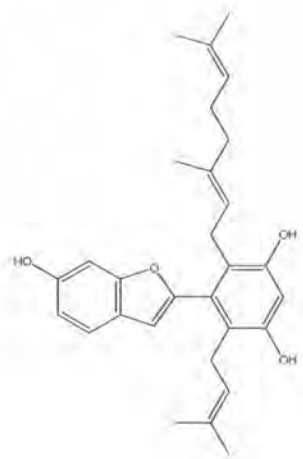
Chemical compounds	Plant part	References
Artocarpin 	Heartwood	Venkataraman, 1972
Cycloartocarpin 	Heartwood	Venkataraman, 1972

Chemical compounds	Plant part	References
5-Hydroxy-7,2′,4′-trimethoxy-flavone 	Heartwood	Pavaro and Reutrakul, 1976
Galangin-3-*O*-α-L-(-)-rhamnopyranoside 	Root bark	Chauhan and Kumari, 1979
Galangin-3-*O*-β-D-(-)-galactopyranosyl-(1-4)-α-L-rhamnopyranoside 	Root bark	Chauhan, Kumari and Sarawat, 1979
Kaempferol-3-*O*-β-D-xylanopyranoside 	Root bark	Chauhan *et al.*, 1982

Chemical compounds	Plant part	References
Norcycloartocarpin 	Heartwood	Venkataraman, 1972
Quercetin-3-*O*-α-L-rhamnopyranoside 	Root bark	Chauhan, *et al.*, 1982
Amyrin acetate 	Bark	Kapil and Joshi, 1960
Cycloartenol 	Bark	Pavanasasivam and Sultanbawa, 1973

Chemical compounds	Plant part	References
Lupeol 	Root bark	Chauhan and Kumari, 1979
Lupeol acetate 	Bark	Kapil and Joshi, 1960
Artocarpus lakoocha lectin	Seed	Chatterjee, Sarkar, and Rao, 1982
Lymphoagglutinin	Seed	Arora *et al.*, 1987
Oxyresveratrol 	Heartwood	Venkataraman, 1972; Mongolsuk *et al.*, 1957, Likhitwitayawuid *et al*, 2005

Chemical compounds	Plant part	References
Resveratrol 		Venkataraman, 1972
β-Sitosterol 	Root bark	Chauhan and Kumari, 1979
Lakoochin A 	Root	Puntumchai *et al.*, 2004

Chemical compounds	Plant part	References
Lakoochin B 	Root	Puntumchai *et al.*, 2004

2. Oxyresveratrol

Oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene) is also known to inhibit cyclooxygenase and ATPase (Shin *et al.*, 1998 a; Nimmanpisut, Chudapongse and Ratanabanangkoon, 1976). For tyrosinase, it inhibits the DOPA oxidase activity of the enzyme. Pharmacological studies have shown that the compound can be transported to tissues at high rates, resulting in a bioavailability of about 50% (Qiu *et al.* 1996). Oxyresveratrol has been used as an active ingredient in dermatological products (Kim *et al.*, 2002). It should be mentioned that the compound may also have potential uses as a neuroprotective agent (Andrabe *et al.*, 2004) or as a starting material for the synthesis of clinically useful antiviral agents.

3. Tyrosinase

Tyrosinase is a polyphenol oxidase copper-containing enzyme. The enzyme is widely distributed in microorganisms, animals and plants (Britton, 1983). Mushroom tyrosinase has been regularly used as a tool in preliminary studies prior to more detailed *in vitro* and *in vivo* investigations.

3.1. Domain structure of mushroom tyrosinase

Tyrosinase from *Agaricus bisporus* was reported to be a heterotetramer comprising two heavey (H) and light (L) chains with a molecular mass of 120 kDa. The central domain contains two Cu binding sites, called CuA and CuB (Figure 3). Several conserved sequences are found to be present in tyrosinases from different sources as shown (Figure 2). In fact, when all tyrosinase sequences were compared, the only conserved domain seems to be the central copper-binding domain, which also shares sequence homology with hemocyanins, copper-containing oxygen carriers from the hemolymph of many molluscs and arthropods. Six conserved histidine residues bind a pair of copper ions in the active site of the enzyme tyrosinase, which interact with both molecular oxygen and its phenolic substrate. The location of cysteine (Cys) also plays an important role in the formation of disulfide linkages, which stabilize the protein structure. The number of Cys residues varies from one organism to another, as along the N-terminal and central part of the protein, Human and mouse tyrosinases have 17 Cys residues and plants have 11, whereas the C-terminal domain contains 1 Cys residue. (Seo, Shama and Shama, 2003)

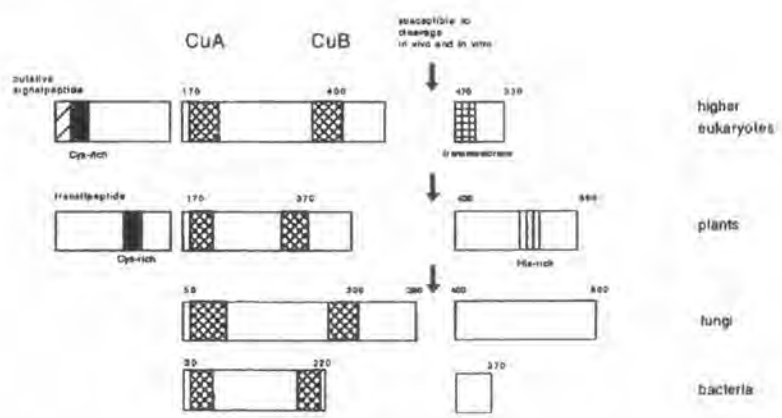


Figure 2: Domain structure of tyrosinase from different groups of species (Van Gelder, Flurkey and Wichers, 1997)

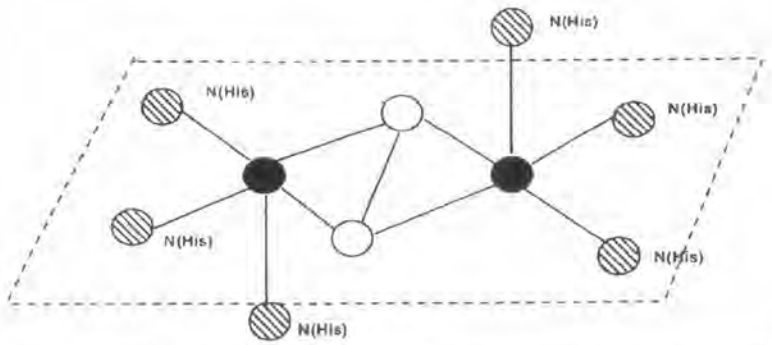


Figure 3: Schematic representation of the binuclear copper center (Van Gelder, Flurkey and Wichers, 1997)

The oxygen is bound as peroxide and each Cu ion is bound to three histidine nitrogen atoms. Black symbols--Cu-ions; white symbols--oxygen; symbols dashed vertically-His-N.

Pigmentation is one of most obvious phenotypical characteristics in the natural world. Of the pigments, melanin is one of the most widely distributed and is found in bacteria, fungi, plants and animals. Melanins are heterogeneous polyphenol-like biopolymers with a complex structure and color varying from yellow to black. Their biosynthesis can be observed by anyone who leaves the surface of a cut apple, potato or banana exposed to air. The color of mammalian skin and hair is determined by a number of factors, the most important of which is the degree and distribution of melanin pigmentation. Melanin is formed in specialized pigment-producing cells known as melanocytes, which originate in the neural crest during embryogenesis and are distributed through the embryo during its development. The migration pathways followed by the melanocytes are under strict genetic control and lead to some interesting results if their final distribution in the skin is not uniform. The characteristic skin patterns of zebras, giraffes and piebald animals in general are due to this uneven distribution of melanocytes. At a cellular level, these compounds are biosynthetized in the membranous or organelles named melanosomes. The mature melanosomes located in the dendrites of melanocytes are then phagocytosed by the surrounding keratinocytes, and it is this process which is responsible for the variety of colors in human skin, hair and eyes (Sanchez-Ferrer *et al.*, 1995)

The melanin biosynthesis is caused by tyrosinase enzyme. Sometimes this tyrosinase process is involved in abnormal accumulation of melanin pigments. Therefore, tyrosinase inhibitors have established as important constituents of cosmetic materials and depigmenting agents for hyperpigmentation.

Figure 4 depicts the biosynthetic pathway of melanin (Britton, 1983). First, tyrosine is oxidized in a two-step reaction to give DOPAquinone. This compound is then transformed into an intermediate named DOPAchrome, a purple-blue compound which has been used as the target for the measurement of tyrosinase inhibition in several *in vitro* assays (บุญชู ศรีตุลารักษ์, 2541; Iida *et al.*, 1995; Mim *et al.*, 2002;). In animals, DOPAchrome is converted through several biochemical reactions to indole 5,6-quinone which is subsequently polymerized to form melanin.

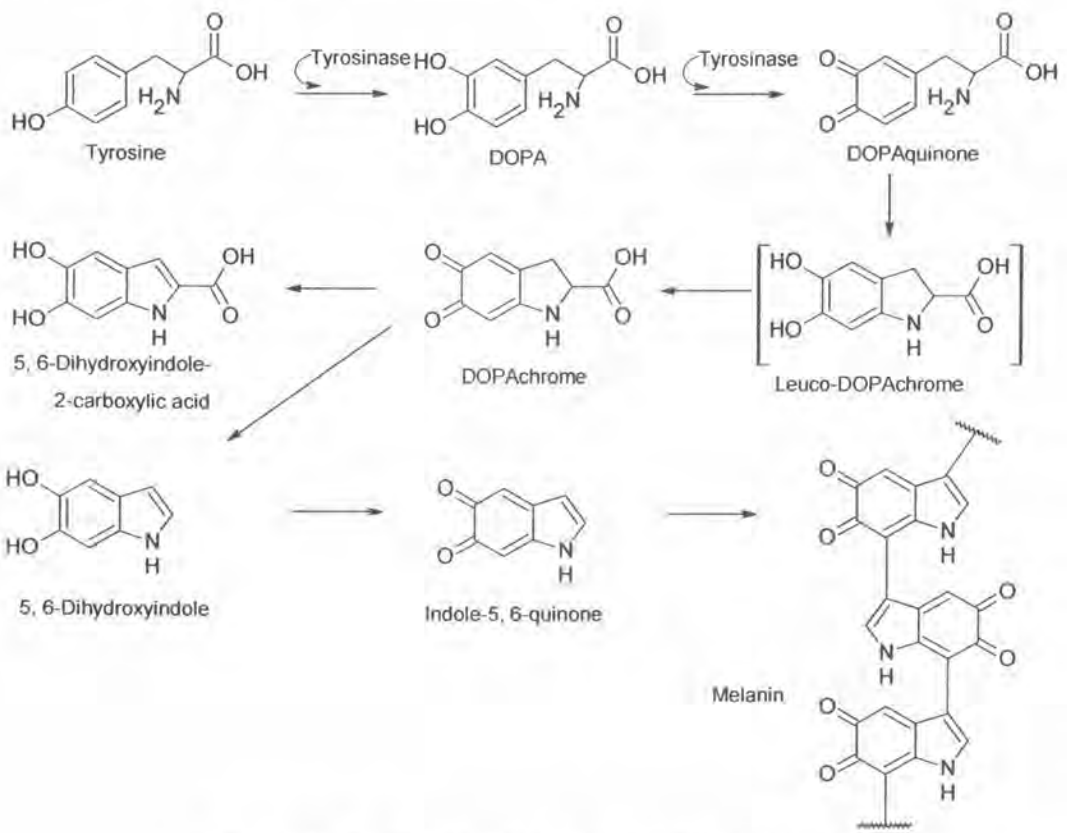


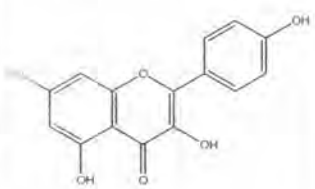
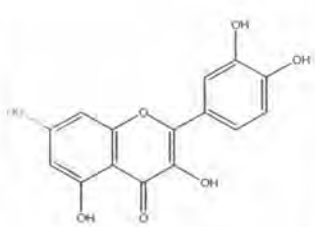
Figure 4: The Raper-Mason scheme of melanogenesis

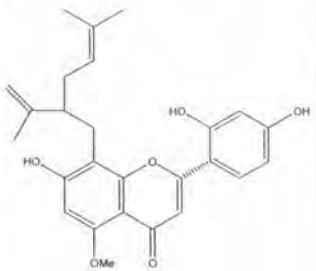
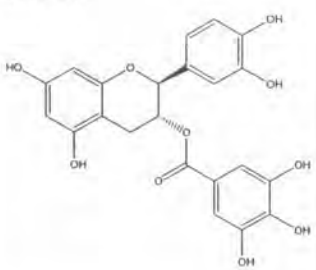
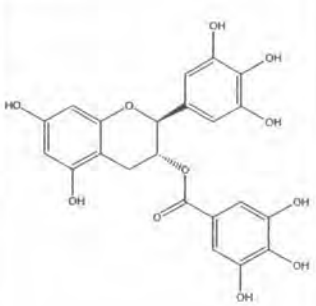
3.2. Study of mushroom tyrosinase

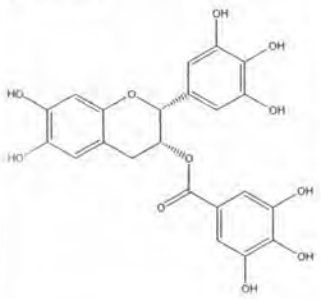
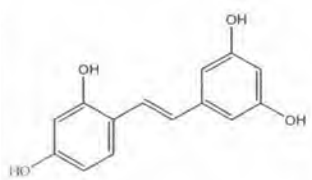
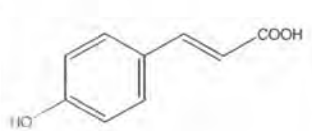
Usually, mushroom tyrosinase is used in the preliminary *in vitro* investigation for the study of tyrosinase inhibition. Compounds with promising activity will then be examined further in detail for possible applications in the food, cosmetic or medical field.

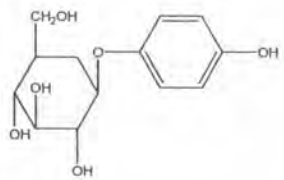
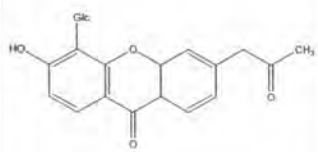
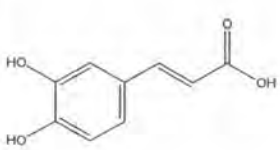
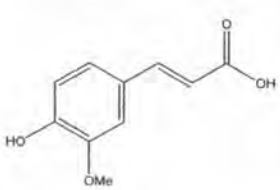
As summarized in Table 2, a number of natural and synthetic compounds have been reported to inhibit mushroom tyrosinase. Since different methods of evaluation have been used in different investigations, it would be difficult to make a direct comparison of the inhibitory activity of these compounds based on the reported ID_{50} values.

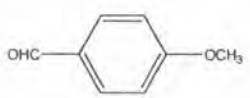
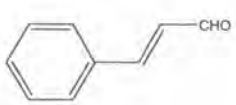
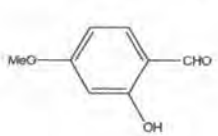
Table 2: Compounds inhibiting mushroom tyrosinase enzyme

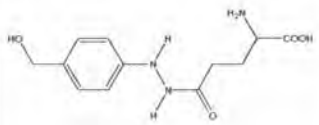
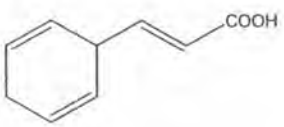
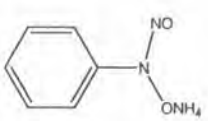
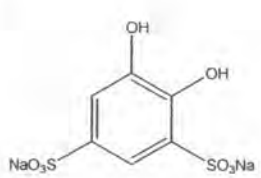
Inhibitor	Source	ID_{50} (mM)	Reference
Kaempferol 	*Crocus sativus*	0.230	Kubo and Kinst-Hori, 1999a
Quercetin 	*Heterotheca inuloides*	0.070	Kubo and Kinst-Hori, 1999a

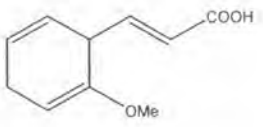
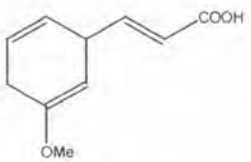
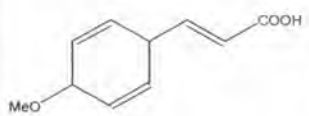
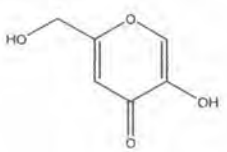
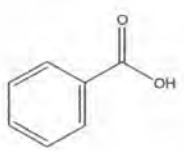
Inhibitor	Source	ID_{50} (mM)	Reference
Kurarinone 	*Sophora flavescens*	0.005	Ha *et al.*, 2001
Epicatechin gallate (ECG) 	Green tea	0.035	No *et al.*, 1999
Gallocatechin gallate (GCG) 	Green tea	0.017	No *et al.*, 1999

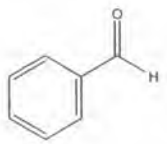
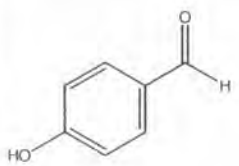
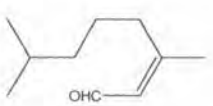
Inhibitor	Source	ID_{50} (mM)	Reference
Epigallocatechin gallate (EGCG) 	Green tea	0.034	No *et al.*, 1999
Oxyresveratrol 	*Morus alba*	0.001	Shin *et al.*, 1998a
Anacardic acid 	*Anacardium occidentale*	3.65	Kubo *et al.*, 1994
p-coumaric acid 	*Panax ginseng*	0.04	Lim *et al.*, 1999

Inhibitor	Source	ID_{50} (mM)	Reference
Arbutin 	*Ulva ursi*	0.10	Funyama *et al.*, 1995
Aloesin 	*Aloe vera*	0.97	Yagi *et al.*, 1987
3,4-dihydroxycinnamic acid 	*Pulsatilla cemua*	0.33	Lee, 2002
4-hydroxy-3-methoxycinnamic acid 	*Pulsatilla cemua*	0.05	Lee, 2002

Inhibitor	Source	ID_{50} (mM)	Reference
Cuminaldehyde 	Cumin seed	0.26	Kubo and Kinst-Hori, 1998
Cumic acid 	Cumin seed	0.38	Kubo and Kinst-Hori, 1998
Anisaldehyde 	Anise oil	0.68	Lee, 2002
Trans-cinnamaldehyde 	*Cinnamomum cassia*	1.3	Lee, 2002
2-hydroxy-4-methoxybenzaldehyde 	*Mondia whitei*, *Rhus vulgaris*, *Scleroca caffra*	0.03	Kubo and Kinst-Hori, 1999

Inhibitor	Source	ID_{50} (mM)	Reference
Agaritine 	*Agaricus bisporus*	0.22	Espin *et al.*, 1998
Cinnamaldehyde 	Synthesis	0.97	Lee, 2002
Cinnamic acid 	Synthesis	0.70	Lee, 2002
Cupferron 	Synthesis	0.001	Shiino, *et al.*, 2001
Tiron 	Synthesis	400	Kahn and Andrawis, 1987

Inhibitor	Source	ID_{50} (mM)	Reference
2-methoxycinnamic acid 	Synthesis	0.34	Lee, 2002
3-methoxycinnamic acid 	Synthesis	0.35	Lee, 2002
4-methoxycinnamic acid 	Synthesis	0.34	Lee, 2002
Kojic acid 	Synthesis	0.014	Kim *et al.*, 2002
Benzoic acid 	Synthesis	0.64	Kubo and Kinst-Hori, 1998

Inhibitor	Source	ID_{50} (mM)	Reference
Benzaldehyde 	Synthesis	0.82	Kubo and Kinst-Hori, 1998
p-hydroxybenzaldehyde 	Synthesis	1.2	Kubo and Kinst-Hori, 1999b
Citral 	Synthesis	1.5	Kubo and Kinst-Hori, 1999b