Vegetables as Chemical Reagents⊥

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Biocatalysis is an important adjunct to the chemical armamentarium that organic chemists may bring to bear for the synthesis of important intermediates and finished pharmaceutical and other commercial products. For most of the world however, such catalytic reagents are not an option due to their high cost and import limitations. Recent studies indicate that the use of locally available vegetables may offer an alternative opportunity for countries to investigate their local resources for the effective conduct of key synthetic transformations with significant economic and ecological implications. This review offers a brief overview of the field of microbial and plant biocatalysts, discusses the studies thus far on the use of intact plant materials for conducting synthetic chemical reactions, and considers some opportunities for future development.

Introduction

Secondary metabolism is a series of chemical transformations in which the primary metabolites, acetate, isoprene precursors, selected amino acids, and several small molecules (e.g., *S*-adenosyl methionine) combine together through several well-established, principal pathways, and under enzymatic control, to produce the parent molecules, which are then further modified to afford the diverse array of known plant-derived natural products. Approximately 140 000 of these metabolites are structurally determined at this time, and they represent more than 5750 skeletal types.[1] The functional diversity within structural classes and subclasses is actually quite limited and typically focuses on oxidation, reduction, esterification, hydroxylation, and methylation reactions. It is presumed that as plants evolved to the 300 000−320 000 species that are regarded as being on the planet today,[2] these metabolic processes also evolved. Thus, one frequently observes more complex metabolites in more evolved plants within plant families. Of course, the ginkgolides, derived from one of the oldest and most primitive plants, *Ginkgo biloba* L. (Ginkgoaceae), constitute some of the most architecturally complex plant products.[3]

The evolutionary time of these secondary metabolic processes is not known, but is assumed to have initiated over 1700 million years ago. These processes evolved in metabolic capability for a diversity of ecological reasons, including those related to allelopathy, the need to produce secondary metabolites, sometimes very rapidly, for defense against predators or against bacterial, fungal, and viral diseases in order to survive.[4,5] At the same time, it must be said that a comprehensive study of these processes, and thus the total potential metabolic profile, of even a single plant on the planet is completely unknown at this time. Phytochemists are invariably surprised at the changes in metabolic profiles that occur when plants are collected in different seasons, grown under different climactic conditions, or placed in tissue culture. The need to understand the operational aspects of these biosynthetic molecular switches is profound as the natural product sciences evolve.[6−8]

Synthetic organic chemistry is a young science by comparison, beginning in the middle of the 19th century and evolving today into a complex labyrinth of reagents and reactions capable of modulating a wide range of precursors in a (usually) well-proscribed manner to afford an anticipated product. As synthetic organic chemistry has matured in the past 50 years, there have been four main areas of emphasis for enhancing selectivity: (i) improvement in the range and scope of reactions (chemo- and regioselectivity); (ii) improvement in the yield of reactions; (iii) modulating reaction conditions; and (iv) developing processes that can mimic the frequent chirality of the products of natural processes (enantioselectivity) or required for optimal biological effectiveness. These are vast challenges, and the resources provided through industrial and government support to achieve the tremendous advances that have been made are correspondingly very substantial. Indeed, whole sections of the chemical industry are devoted to the small- and large-scale production of such reagents and chemicals. Unfortunately, by comparison, efforts to understand the selectivity involved in the corresponding natural synthetic processes of secondary metabolism have received scant intellectual and fiscal attention. Such biosynthetic studies are now an extremely important component of synthetic organic and natural product chemistry.

Chirality has therefore become a driving force for synthetic organic chemistry, and the investigation of any reaction that has the capacity to introduce chirality is therefore of substantial interest. The ability of natural sources to induce chirality has been known for almost 150 years, since the first microbial chiral resolution was probably that of Pasteur in 1858 forming (−)-tartaric acid from the racemate.[9] However, it was not until 1894 that Fischer described the uses of emulsin and maltase as the first plant-based enzymes to be studied.[10,11]

Since that time, plant enzymology has evolved in three directions: (i) understanding the basic processes by which plants thrive; (ii) understanding how the sequence of processes of secondary metabolism work together to elicit the products of a particular pathway, for example, morphine biosynthesis,[12] and (iii) obtaining selected enzymes for the particular reproduction of secondary metabolic processes on a more diverse array of substrates. These two concepts of selectivity in organic synthesis and plant and fungal enzyme knowledge are intellectually and practically fused in the realm of biocatalysis.

Biocatalysis employing microorganisms dates back over 4000 years to the early Sumerian records of brewing beer and has been an integral aspect of human cultures in the development of numerous foods and beverages globally since that time. Because of the burgeoning need for chiral drugs derived from prochiral precursors, interest in using enzymes for such transformations has increased steadily as the agrochemical and pharmaceutical industries

⊥ Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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have grown. A recent study based on the synthetic pathways used by three British drug companies for 128 drugs showed that 69 of these were chiral compounds, each having about two chiral centers.[13] It was found that the predominant method for the introduction of chirality continues to be resolution through salt formation, followed by dynamic kinetic, chromatographic, and enzymatic methods.[13] Required chirality is typically introduced early in the overall synthetic process for reasons of ease and integrity of manipulation. The dominant required functional group interconversions were of an alcohol to a halide, of an amide to an imidoyl chloride, and of an acid to an acid chloride. Protection and deprotection reactions in these drug syntheses accounted for over 20% of all the synthetic reaction steps.[13] Comments were made that "The development of a mild catalytic hydrogenation...would find widespread industrial application" and "The discovery of new chemoselective oxidations, particularly if catalytic, would greatly increase flexibility in synthetic design", and finally, "Development of catalytic, low-waste acylation methods would significantly improve the environmental performance of many syntheses".[13]

These are important challenges to which academic and industrial laboratories can apply intellectual and fiscal resources. Part of the challenge is developing high-throughput technologies to find and optimize such catalysts. Wells from AstraZeneca has recently discussed the nature of biocatalysts and the development of industrial biocatalysts from the screening phase to the use of resins on a large scale to enhance surface availability.[14] In the development phase, the deployment of directed evolution strategies may be necessary in order to optimize the catalytic activity, the enzyme stability, and the selectivity of the enzyme. Directed evolution involves creating a diversity of mutant genes and then, based on their corresponding phenotype, sorting these. Delcourt and co-workers[15] have recently discussed some of the approaches to both the library creation and the screening/selection phases for the development of optimum mutants of enzymes.

Applications for these developmental processes have been principally for the improved synthesis of pharmaceuticals (typically key chiral intermediates)[16] or for the exploration of chirality in enhancing biological reactivity. While much of the research in this area, particularly the screening of potential enzyme sources, has been conducted outside of the pharmaceutical industry, there is a recognition that the need for such process development on the small and larger scales needs to be brought in-house. Consequently, a number of the major companies (Bristol-Myers Squibb, Merck, Pfizer, and Glaxo SmithKline) have dedicated research efforts for the exploration of the use of biocatalysts to improve their drug synthesis processes.[17] In addition, there are a number of smaller companies, some of them spin-offs from the larger pharmaceutical houses, that are exploring ways to potentiate the discovery and use of enzymes in drug synthesis. Shaw and co-workers of Lonza have reviewed the importance of biocatalytic processes for a custom organic synthesis manufacturer.[18] Such processes are also important for other aspects of the chemical industry. For example, long-chain fatty acid esters, such as cetyl ricinoleate, have been synthesized enzymatically for the cosmetic industry.[19]

Pharmaceuticals in the World

Over 20 years ago it was estimated that 64% of the world's population uses plants as their primary form of health care.[20] This number is probably higher today now that Earth's population has gone from 4.79 billion to 6.65 billion in that time. Elsewhere, one of us has discussed the extremely urgent need to develop traditional medicines on a standardized basis as a response of the natural product science community to this burgeoning global public health crisis.[8,21−24] The capacity to synthesize organic chemicals on a fine or bulk scale in order to make pharmaceuticals globally available does not extend to the rest of the world. An associated issue within the "Great Divide" that exists in the world today with respect to the availability of pharmaceutical products is the cost of such imported products in less-developed countries. One of the solutions to this health crisis has been the development in a few selected countries of fine chemical industries capable of accessing needed chemicals and reagents for the internal production of particular organic compounds of high commercial interest. However, for both academic and industrial needs there remains a requirement for access to cheaper reagents that can contribute to the development of affordable bulk and fine chemicals.

It is now more widely realized that, from a pharmaceutical perspective, it will not be possible to maintain the current commitment to synthetic drugs without strategic philosophical and process changes.[25,26] As a consequence, the more affluent part of the developed world has seen an increasing research and development investment in the concern for processes that are "green". While this has been defined in a number of ways,[25−27] there are a set of 12 principles that have been developed to embrace the concepts and practice outcomes anticipated as a result of employing "green" strategies.[28] Succinctly, these principles can be coalesced into six aspects related to synthetic organic chemical reactions: (i) recyclable and safer solvents; (ii) more energy-efficient reaction conditions; (iii) recyclable (or at least catalytic) reagents; (iv) renewable feedstocks that do not deplete the resources of the planet; (v) avoiding unnecessary reaction steps (such as protection reactions) and aiming for atom economy, and (vi) reaction byproducts that are environmentally friendly and of minimal toxicity. This effort has led to some remarkable changes in how certain industrial chemicals are produced and, thus, the environmental impact of the overall processes.[25−27]

In a less-developed country, particularly for academic and industrial organic chemical research laboratories, one of the major challenges to a productive research environment is the acquisition of reagent chemicals and solvents. Delays in shipping, delays in customs inspection, and high taxes on the importation of chemicals are serious deterrents to the initiation and development of certain types of chemical research programs, particularly synthetic organic chemistry programs.

These two areas of concern, the greening of organic chemistry and the need to have cheap and effective reagents available in the less-developed world, coalesce in the concept that plants, as well-established sources of secondary metabolites, most of which incorporate one or more chiral centers, may have the capacity to achieve required synthetic chemical transformations if they mimic established biosynthetic steps. Given the ubiquitous nature of certain biosynthetic steps and their relationship to needed "green" organic chemical reactions, the potential is abundantly clear. For example, there are numerous natural products in which a double bond is reduced under enzymatic control and, thus, probably in a chiral manner. There are many examples where esterification, hydrolysis, glucosylation, deglycosylation, or *O*-, *N*-, or *C*- alkylation occurs regiospecifically. In addition, there are many instances where, regiospecifically and stereoselectively or stereospecifically, functional groups are transformed oxidatively or reductively. In Nature, each of these processes is under exquisite enzyme control. The challenge is to explore and harness these enzymatic capabilities and to potentiate their use as reagents for organic synthesis. This brief review will discuss some of the uses of microbial enzyme and plant cell systems for chemical transformations and present some of the recently evolving work on the use of whole plants, particularly foodstuffs, for the conduct of certain chemical reactions in an enantioselective or stereoselective manner. This review is not intended to be comprehensive, and we apologize in advance to any researchers whose work in these areas is not discussed herein.

Biocatalysts in Industry

For the past 10 years or so, many fine chemical companies have been exploring the use of biocatalytic processes in the synthesis of

their products or for the custom synthesis of particular client-defined compounds. Frequently, these studies have frequently been driven by a regulatory need, as far as pharmaceutical entities are concerned, to incorporate chiral centers in drugs and drug candidates. As a result, a number of classes of enzymes have evolved,[17,29] including hydrolases, oxidoreductases, transferases, lyases, ligases, and isomerases to effect selected organic transformations. The use of the enzymes from various eukaryotes as biocatalysts for industrial uses has been reviewed recently.[30]

The ability to induce chirality in a molecule through synthesis has also been examined for several reaction processes. An example would be the enantioselective hydrolysis of a racemic ester as described for the formation of (*S*)-2-ethoxy-3-(4-hydroxyphenyl)-propanoic acid (**1**), which is used in the synthesis of PPARα and -γ agonists.[31] The reaction on the ethyl ester was operated on a 44 kg scale and afforded yields of 43−48% with ee in the range 98.4−99.6%.

The significance of chiral epoxide formation has been explored using halohydrin dehalogenases derived from *Arthrobacter* sp. AD2, *Mycobacterium* sp. GP1, and *Agrobacterium radiobacter* AD1. In the presence of cyanide these reactions yield the corresponding cyanohydrin in moderate yield and enantiomeric excess (ee).[32] There is a need for enantiopure cyanohydrins, and these can be produced in good enantiomeric excess with the appropriate (*R*)- or (*S*)-hydroxynitrile lyase (HNL). This area of developing methods for the formation chiral cyanohydrins has been extensively reviewed by Griengl and co-workers[33−36] and Effenberger.[37] One area of concern has been the stability of these systems, given that in the partially purified enzyme systems other reactions could also occur. It was found that flavonoids, such as rutin and hyperoside, at about 5 ng/mL could improve enzyme stability up to 50%.[38]

The (*S*)-hydroxynitrile lyase (HNL) from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. has been examined for effectiveness and selectivity against a series of α- and β-substituted alkyl and alkoxy aldehydes. It was found that there was no chiral discrimination between the enantiomers of the racemic substrates, and therefore the system could not be considered for kinetic resolution.[39] A comparison with the (*R*)-HNL derived from *Prunus amygdaloides* Schltr. showed that the *Hevea* HNL gave a higher diastereomeric excess (de), but was very negatively influenced by the presence of oxygenated substituents at the α- or β-positions. A HNL derived from *Manihot esculenta* Crantz has been used for the enantioselective HCN addition to form (*S*)-ketone cyanhydrins (85−97% yield and 69−98% ee). Acylation afforded an (*S*)-acyloxynitrile intermediate that could be cyclized by lithium hexamethyldisilazanide (LHMDS) to afford 4-amino-2(5*H*)-furanones without racemization, which are of biological interest.[40]

A recent example that has been scaled up to produce kilogram quantities of product has been the synthesis of (*R*)-2-amino-1-(2-furyl)ethanol (**2**) using a HNL derived from *Hevea brasiliensis*, followed by borohydride reduction of the nitrile to the amine. Overall yields were in the 82−95% range in repeated runs with average ee of 99.7%.[41] Weis and co-workers have described the development, through high-throughput screening, and the use of a recombinant almond *R*-HNL isoenzyme 5, which is overexpressed in the yeast *Pichia pastoris*, for the synthesis of (*R*)-cyanohydrins based on natural and unnatural substrates.[42] High-throughput screening has also been used to search for nitroreductases for the synthesis of amines, such as anilines and chiral amines.[43] An oxidation reaction that has been scaled up is a catalytic Baeyer−Villiger oxidation of acyclic ketones to lactones using a cyclopentanone monooxygenase from *Comamonas* NCIMB 9872 supported on a resin.[44]

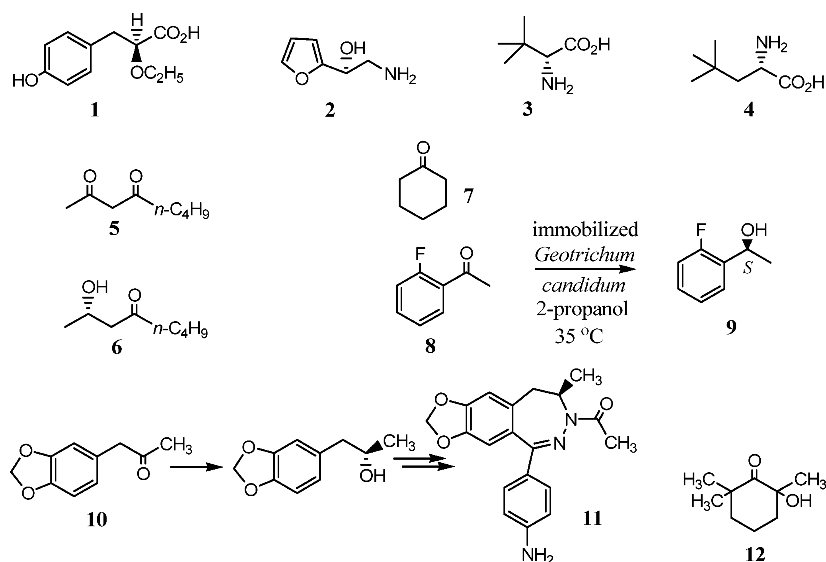
A prominent center for the study and development of industrial biocatalytic processes is the Research Centre for Applied Biocatalysis at the University of Graz, Austria. One of their recent studies has focused on the biocatalytic asymmetric hydrogen transfer process, which offers an oxidation of racemic *sec*-alcohols to ketones using an alcohol dehydrogenase ADH-'A' from *Rhodococcus ruber* DSM-44541. This system affords the ketone (leaving the chiral *sec*-alcohol in this case with the (*R*)-configuration) in aqueous medium at room temperature and is carried out as a coupled substrate approach in the presence of acetone. Reaction times were 24−38 h, and yields were in the 50% range with ee values of >99% for low molecular weight *sec*-alcohols. For more complex substrates, yields and ee dropped substantially.[45] Faber and co-workers have reviewed the biocatalytic oxidation of primary and secondary alcohols[46] and also the oxidation of alcohols and the reduction of ketones.[47] The oxidations typically involve an alcohol dehydrogenase, alcohol oxidases, peroxidases, monooxygenases, or whole cell systems. The systems are typically microbially based, and some were indicated to be commercially available.[46] No plant materials were mentioned for oxidative purposes other than a glycolate oxidase from spinach (*Spinacia oleracea* L.).

Aldolases can catalyze selective carbon−carbon bond forming reactions, but limited substrate ranges have hampered their development. Recently, a Dutch collaboration has reported on the development of a modified (through directed evolution)[15] *E. coli*-based, 2-deoxy-D-ribose 5-phosphate aldolase to produce an intermediate for atorvastatin synthesis in a considerably higher yield.[48]

Another important reaction of substantial interest is the reductive amination of an α-keto acid or ester to afford the corresponding chiral amino acid, which may be accomplished chemically through the Strecker reaction.[49] This process can be achieved enzymatically through the use of a leucine dehydrogenase and a formate dehydrogenase, where the latter provides a hydride ion to recycle the NAD^+ to NADH. However, the need for isolated enzymes and expensive cofactors limits the scalability, and thus an approach using a recombinant whole cell system was developed for the formation of L-*tert*-leucine (**3**)[50] and the bulky amino acid L-neopentylglycine (**4**).[51] In this case, *E. coli* was used as the host for a leucine dehydrogenase from *Bacillus cereus* and a mutant of a formate dehydrogenase from *Candida albicans*. The yield in the presence of ammonium formate after 25 h, with no cofactor added, was greater than 95% with >99% ee.

A common functional group transformation is the reduction of a ketone to a chiral secondary alcohol, and strategies for such transformations have been developed, including systems that involve a two-enzyme strategy involving an alcohol dehydrogenase and either a formate dehydrogenase or a glucose dehydrogenase.[17] Ketoreductases are also available (see later in this review), but these, like alcohol dehydrogenases, require nicotinamide adenine dinucleotide (NADH) or NAD phosphate (NADPH) as the hydrogen source. Strategies have evolved to regenerate these cofactors *in situ*.[17] A more challenging target is the regioselective reduction of a carbonyl group in the presence of polyfunctionalized systems, without protecting groups. Correspondingly, there is the need to be able to selectively oxidize chemically equivalent or similar diols to the chiral keto-alcohol, which is a common feature in natural product structures. Faber and co-workers[52] have described strategies for these transformations using the lyophilized cells of *Rhodococcus ruber* DSM 44541 containing ADH-'A' as the catalyst.[52] The system worked well (90% yield, >99% ee) for 2,4-octanedione (**5**) to afford the 2*S*-alcohol **6**, but was less successful in terms of yield for smaller diketones.[52] For the oxidation process, selectivity between hydroxyl groups in a diol was observed with little double oxidation to the diketone; yields of the chiral hydroxy ketone varied between 27 and 62%.[52] Selectivity was also observed for a secondary versus a primary alcohol group in the same molecule, which could have synthetic use. In 2004, the Graz group reported on the regio- and stereoselective reduction of α,β-unsaturated ketones using lyophilized cells from *R. ruber* DSM-44541 to afford the corresponding allylic alcohols.[53] This system afforded the (*S*)-products in the range 50−92% with >99% ee after 20−22 h. This

Chart 1



biocatalyst, which recycles the cofactor NADH in the presence of 2-propanol as a solvent and a cosubstrate, had been used previously for the reduction of aliphatic, aromatic,[54] and heteroaromatic ketones.[55] Immobilized cells of the fungus *Geotrichium candidum* were used in a continuous flow process using supercritical carbon dioxide for the reduction of ketones in the presence of 2-propanol.[56] The system worked effectively for the reduction of cyclohexanone (**7**) and also reduced 2-fluoroacetophenone (**8**) to the (*S*)-alcohol **9** with >99% ee. Volumetric productivity was higher than a batch process.

Yeast systems have been used for the reduction of benzyl ketones, as in the reduction of the key intermediate **10** in the formation of the 5*H*-2,3-benzodiazepine LY300164 (**11**) by a group at Eli Lilly and Company using a NAD(P)H-dependent oxidoreductase from *Zygosaccharomyces rouxii*.[57] The substrate was absorbed onto a resin and then added to an aqueous suspension of the yeast up to levels of 80 g/L. Yields were around 99.6% with >99.9% ee. *Saccharomyces cerevisiae* 013 immobolized on sintered glass has been used to reduce aldehydes and ketones, but the yields were modest to good (27−87%).[58] A baker's yeast system was used for the reduction of racemic 2-hydroxy-2,6,6-trimethylcyclohexanone (**12**), but the yields were low and the ee only about 90%.[59] The recycling mechanism for the cofactors that transfer hydride in the yeast systems has been studied, but without completely clarifying the process.[60]

Speicher and co-workers have described the use of bryophytes, in particular liverworts grown in cell culture or as a suspension in a phosphate buffer for reductive transformations.[61] Cultures of *Marchantia polymorpha*, *M. plicata*, *Riccia fluitans*, and *Asterella blumeana* were developed and used for the reduction of a variety of aliphatic and aromatic ketones and benzaldehyde; a number of keto-esters were also examined. For acetophenone (**13**), the reduction reaction was moderately effective, with yields of the (*S*)-alcohol **14** in the range 34−74% ee and a maximum chemical yield of 74% after 10 days. Somewhat better results were obtained for the reduction of ethyl and *tert*-butylacetoacetates (**15**, **16**), where *R. fluitans* and *A. blumeana* gave ee values of 90 to >95% and yields of 70−90% for the 3*S* product (e.g., **17**). The reduction of ethyl 2-methyl-3-oxo-butanoate (**18**) by *M. plicata* gave good yields (86−92%) and good to high ee (92−96%) for the *anti*-(2*S*,3*S*)-product **19**. (−)-(4*R*)-Carvone (**20**) was used as a substrate to examine the reduction of the double bond. The results were comparable or slightly better than earlier results with *Nicotiana tabacum* L.[62] and *Medicago sativum* L.,[63] occurring with high regio- and diastereoselctivity to afford (+)-*n*-dihydrocarvone (**21**).[61]

Pollard and co-workers at Merck have described a process for the development of ketoreductases that could effect the formation of either the (*S*)- or (*R*)-isomers of 3,5-bistrifluoromethylphenyl ethanol (**22** and **23**, respectively),[64] and the development of one system in particular capable of being scaled up industrially. The enzymes were derived from *Rhodococcus erythropolis*, *Candida parapsilosis*, and *Candida boidinii*, which yielded the (*S*)-enantiomer, and *Lactobacillus brevis* and *Lactobacillus kefir*, which yielded the (*R*)-enantiomer. Effects of pH, temperature, and substrate concentration were followed by selective cofactor addition (formate and glucose dehydrogenases) in developing a pilot-scale process.[64]

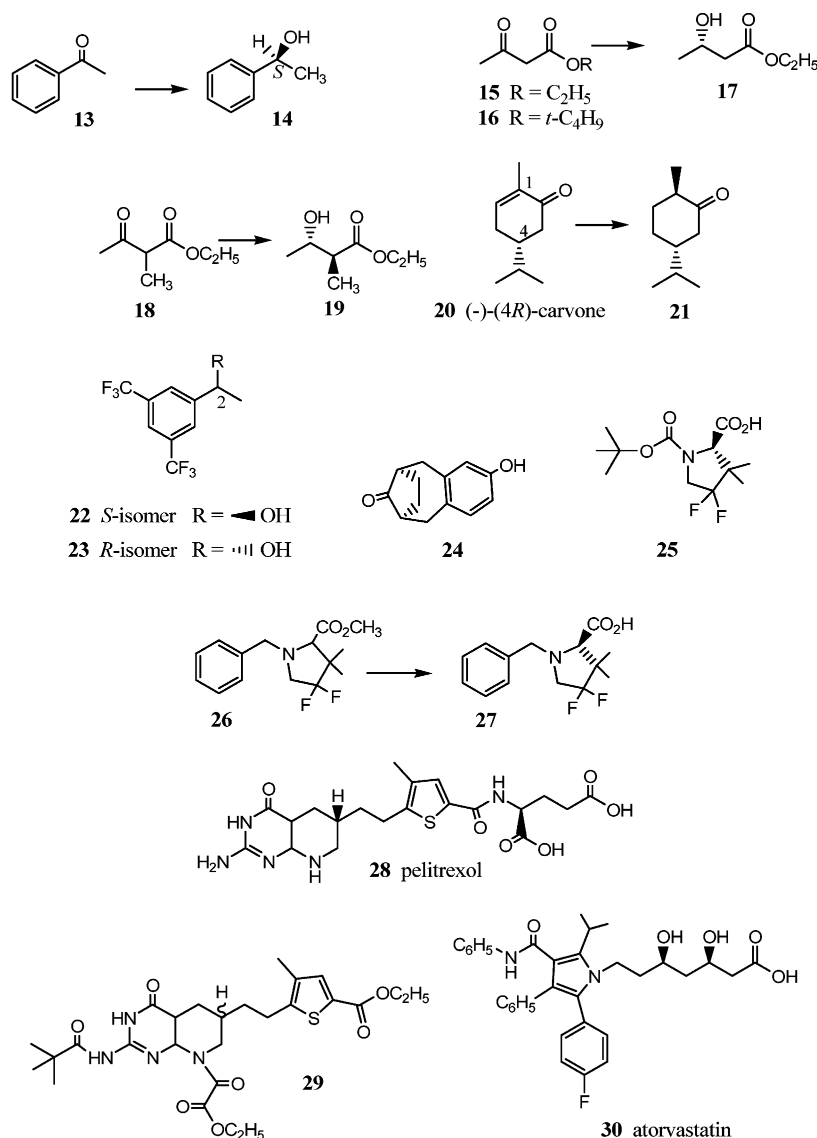
The same group at Merck has also described the use of a commercial ketoreductase for the resolution of a bicyclic ketone intermediate in which the (6*R*,9*S*)-enantiomer of 11-oxo-5,6,7,8,9,10-hexahydro-6,9-methanobenzocyclooctene was selectively reduced, leaving the required (6*S*,9*R*)-enantiomer **24**.[65] This is not the first example of using such systems for the chiral resolution of polycyclic molecules, but in this case the yields were substantially higher following studies aimed at enhancing substrate solubility using dextrin and optimizing pH and temperature. Somewhat surprisingly, the optimum temperature was found to be 10 °C.

A group at Pfizer has developed a protease-catalyzed enantioselective hydrolysis as an intermediate step in the synthesis of **25**, which is a crucial intermediate in the synthesis of several HIV protease inhibitors.[66] This followed a screening process of 94 hydrolases, including lipases, proteases, esterases, and acylases. Eventually a pig liver esterase was used on a 1 kg scale for the hydrolysis and resolution of the racemic intermediate methyl ester **26** to afford the acid **27** in 44% isolated yield and >99% ee.[66] Workers at Pfizer have also examined the chemoenzymatic synthesis of pelitrexol (**28**), a GARFT inhibitor.[67] An oxalamic ester was introduced, and this aided the enantioselectivity for the hydrolysis of the ethyl ester **29** at a distant location in the molecule using a *Candida antartica* lipase B (CAL-B) preparation.[67]

The tremendous interest in the synthesis of intermediates for the formation of Pfizer's cholesterol-lowering drug atorvastatin (**30**), particularly of the (3*R*,5*S*)-dihydroxyhexanoate side chain, has spurred a number of biocatalytic approaches from at least five pharmaceutical companies.[68] Depending on the precursors and the subsequent chemical transformations, alcohol dehydrogenases, carbonyl reductase, glucose dehydrogenase, formate dehydrogenase, and deoxy-5-phosphate aldolase systems have been deployed.[68]

The availability of ketoreductases has increased with the development of companies that market such products where the

Chart 2



whole system merely requires the addition of water. These systems are very expensive (typically more than \$2000/g), their source is not discussed, and their range of substrate specificity is not described in the advertising or at the corporate website. This makes targeting the use of such products extremely speculative and their strategic inclusion in synthetic pathways unattainable for chemists in less-developed countries. On the other hand, the availability of these diverse systems has offered opportunities for academic–industrial collaboration. One such recent report concerns the recombinant ketoreductases available from Biocatalytics in the KRED101–131 series.[69] These enzymes of undescribed origin effect the reduction of substituted aryl ketones to the corresponding alcohol[69] and the reduction of β-ketoesters[70] in good to excellent yield and in high ee. Reactions were dependent on substrate structure, and yields dropped for systems in which the size of the α-substituent of ethyl 3-oxo-butyrate (**31**) was steadily increased.[69] The first set of β-ketoesters varied in alkyl chain length and substitution, whereas the second set was substituted at the α-position. Most of the panel of ketoreductases were effective, affording the L-enantiomer of the alcohol with excellent ee. As the branching increased, some of the enzyme systems decreased in conversion rate, while others gave nonpredictable results; some enzymes gave high conversions to the D-enantiomer.[70] For the second set, as the size of the α-substituent increased, enzyme activity decreased. Although the second set could potentially yield four diastereomers, in many cases only one diastereomer was obtained with very high diastereoselectivity, and this was particularly true with substrates **18**, **32**, **33**, and **34**.[70]

Yeast systems have also been used for the transformation of other functional groups. For example, baker's yeast has been used for the reduction of aryl azides to afford the corresponding amine for the synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepine antibiotics,[71] and yeasts have also been used for the reduction of alkenes.[72,73] An immobilized yeast system derived from *Rhodotorula rubra* CBS 6469 was used for the enantioselective reduction in good yield of an α-thio-cinnamide group in 5-benzylidenethiazolidene-2,4-diones.[72] Retaining enantioselectivity was an issue due to the stereochemical instability of the product. Reducing the pH of the system to 3.5 for a 4 h reaction gave 93% conversion to **35** as the (*R*)-enantiomer (95:5 ratio with the (*S*)-enantiomer). Bruyn, in 1954, showed that *Candida lipolytica* grows on hexadec-1-ene as a sole carbon source,[74] and subsequent studies revealed that various fungi and bacteria could metabolize alkenes through epoxidation and hydroxylation.[73] Vlahov and co-workers examined the biocatalysis of four alkenes (cyclohexene, non-1-ene, 2-methylbut-2-ene, and 2,4,4-trimethylpent-2-ene) by three *Candida* species, *R. rubra*, *Rhodococcus*, and *Pseudomonas* species.[73] None of the yeasts could transform non-1-ene, but *C. lipolytica* gave a 31% yield of cyclohex-2-en-1-one and *Candida guilliermondii* gave a 53.7% yield of

2-methyl-2,3-epoxybutane (chirality not determined) after 48 h at pH 6 in a two-phase system. Transformations by the bacteria were very poor.[73]

The Use of Plant Systems for Biocatalysis

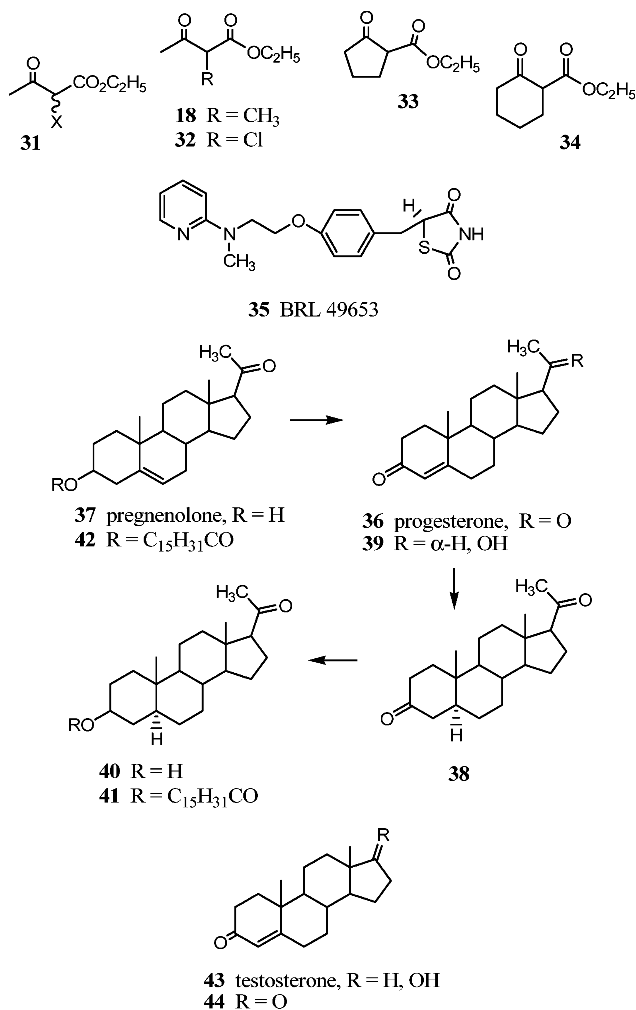
For the most part, the systems described above were generated from microbial (fungal, bacterial, and yeast) sources. By comparison, less attention has been given to the use of plants as potential sources of enzymes that could conduct selected organic reactions. Several years ago, Reinhard and Alfermann[75] provided a very interesting review of the biotransformations induced by substrate addition to plant cell cultures, with a particular emphasis on aromatic compounds, coumarins, alkaloids, terpenoids, and steroids. It was noted that a number of systems were able to perform transformations that did not relate to known substrates and isolates of the intact plant (e.g., the conversion of tryptophan to harman and nor-harman by *Phaseolus vulgaris* L. cultures). This is clearly of critical importance from the aspect of substrate diversity. As the use of plants for these transformations has progressed, three distinct systems have been utilized for substrate modification: cell cultures, plant-derived enzymes, and intact plant materials. Some of the representative studies that have been conducted in the past 30 years in this area are mentioned below.

Cell Cultures. The first use of plant cell cultures to transform exogenous metabolites was probably the work of Stohs and Staba using digitoxin and digitoxigenin and *Digitalis* suspension cultures.[76] But it was the subsequent studies of Graves and Smith[77] that hinted at the potential selectivity of the biotransformation processes. They showed that both progesterone (**36**) and pregnenolone (**37**) could be transformed by several plant species to afford products. Thus, **37** was transformed by *Digitalis purpurea* L., *Digitalis lutea* L., and *Nicotiana tabacum* to progesterone (**36**), and by *D. lutea* to further yield 5α-pregnan-3,20-dione (**38**) after 7−14 days. When progesterone (**36**) was used as a substrate, *Parthenocissus* sp., *Rosa* sp., and *N. tabacum* afforded pregn-4-en-20α-ol-3-one (**39**), in which the C-20 ketone was regio- and stereoselectively reduced, whereas *Solanum tuberosum* L., *D. purpurea*, *D. lutea*, *Atropa belladonna* L., *N. tabacum*, *Nicotiana rustica* L., and *Hedera helix* L. all yielded **38** and 5α-pregnan-3β-ol-20-one (**40**), in which the regio- and stereoselectivity of reduction was at C-3.[77] *Ipomoea* sp. (sweet potato) and *Malus pumila* Mill. (apple) also metabolized the substrates, but the products were not identified.[77]

This work was followed up by Furuya and co-workers, who showed that progesterone (**36**) could be transformed to 5α-pregnanolone palmitate (**41**) by both *Nicotiana tabacum* (17% yield) and *Sophora angustifolia* Siebold & Zucc. cells after 4 and 5 weeks, respectively.[78] Pregnenolone (**37**) was also transformed into **41** and pregnenolone palmitate (**42**). For the formation of **41**, it is worth noting that at least three steps are involved: regio- and stereospecific reduction of the carbonyl group at C-3, stereospecific reduction of the 4,5-double bond, and esterification. Hirotani and Furuya then turned their attention to the biotransformation of testosterone and some androgen derivatives using *N. tabacum* cell cultures.[79] Testosterone (**43**) afforded nine different metabolites, and androst-4-ene-3,17-dione (**44**) afforded eight metabolites, which reflected the same three reactions observed previously,[78] followed by esterification by palmitic acid or glucosylation. A time-course study showed that the 5α-androstane-3β,17β-diol fraction and the epi-androsterone fraction each peaked after about 5 days and then declined, while the palmitate and glucoside fractions increased steadily and then stabilized after 11 days.[78]

Furuya and co-workers also showed that *Digitalis purpurea* callus tissue cultures, containing no cardenolides, when shaken with digitoxin (**45**) for 26 days, produced a number of metabolites, including gitoxin (**46**), purpurea glycoside A (**47**), and purpurea glycoside B (**48**).[80] The dominant metabolite was **47**, reflecting

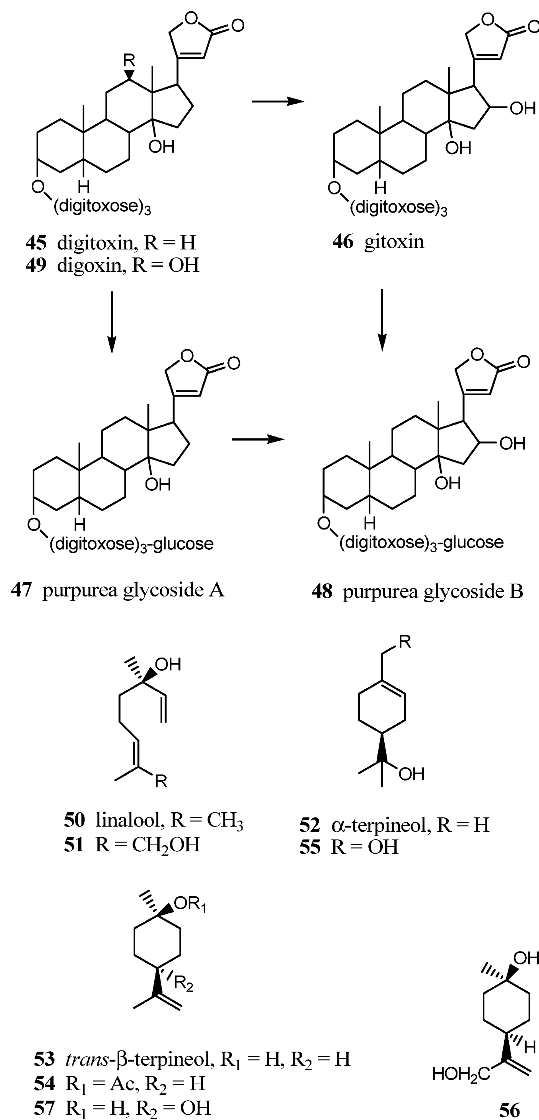
Chart 3



glucosylation of **45**, whereas **46** and **48** represent 16β-hydroxylation, akin to that produced by the action of fungi on cardenolides.[81] Similar results were subsequently observed by Reinhard and co-workers using immobilized cells of *Digitalis lanata* Ehrhart to transform cardiac glycosides.[82] In this instance, digitoxin (**45**) was transformed to purpurea glycoside A (**47**), and 16-hydroxylation to digoxin (**49**) also occurred. The metabolic activity remained constant for at least 61 days.[82]

Suga and co-workers were the first to examine the biotransformation of simple monoterpenes by plant tissue cultures when they cultured *Nicotiana tabacum* "Bright Yellow" with a series of monoterpenes for 7 days. The dominant reaction was regioselective oxidation of the *E*-methyl group to a hydroxymethyl group.[83,84] For example, linalool (**50**) was transformed to 8-hydroxylinalool (**51**) (16.5% yield), and the dihydro derivative of **50** was correspondingly metabolized (14.9% yield). The study was extended using the same callus culture material with three monocyclic monoterpenes, α-terpineol (**52**), *trans*-β-terpineol (**53**), and *trans*-β-terpinyl acetate (**54**).[85] Yields were in the range 3.9−15.0%, but some interesting inferences were drawn: **52** afforded 15% of the 7-hydroxy-α-terpineol (**55**), while *trans*-β-terpineol (**53**) gave the 10-hydroxy- (**56**) and 4-hydroxy- (**57**) derivatives, indicating stereoselective allylic oxidation at C-4; similar results were observed for **54**. The stereoselectivity of the hydroxylation of a carbon−carbon double bond, noted for **54** above, was investigated with α-terpinyl acetate (**58**) over a 9-day period of culture with the *N. tabacum* system.[86] Eight products were observed during the time period, in which **59** dominated (27%) with none of the 2,3-isomer being formed, indicating the stereospecific formation of the *trans*-diaxial diol.

Chart 4



In 1983, Suga and co-workers reported on the interconversion of five- to eight-membered cycloalkanones (**7**, **60**−**62**) and the corresponding cycloalkanols (**63**−**66**) by *Nicotiana tabacum* cell suspension cultures.[87] The equilibrium balance depended on the ring size. For cyclohexanone (**7**), the equilibrium lay 4:1 in favor of the alcohol **64**, whereas cyclopentanol (**63**), cycloheptanol (**65**), and cyclooctanol (**66**) were quantitatively converted to the respective ketones (**60**, **61**, and **62**).[87]

These studies were followed up by an examination of the oxidation of a series of bicyclic monoterpene alcohols, including borneol (**67** and **68**), isoborneol (**69** and **70**), and isopinocampheol (**71** and **72**) using cultured suspension cells of *Nicotiana tabacum* "Bright Yellow".[88] Clear distinctions between the rates and extent of oxidation were observed. For example, (+)-borneol (**67**) afforded (+)-camphor (**73**) after 10 days, whereas the (−)-isomer (**68**) was almost unaffected. A similar enantioselectivity for the oxidation reaction was observed for (−)-isoborneol (**69**) to **73** (100% yield) and to a lesser extent for (−)-isopinocampheol (**71**) to (−)-isopinocamphene (**74**) in 44% yield. The results offer the possibility that a plant cell system could serve as a bioreactor capable of effecting optical resolution of racemic cycloalkanols.[88]

The ability of plant cell cultures to distinguish between two diastereomers of dibenzylbutanolides (e.g., **75**) to afford a single isomer (e.g., **76**) in quantitative diastereomeric yield has been described by Takemoto and co-workers.[89] Four different plant materials were used (reaction time to completion): *Catharanthus roseus* (L.) G. Don (5 days),[90] *Camellia sinensis* (L.) Kuntze (18 h), *Nicotiana tabacum* (6 h), and *Daucus carota* L. (3 days).[89]

Plant cell systems have also been used to conduct other types of oxidation reactions. Thujopsene (**77**) was oxidized to mayurone (**78**) in 63% yield after 20 days using cultured cells of *Hibiscus cannabinus* L. *Nicotiana tabacum* and *Catharanthus roseus* were also used to afford **78** in 85% and 78% yield, respectively, after 14 days.[91] The reaction was proposed to proceed through an intermediate diene. Enzymes derived from plants in the Asteraceae have been used for the regio- and stereospecific oxidation of sesquiterpene olefins.[92] An interesting application of the use of plants as biosensors is the determination of catechol through reduction at a glassy carbon electrode following biocatalytic oxidation using a coconut-based reactor.[93] The system was stable for at least a week and could analyze, in the micromolar range, 60−90 samples per hour. Some years earlier, a number of fruits and vegetables (eggplant, potato, manioc, yam, apple, dwarf banana, apple-flavored banana, pear, and peach) were examined for their ability to serve as a biocatalytic oxidizing agent for phenols.[94] The yam (*Alocasia macrorhiza*) was the best source of a polyphenol oxidase, and an amphoteric biosensor was constructed through immobilization of crude yam extract with glutaraldehyde and bovine serum albumin onto an oxygen electrode. Detection levels of 10^{-5} M and lower were achieved for pyrogallol, catechol, cresol, and phenol with a response time of 1−4 min;[94] applications for phenol detection in industrial wastewaters were described.

Decarboxylation reactions are of considerable synthetic interest, but are often difficult to achieve cleanly. In the case of styrenes, these reactions typically require extremely vigorous conditions, such as heating in quinoline at 200−300 °C for 4−5 h in the presence of Cu powder. Many years ago, decarboxylase enzymes from *Aspergillus*[95] and *Aerobacter*[96] were used to decarboxylate *trans*-cinnamic acids. Takemoto and co-workers have also shown that cinnamic acid derivatives (e.g., **79** and **80**) could be decarboxylated quantitatively, employing plant cell cultures at room temperature.[97] Using systems derived from *Catharanthus roseus*, *Nicotiana tabacum*, *Daucus carota*, and *Camellia sinensis* they showed that *C. sinensis* was ineffective in the decarboxylation of ferulic acid (**79**), but that quantitative decarboxylation could be realized under three different conditions with *C. roseus* cells, and under two different conditions by treatment with *N. tabacum* cells for 3−5 days. Typical preparations included suspended cells after a 10-day growth period and homogenized plant cells in phosphate buffer at pH 6.0 or 6.4. Yields dropped substantially for a more diverse array of substrates. For two substrates, *β*-furyl acrylic acid (**81**) and 3-nitrophenylacrylic acid (**82**), *C. sinensis* afforded the corresponding decarboxylated product in quantitative yield after 10 days.[97]

Takemoto and Achiwa[98−100] have described the use of *Catharanthus roseus* cell cultures for the deracemization of pyridyl ethanols. Thus, when racemic 3-pyridylphenylmethanol (**83**/**84**) was treated with *C. roseus* cells at room temperature for 17 days, the corresponding (−)-**84** was obtained in 93% yield with 100% ee. Over an extended period (32−52 days) conversion to the ketone **85** occurred in 45% yield. Immobilized cells of *Nicotiana tabacum* were not effective in performing this transformation.[100] The mechanism appears to be that (+)-**83** is rapidly oxidized stereoselectively to **85** and then rapidly reduced to (−)-**84**, whereas (−)-**84** is only very slowly oxidized to **85**. Studies were also conducted by the same group using cell cultures and immobilized cells of *N. tabacum* in which the bioreduction of three benzoylpyridines was evaluated.[101] Yields were in the range of 80% with an ee of 48−71% after 12−30 days. The results were extended using the 2- and 4-isomers of *x*-(4-chlorobenzoyl)pyridine (**71**).[102] The best results were obtained with an immobilized carrot cell preparation, which retained effectiveness for at least four reuses (94% yield, 86% ee for the (*R*)-isomer after 15 days). Yields for *N. tabacum* and *C.*

Chart 5

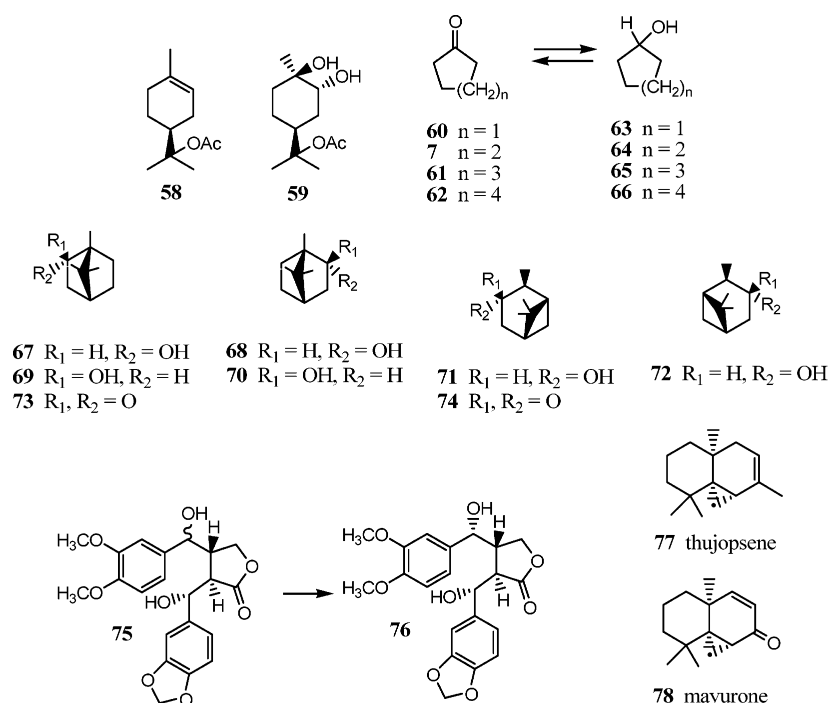
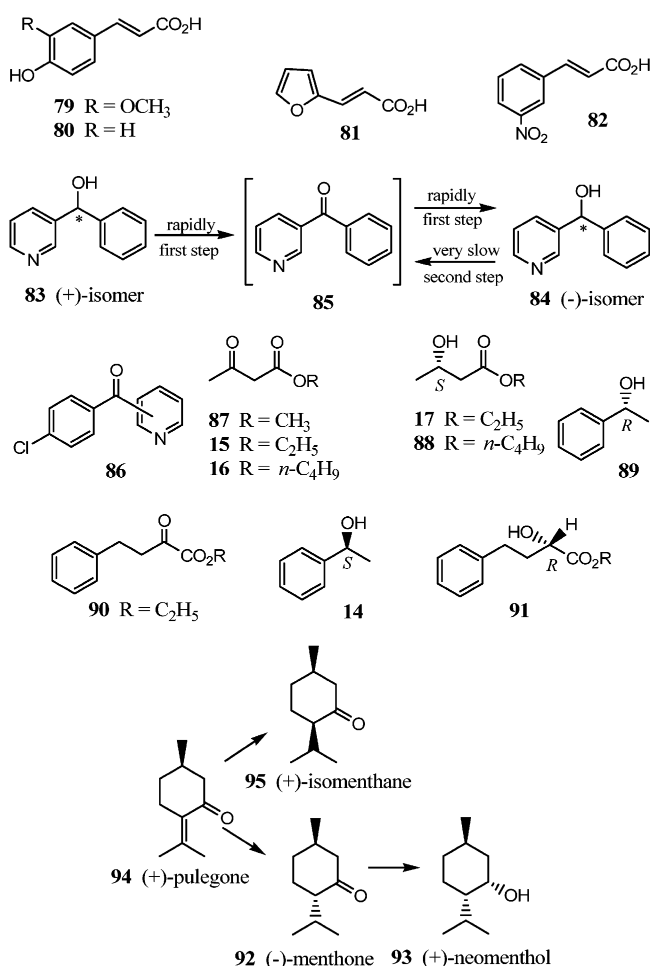


Chart 6



roseus cell systems were lower, at 10% and 79%, respectively. In 1989, Naoshima and co-workers showed that immobilized cells of *N. tabacum* were shown to regio- and stereoselectively reduce some 3-oxobutanoates (e.g., **15**, **16**, **87**) to the corresponding (*S*)-3-hydroxybutanoates (e.g., **17**, **88**) in relatively high optical yields.[103,104]

In 1991, Naoshima and Akakabe reported the use of immobilized cells of *Daucus carota*, *Nicotiana tabacum*, and *Gardenia jasminoides* J. Ellis entrapped in calcium alginate beads for the reductive biotransformation of four aromatic ketones.[105] *N. tabacum* cells gave yields in the range 6−37%, and *G. jasminoides* yields in the range 13−25%. On the other hand, the immobilized *D. carota* cells gave yields in the range 54−70% with ee values of 89−99%. Incubation times varied from 2 to 13 days for the *D. carota* system. Attempts to use free and immobilized baker's yeast were lower than the plant cell system and were reviewed by these researchers.[106] These preliminary data were expanded by Naoshima and co-workers using a variety of keto-esters, aromatic ketones, and heterocyclic aromatic ketones, affording the corresponding secondary alcohols with a yield of 30−63% and in an ee of 52−99% using immobilized *D. carota* cells on alginate beads at room temperature for 5 h to 6 days.[107] Electron-deficient acetophenones were reduced to a greater extent than electron-rich acetophenones or the 2-, 3-, or 4-acetopyridines.

The mechanism of the reduction of acetophenone (**13**) by *Gardenia jasminoides* immobilized cells, to afford exclusively the (*R*)-alcohol **89**, was shown to be a two-step process, in which the initial step is nonstereospecific reduction to afford the racemic alcohol followed by stereoselective oxidation of the (*S*)-alcohol **14** and reduction, in a repeating cycle.[108] Further studies using the immobilized cells of *Daucus carota*, *Nicotiana tabacum*, and *G. jasminoides* showed 99% ee in the reduction of acetophenone by *D. carota* to give (*S*)-**14**, whereas *G. jasminoides* afforded the (*R*)-**89** with 90% ee.[108] *N. tabacum* immobilized cells on the other hand gave 88% ee of (*S*)-**14** in 50% yield after 24 days and a very poor oxidation of the racemic alcohol **14**.[108] There was no indication for *D. carota* as to whether the overall stereoselective reduction was a one-step or a two-step process.[102]

Chadha and co-workers examined the use of immobilized *Daucus carota* cells for the reduction of 4-aryl-2-oxobut-3-enoic carboxylic esters to the 2-hydroxy derivatives.[109] The same group had earlier reported on the reduction of 2-oxo-4-phenylbutanoic acid ethyl ester (**90**) in >99% ee by plant cell cultures of *D. carota*.[110] The 4-aryl-2-oxobut-3-enoic acid ethyl and methyl esters were substituted by Cl or CH_3. Each was incubated with the tissue culture cells of *D.*

carota for 10 days. Conversion to the (*R*)-enantiomer (**91**) occurred with yields of 62–73% and 92–>99% ee.

Hamada's group has also studied the reductive biotransformation of three linear decan-*x*-ones ($x = 2$, 3, and 4) using immobilized cells of *Nicotiana tabacum*.[111] Cells were entrapped in calcium alginate, and the beads were incubated with the substrate at 25 °C for 7–10 days. Products were typically obtained in ca. 90% yield and 98.5–99.2% ee. The reaction rate depended on the proximity of the carbonyl group to the end of the chain. In 1985, Galun and co-workers[112] described the use of 500 Gy γ-irradiated plant cells that were capable of conducting reduction reactions. Irradiated *Nicotiana sylvestris* Speg. & S. Comes and *Mentha* cells maintained the ability to convert (−)-menthone (**92**) to (+)-neomenthol (**93**) in suspension after 14 h. The system was stable and retained the same level of effectiveness after immobilization following entrapment in a glyoxal cross-linked polyacrylamide-hydrazide (PAAH) support. Preliminary work in this area was initiated by Galun's group several years earlier.[113−116] In 1978, they reported on the stereospecific reduction of (+)-pulegone (**94**) to (+)-isomenthone (**95**) by *Mentha* strains over a 24 h period and in essentially quantitative yield.[113] Cell lines derived from plants that did not normally produce pulegone still had this reactivity. The rate of conversion was found to be dependent on incubation time, precursor concentration, and cell density.

Subsequently, a selection of α,β-unsaturated ketones was also examined, including mesityl oxide (**96**), 2-isopropylidene cyclohexanone (**97**), and *trans*-6-methyl pulegone (**98**).[115] Of the compounds tested, only the latter two were reduced by three of the cell lines. The next monoterpene studied was (−)-menthone (**92**), in which the ketone unit was stereospecifically reduced to afford (+)-neomenthol (**93**) by a specific *Mentha* cell line.[114] Optimum conversion was noted after about 12 h, followed by a decline in yield. (+)-Isomenthone (**95**) was not reduced. A cross-linked polyacrylamide-hydrazide (PAAH) system was then used to immobilize the six *Mentha* cell lines, and the conversions of (−)-menthone (**92**) to (+)-neomenthol (**93**) and of (+)-pulegone (**94**) to (+)-isomenthone (**95**) were studied.[116] The first conversion was complete at room temperature after 24 h and to the extent of 63% for the second conversion. Further reactions were not observed using the entrapped cells, whereas using the suspended cells secondary reactions tended to occur.

Suga and co-workers continued their studies on the biocatalytic capabilities of *Nicotiana tabacum* cells, demonstrating that they had the ability to regioselectively reduce the α,β-unsaturated double bond of (+)- and (−)-carvone (**20** and **99**, respectively) and subsequently the ketone group.[62] (4*R*)-(+)-Carvone (**20**) gave (1*R*,2*S*,4*R*)-(+)-neodihydrocarveol (**100**) as the major product in 10.7% yield. The corresponding (4*S*)-isomer (**99**) afforded (1*R*,4*S*)-(−)-isodihydrocarvone (**101**) and (1*R*,2*S*,4*S*)-(−)-neoisodihydrocarveol (**102**) in 13.7% and 7.9% yields, respectively. The α,β-unsaturated double bond of carvone was reduced initially on the *si*-face at C-1, followed by reduction at the *re*-face of the carbonyl at C-2.[62] This inference was subsequently demonstrated through the bioreduction of (4*R*)-[6-^{2}H]-(−)-carvone (**103**) to afford neodihydrocarveol (**104**).[117]

In 1987, Suga and co-workers described additional studies on the use of cultured cells of *Nicotiana tabacum*.[118] Two reactions were reported: the reduction of verbenone (**105**) and the oxidation of neoisopinocamphol (**106**/**107**).[118] Verbenone (**105**) afforded the (1*S*,5*S*)-enantiomer of the dihydro derivative (**108**), whereas the (1*R*,5*R*)-isomer was essentially unaffected. The hydroxyl group of neoisopinocampheol (**106**/**107**) was oxidized enantioselectively to afford the (1*S*,2*S*,3*R*,5*R*)-enantiomer (**74**). Turning their attention to menthane derivatives,[119] analogous results were obtained. Thus (1*R*,4*R*)-(+)-carvomenthone (**21**) was quantitatively converted to (1*R*,2*S*,4*R*)-(+)-neocarvomenthol (**109**), whereas the (−)-enantiomer gave a mixture of products. Stereoselectivity in the oxidation of various menthol derivatives was also explored. Yields were low (20%), even over prolonged (10 day) periods. These data were reviewed by Hamada.[120]

Enzymes. Some of the fundamental enzymes involved in lipid modification have been studied, and these activities have been summarized. Mukherjee has reviewed the use of plant-derived lipases for the biocatalytic transformation of lipids, particularly the triacylglycerol acylhydrolases that hydrolyze ester bonds during the storage of seeds,[121] and Gardner and Grechkin have reviewed the use of lipoxygenase isoenzymes for the modification of natural and synthetic fatty acids to generate hydroperoxide derivatives regio- and stereospecifically.[122] Giri and colleagues[123] have discussed the general use of plant-based systems (cells, organ cultures, and enzymes) for biocatalysis. They described the key reactions that are possible and the strategies involving the cloning and overexpression of genes for the required enzymes, as well as the opportunities derived from site-directed mutagenesis and gene manipulation for expanding substrate specificity.[123]

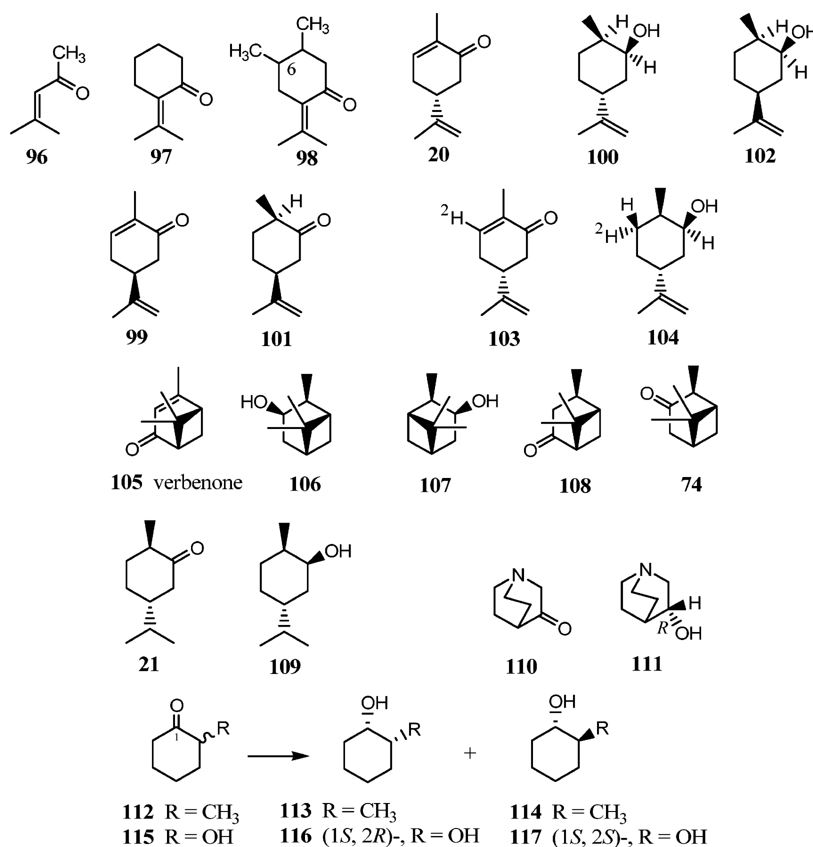
Another example of stereospecificity that may extend to more diverse substrates relates to tropane alkaloid biosynthesis. It is well-established that in the biosynthesis of the atropine/scopolamine alkaloids in *Datura*, *Atropa*, and *Hyoscyamus* the ketoreductase affords the α-isomer at the C-3 position. On the other hand, in cocaine biosynthesis, the chirality at C-3 is of the β-configuration. These reductases, tropinone reductase I and tropinone reductase II, have been studied extensively.[124] One of these enzymes has been applied for the reduction of 3-quinuclidone (**110**) to the corresponding (*R*)-alcohol **111** on an industrial scale.[17]

Intact Plant Systems. Recognizing the inherent difficulties associated with using cell systems, Baldassare and co-workers in 2000 were one of the first groups to use fresh plant material, without any processing, for reactions on exogenous organic substrates.[125] The carrot (*Daucus carota*) root material was simply shredded with a kitchen peeler and the substrate added to a stirred suspension of the roots. The ratio of substrate to plant material was 1:200. After 50 h, racemic 2-methylcyclohexanone (**112**) was reduced to a 1:1 mixture of (1*S*,2*R*)-**113** and (1*S*,2*S*)-**114** with >99% ee, and after 54 h the product from racemic 2-hydroxycyclohexanone (**115**) was a 35:65 mixture of (1*S*,2*R*)-**116** and (1*S*,2*S*)-**117** (>95% ee), respectively. This study prompted investigations on the use of the fresh, intact plant material as a reagent rather than cell systems, suspended or immobilized, or any derived enzymes. These investigators described aspects of their studies in an Italian patent.[126] The invention was related to the ring opening of epoxides and the reduction of ketones using parts of plants or animal organs. The only reaction presented in the abstract was the carrot-catalyzed reduction of racemic 2-methylcyclohexanone (**112**), which was reduced to a 1:1 mixture of (1*S*,2*R*)-**113** and (1*S*,2*S*)-**114** with >99% ee, in 40% conversion after 40 h.[126]

Yadav and co-workers examined the use of *Daucus carota* root material for the asymmetric reduction of azidoketones, which are intermediates for the formation of pharmaceutically important chiral β-amino aryl ethanols.[127] In particular, they reported on the synthesis of (*R*)-(−)-denopamine (**118**), (*R*)-(−)-tembamide (**119**), and (*R*)-(−)-aegeline (**120**). The key step is the conversion of an azidoacetophenone (e.g., **121**) to the (*R*)-(−)-azidobenzyl alcohol (e.g., **122**). The yields were slightly higher using *D. carota* than using baker's yeast, with ee values of >99% and isolated yields of 85–92%.[127]

The same group subsequently reported the use of carrot root to directly effect the enantioselective reduction of a variety of aliphatic and aromatic ketones and β-ketoesters.[128] Forty-one different substrates were used, and ee values of least 90% were consistently obtained with yields of 50–82%. Typical conversion times were 40–70 h. For the acetophenones, it was observed that electron-donating substituents slowed the reaction down. With two of the racemic β-ketoesters, the (*R*)-isomer was reduced faster than the

Chart 7



(*S*)-enantiomer, yielding a (1*R*,2*S*)-product. Of interest, because it is a chemically difficult process, was the chiral reduction of several simple open-chain ketones with carrot root. Yields were low to moderate (32−50%), ee values were in the range 71−92%, and reaction times were longer (80−102 h) than other substrates.[128]

Andrade and co-workers extended the use of *Daucus carota* root material to the synthesis of a range of organochalcogeno-α-methylbenzyl alcohols (e.g., **123**).[129] No reaction was observed when the selenium substituent was at the *ortho*-position, and a low yield was observed for an *ortho*-sulfur substituent. Otherwise, after 48−72 h, conversions were in the range 72−97% and ee values were >99% for (*S*)-configured products.

In 2006, Andrade and co-workers reported on a more systematic study regarding the use of several vegetables to effect the reduction of a range of ketones (**13**, **124**−**127**) and to oxidize three racemic 1-phenylethanol derivatives (**128**−**132**).[130] The plant materials used were burdock root (*Arctium lappa* L.), sweet white potato tubers [*Ipomeoa batatas* (L.) Lam.], sweet red potato tubers [*Ipomeoa batatas* (L.) Lam.], potato tubers (*Solanum tuberosum*), beet roots (*Beta vulgaris* L.), yam tubers (*Dioscorea alata* L.), chive bulbs (*Allium shoenoprasum* L.), coriander roots (*Coriandrum sativum* L.), ginger roots (*Zingiber officinale* Roscoe), taro roots [*Colocasia esculenta* (L.) Schott], lotus roots (*Nelumbo nucifera* Gaertn.), manioc roots (*Manihot esculenta* Crantz), arracacha roots (*Arracacia xanthorrhiza* Bancroft), turnip roots (*Brassica rapa* L.), radish roots (*Raphanus sativus* L.), and yacon roots (*Polymnia sonchifolia* Poepp.).

The biooxidation of racemic 1-phenylethanol (**128**) was evaluated after 3 and 6 days using 15 different vegetables. For *Arctium lappa*, *Brassica rapa*, *Ipomeoa batatas* (red), *Polymnia sonchifolia*, and *Zingiber officinale* there was no ketone **13** produced.[130] However, *Z. officinale* produced a quantitative yield of the (*S*)-alcohol (**14**) with >98% ee and *P. sonchifolia* gave 99% of the (*S*)-alcohol (**14**) with 93% ee. *Arracacia xanthorrhiza* gave the highest yield (88%) of the ketone **13**, leaving 12% of the (*S*)-alcohol (**14**), followed by *Raphanus sativus* (74%), leaving 26% of the (*R*)-alcohol (**89**) with 98% ee, *Colocasia esculenta* (67%) leaving 33% of the (*R*)-alcohol (**89**) with 41% ee, and *Allium shoenoprasum* (54%), leaving 46% of the (*R*)-alcohol (**89**) with >98% ee.[130] *A. shoenoprasum* therefore provides a kinetic resolution of **128**, and the behavior of *Z. officinale*, *P. sonchfolia*, and *A. xanthorrhiza* suggests the operation of a two-step racemization process, as discussed previously for *Gardenia jasminoides*.[130]

The biooxidation reaction was also pursued with four further substrates, **129**−**132**, using 12 plants.[130] For the racemic alcohol **129**, the best catalyst was *Dioscorea alata*, which gave ketone **124** in 53% yield and (*S*)-**133** with 83% ee. *Allium schoenoprasum*, *Coriandrum sativum*, and *Solanum tuberosum* provided complete oxidation of alcohol **130** (96−100% yield). For racemic 1-(4-nitrophenyl)ethanol (**131**), in addition to alcohol oxidation, nitro group reduction to an amine was also observed. Thus, *Arracacia xanthorrhiza* and *Beta vulgaris* provided 4′-aminoacetophenone (**134**) in 86 and 92% yield, respectively. Nitrobenzene was reduced by *B. vulgaris* to aniline in 52% yield after 8 days. *Raphanus sativus* and *Zingiber officinale* were highly effective (quantitative yields) for the oxidation of 1-(4-methylselenophenyl)ethanol (**132**) to the ketone **127**.[130]

The bioreduction of acetophenone (**13**) was also evaluated after 3 and 6 days in the presence of each of the 16 plants.[130] *Arracacia xanthorrhiza* gave the (*S*)-alcohol **14** (91% yield, 66% ee), whereas *Beta vulgaris* afforded the (*R*)-alcohol **89** (83% yield, 87% ee). *Dioscorea alata* provided the (*S*)-alcohol **14** (47% yield, 99% ee), but all of the other plants were ineffective for this reduction reaction (yields 0−27%).

Ten of the plants were then used to evaluate the bioreduction of 4′-bromo- (**124**), 4′-methyl- (**125**), 4′-nitro- (**126**), and 4′-methylselenoacetophenone (**127**).[130] For **124**, *Manihot esculenta* gave the best conversion (55%) to (*R*)-**135** with 89% ee. For **125**, *Beta vulgaris* gave (*R*)-**136** with 86% ee in 22% yield; other plants gave even poorer yields. In the case of **126**, (*S*)-**137** was produced by

Chart 8

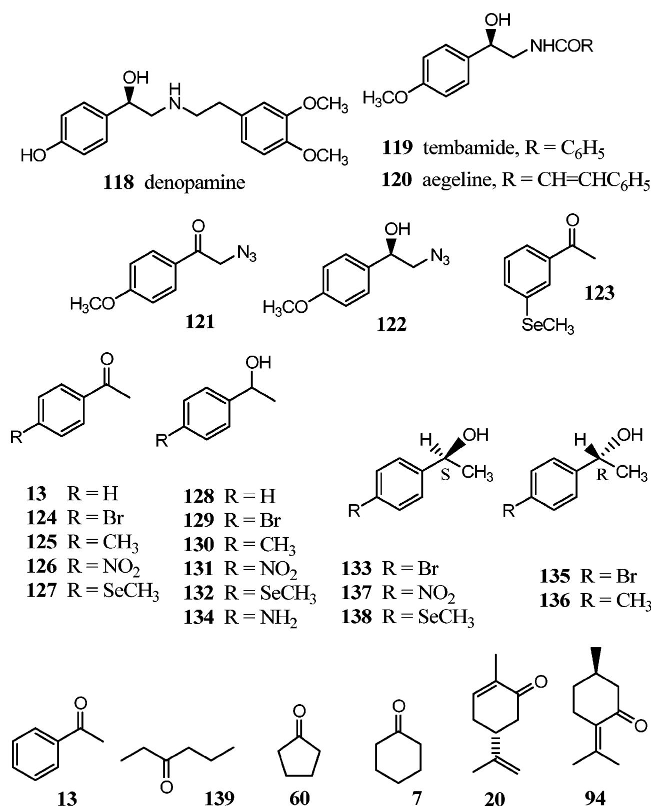
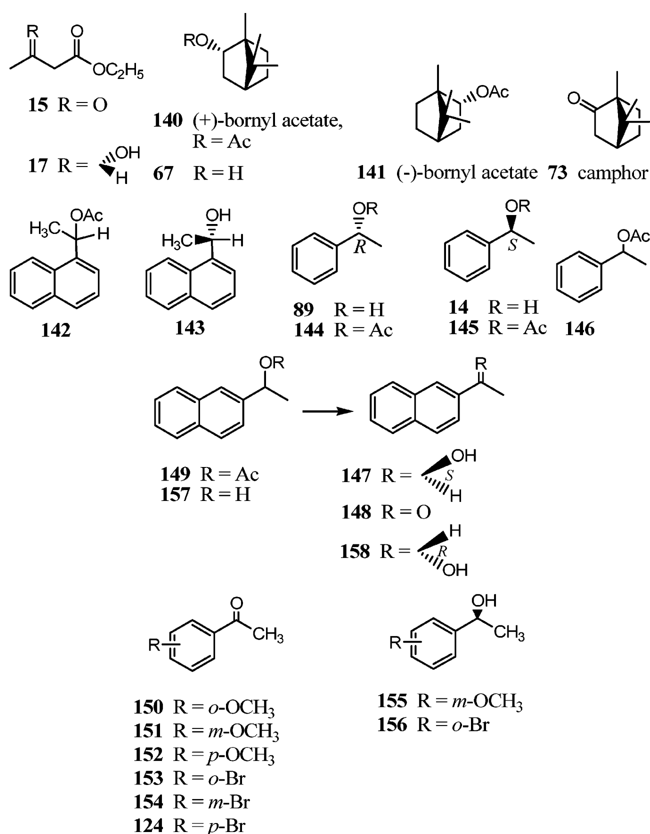


Chart 9



Arracacia xanthorrhiza in 79% yield and 95% ee. *Colocasia esculenta* provided the complete reduction of the nitro group after 6 days to afford 4′-aminoacetophenone (**134**) in quantitative yield. For the 4′-methylseleno derivative **127**, *A. xanthorrhiza* gave only a 21% yield of the (*S*)-alcohol **138**, but with >98% ee.[130]

Recently, we have reported some of our preliminary data.[131] The first set of experiments aimed to examine six species of vegetable for their ability to reduce acetophenone (**13**) to a chiral phenyl ethanol. The vegetables used were eggplant (aubergine) (*Solanum melongena* L.) (42.1% yield of **14**), sweet manioc [*Manihot dulcis* (J.F. Gmel.) Pax] (85.5%), cassava, manioc, tapioca (*Manihot esculenta*) (89.3%), carrot (*Daucus carota*) (46.3%), taro (*Colocasia esculenta*) (55.8%), and sweet potato (*Ipomoea batatas*) (42.6%). Given the high yields observed, the two *Manihot* species were used subsequently for reactions on the reduction of acetophenones, benzaldehydes, cinnamaldehydes, and furfuraldehyde at room temperature for 3 days. The product yields were in the range of 84 to 100% with ee values of >94.0% where applicable. Regioselectivity was observed in the case of two cinnamaldehyde derivatives, where reduction occurred only at the carbonyl group. The reaction was then extended using *M. esculenta* and *M. dulcis*, to aliphatic, cyclic, and α,β-unsaturated ketones, and simple carboxylic acid derivatives, including β-ketoesters, a nitrile, and an amide. Excellent yields were obtained with hexan-3-one (**139**) (97.5%; 96.7% for the two species, respectively), cyclopentanone (**60**) (92.3%; 93.4%), and cyclohexanone (**7**) (97.3%; 91.7%). Alcohols with the (*S*)-configuration were produced with ee values of 93−98%. Much lower yields were obtained with pulegone (**94**) and carvone (**20**), and no reaction was observed with two steroidal 3-ones. When esters were utilized, the dominant reaction was hydrolysis, but the β-keto ester **15** afforded the 3-(*S*)-hydroxybutyrate ethyl ester (**17**) in 95.4% yield from *M. dulcis* and an ee of 98%. Benzamide and benzonitrile were not affected by these systems.[131] Cassava wastewater ("manipueria") has been used as a substrate to develop a crude lipopeptide surfactant based on two *Bacillus subtilis* strains.[132,133]

Esterase activities have, perhaps not surprisingly, been studied using a number of plant species, some in cell culture and some as intact plant parts, including *Nicotiana tabacum*,[134] the pulp of potato (*Solanum tuberosum* cv. Saturna) and topinambur tubers (*Helianthus tuberosus* Jacq., artichoke),[135,136] suspension cultures of *Spirodela punctata* (G. Mey.) C.H. Thomps., *Nephrolepis exaltata* (L.) Schott, *Cyrtomium falcatum* Ching & K.H. Shing, *Nephrolepis cordifolia* (L.) C. Presl, *Helianthus tuberosus*, *Daucus carota*, and *Petunia hybrida* Vilm.,[137] and apple pulp (*Malus sylvestris* Mill.).[135] For the topinambur, it was noted that the grated bulb was more effective with a variety of substrates than the suspension culture.[135] In 1986, Suga and co-workers observed that cultured cells of *N. tabacum* had the ability to distinguish between (+)- and (−)-bornyl acetates (**140** and **141**) and the corresponding isobornyl and isopinocampheyl acetates.[134] Preference was observed for the (*R*)-enantiomer. Thus (+)-bornyl acetate (**140**) afforded camphor (**73**) through hydrolysis and oxidation, whereas no camphor was produced from **141**. The intermediate alcohol, (+)-borneol (**67**), peaked after about 4 days, but the yield of **73** did not exceed 25%.[134]

Following earlier studies,[135] Mironowicz showed that potato tuber (*Solanum tuberosum*) and topinambur (artichoke, *Helianthus tuberosum*) pulp had differential rates of hydrolysis for the racemic mixtures of chiral esters and that the resulting alcohols were slowly converted into ketones.[136] For example, after 48 h the acetate **142** was converted into the alcohol (*R*)-**143** in 73% yield with 37% ee with potato and in 43% yield and 68% ee with topinambur.[136] Whereas the (*R*)-enantiomer of 1-phenylethyl acetate (**144**) was hydrolyzed completely after 20 h, the (*S*)-isomer (**145**) was only 80% hydrolyzed. Comparison of the hydrolyses of a series of aliphatic and aromatic acetates and methyl esters by Mironwicz and co-workers[136] using the seven plants mentioned above indicated quite differential rates of hydrolysis. For example, benzyl acetate was completely hydrolyzed by *Spirodela punctata*, *Nephrolepis exaltata*, *Cyrtomium falcatum*, and *Nephrolepis cordifolia*, but only to the extent of 8% by *Petunia hybrida*. *H. tuberosus* was the least effective hydrolyzing agent. Some selectivity was observed for the

Table 1. Summary of the Use of Foods and Vegetables as Chemical Reagents[a]

food/vegetable		
botanical name	common name	reaction(s) effected[b]
Allium shoenoprasum	chive bulb	BFK[c] [130]
Armoracia lapatifolia	horseradish	CDF[c]K [130]
Apium graveolens var. rapaceum	celery root	BCDFK [142]; K [143]
Arctium lappa	burdock root	B[c]F[c]K [130]
Arracacia xanthorrhiza	arracacha root	BFK [130]
Artemisia vulgaris	wormwood	B [145]
Beta vulgaris	beet root	BFK [130]
Brassica rapa	turnip root	BF[c]K[c] [130]
Colocasia esculenta	taro root	BFK [130]; K [131]
Coriandrum sativum	coriander root	B[c]FK [130]
Cucurbita pepo	pumpkin	B [145]
Cyrtomium falcatum	Japanese holly fern	D [137]
Daucus carota	carrot root	J [125,126]; H [127]; HJKL [128]; K [129,131,143]; CDF[c]K [142]; B [145]
Dendrobium phalenopsis	orchid	D [139]
Dioscorea alata	yam tuber	BFK [130]
Helianthus tuberosus	artichoke	D [135]; CDFK [136]
Hordeum vulgare	wheat	B [145]
Ipomeoa batatas	sweet white potato tuber	B[c]FK[c] [130]; K [131]
Ipomeoa batatas	sweet red potato tuber	B[c]F[c]K[c] [131]
Malus sylvestris	apple	D [135]; CDF[c] [140]
Manihot dulcis	sweet cassava	KGEDL [131]
Manihot esculenta	manioc root	B[c]FK [130]; KGEDL [131]
Nelumbo nucifera	lotus root	K[c] [130]
Nephrolepis cordifolia	fishbone fern	D [137]
Nephrolepis exaltata	Boston fern	D [137]
Phaseolus aureus	green grams, dal	GIJK [144]
Polymnia sonchifolia	yacon root	BK[c] [130]
Raphanus sativus	radish root	BFK[c] [130]
Solanum melongena	eggplant, aubergine	K [131]
Solanum tuberosum	potato tuber	BFK[c] [130]; D [135]; CDF[c]K[c] [136]
Spirodela punctata	duckweed	CD [137]; D [138]
Triticum aestivum	wheat	B [145]
Undaria pinnatifida	wakame seaweed	B [145]
Zingiber officinale	ginger root	BFK[c] [130]

[a] Whole plant preparations only. [b] Reactions. B: Deracemization of an alcohol; C: Deracemization of esters; D: Ester hydrolysis; E: Reduction of an aldehyde to a primary alcohol; F: Oxidation of an alcohol to a carbonyl; G: Reduction of an α,β-unsaturated ketone to an allylic alcohol; H: Reduction of an azidoketone; I: Reduction of a double bond; J: Chiral reduction of a ketone; K: Chiral reduction of an aromatic ketone; L: Chiral reduction of a ketone carbonyl of a β-ketoester. [c] Attempted; poor result.

hydrolysis of the racemates of prochiral alcohols with *S. punctata*,[138] *Dendrobium phalaenopsis* Fitzg.,[139] and *Daucus carota*,[137] not always to yield the same enantiomer. In 1997, Mironowicz[140] reported on the use of apple (*Malus sylvestris*) pulp on the enantiospecific hydrolysis of racemic acetates; two varieties, "Golden" and "Gloucester", were used. A sample of apple pulp was mixed with a small quantity of substrate and the mixture shaken in phosphate buffer for 2 days. The hydrolysis products were also oxidized in low yield to the ketones. One example was the hydrolysis/oxidation of racemic 1-phenylethyl acetate (**146**) to the (*S*)-alcohol (**14**) in 40% yield with 15% of the ketone **13** also produced; 26% of the (*R*)-substrate **89** remained.[140]

This work was followed up by Mironowicz and Kromer with studies involving apple tree shoots and transformed (*Agrobacterium rhizogenes*) carrot (*Daucus carota*) and apple (*Malus pumila*) roots.[141] The apple tree shoots converted racemic 1-phenylethyl acetate (**146**) to the (*S*)-alcohol (**14**) in 98% yield after 5 days. Other substrates were less cleanly modified and at lower yields than either the fruit or transformed apple roots. The latter afforded the alcohol in 95% yield, but with very low ee. On the other hand, the system afforded 61% yield of the alcohol (*S*)-**147** and 39% of the ketone **148** from the racemic acetate **149**. The transformed and nontransformed carrot systems gave very poor conversions to either the alcohols or the ketones with the four substrates used.[141]

The enantioselective hydrolysis of racemic 1-phenyl (and naphthyl) ethyl acetates and the reduction of methylphenyl (or naphthyl) ketones were reported by Mączka and Mironowicz using carrot roots, celery (*Apium graveolens* var. *rapaceum* DC.), and horseradish (*Armoracia lapathifolia* Gilib.).[142] The enzyme effects of hydrolysis, oxidation, and reduction are observed for racemic 1-phenylethyl acetate (**141**) by the celery system such that the initially formed (*S*)-alcohol **14** is oxidized to the ketone **13**, leaving the (*R*)-alcohol **89**. On the other hand, the horseradish system was slower, and after 48 h, it was not able to fully oxidize the intermediate (*S*)-alcohol **14** (62% yield, 41% ee) and left unreacted substrate as the (*R*)-isomer **89** with 66% ee.[142] The same group has also examined the enantioselective reduction of six bromo- and methoxyacetophenone derivatives (**124**, **150**−**154**) using comminuted carrot roots (*Daucus carota*) and celery roots (*A. graveolens* var. *rapaceum*) for 48 h at room temperature and at pH 6.5 and 6.2, respectively.[143] Both the carrot and the celery systems reduced 3-methoxy acetophenone (**151**) to the (*S*)-(−)-(3-methoxyphenyl)ethanol (**155**) in quantitative yield and 98−100% ee. 2-Bromoacetophenone (**153**) was also converted enantioselectively to the corresponding (*S*)-(−)-(2-bromophenyl)ethanol (**156**) with 100% ee but in only 8 and 27% yield, respectively. The other derivatives gave yields in the range 10−54% and ee values in the range 78−95%. Bromo derivatives were typically reduced much faster than the corresponding methoxyacetophenone.[143]

Other plant species have also been evaluated for their ability to reduce prochiral ketones. Green grams (*Phaseolus aureus* Roxb.), also known as "moong dal", is a widely used vegetable in India for the preparation of dal and curries. When acetophenone (**13**) was treated with soaked *P. aureus* at 10−15 °C for 24 h, (*S*)-1-phenylethanol (**14**) was generated with 84% ee in 52% isolated yield. The system was applied to a variety of aromatic and aliphatic

ketones as substrates, and while optical purity values ranged from 72 to 98%, yields varied from 23 to 55%.[144]

A different approach was reported in 2004 by Nagaoka.[145] In these experiments, the crude powdered plant material was immobilized in alginate beads. The plants were young wheat leaves, young barley leaves, *Artemisia vulgaris* L. leaves, wakame seaweed, carrot, and pumpkin; wheat bran, chorella cells, and baker's yeast were also used. The diversity of reagents permitted the chiral resolution of racemic alcohols; for example the racemic alcohol **157** was converted to the (*R*)-isomer **158** by *A. vulgaris* [(*S*)-isomer **147** oxidized and ketone **148** reduced], whereas the (*S*)-isomer **147** was produced from young wheat leaves. In both instances, the yield was 50% and >99% ee. It was important that these systems were activated by apoenzyme and cofactor preincubation. Table 1 summarizes the efforts thus far to examine the use of whole plant systems for the chemical transformation of organic compounds.

Conclusions

There are approximately 7000 vegetable crops used in food and agriculture throughout the world.[146] In addition, there are numerous plants used commercially for various flavor, cosmetic, construction, and other uses. The research briefly described herein represents an effort to illustrate that even the random evaluation of vegetables can lead to some reactions of substantial synthetic interest. Our discovery of the potential of *Manihot* species to conduct chiral reduction reactions, species that are available throughout the tropical world, is a single example of a simple and cheap system that might be made available after local resources are investigated.[131] Such systems offer some interesting advantages with respect to either the standard chemical reagents or plant cell or enzyme preparations. For example, the need for expensive cofactors is eliminated by using the whole plant tissue since the plant automatically provides this requirement. Depending on the vegetable used, either enantiomer may be made available in high yield and high ee, which could be a critical factor from a drug development/bioactivity evaluation perspective. In addition, the lower time factor, the lower cost, and the ease of preparation of the reactive system make the use of the intact plant part a very economic alternative. Furthermore, there is evidence that such systems can be used repeatedly (5−6 times) without substantial loss of activity, thus reducing costs even further. In addition, it is clear that such systems are highly adept in conducting profound transformations on unnatural substrates. The disadvantages of using intact plant systems is that the isolation procedure can be a little more complex, other enzymatic processes may also occur simultaneously, and the length of time involved for the process to evolve requires that the product be stable. There is also the concern about the botanical identification, to the variety or cultivar level, of a particular vegetable. We believe that the advantages outweigh the disadvantages.

Our hope is that other research groups will explore further the opportunities that this exciting area of natural products research offers in two major ways: (i) there may be hundreds of plants in the culinary repertoires of other countries around the world, which could offer even more effective ketoreductase systems; (ii) more importantly, by thinking in biosynthetic terms with respect to a particular chemical reaction of interest, and knowing the secondary metabolites of a plant and their biosynthetic pathways, it might be possible to target plant sources for specific enzyme activities. The evaluation of locally available vegetables, fruits, common plants, and natural waste products for a selection of standard organic chemical reactions of commercial significance could prove to be a very valuable economic endeavor. It may well offer new opportunities to expand the role of natural products as sustainable chemical reagents where high-cost, nonrenewable reagents are presently used. In doing so, such research may enhance the value of "green" chemistry to the pharmaceutical and agricultural industries in less-developed countries in the world, as well as the "North".

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References and Notes

(1) Cordell, G. A.; Quinn-Beattie, M. L.; Farnsworth, N. R. *Phytother. Res.* **2001**, *15*, 183−205.
(2) Prance, G. T.; Beentje, H.; Dransfield, J.; Johns, R. *Ann. Missouri Bot. Gard.* **2000**, *87*, 67−71.
(3) Rovner, S. *Chem. Eng. News* **2005**, *Oct. 10*, 44−49.
(4) Wink, M. Ed. *Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology*; Sheffield Academic Press: Sheffield, 1999; 362 pp.
(5) Wink, M. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, 2000; Vol. 21, pp 3−122.
(6) Cordell, G. A. *Phytochemistry* **2000**, *55*, 463−480.
(7) Cordell, G. A. *Phytochem. Rev.* **2002**, *1*, 261−273.
(8) Cordell, G. A.; Colvard, M. D. *J. Ethnopharmacol.* **2005**, *100*, 5−14.
(9) Pasteur, L. *C. R. Acad. Sci.* **1858**, 615.
(10) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1894**, 2895.
(11) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1894**, 3189.
(12) Weid, M.; Ziegler, J.; Kutchan, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13957−13962.
(13) Carey, J. S; Laffan, D.; Thomson, C.; Williams, M. T. *Org. Biomol. Chem.* **2006**, *4*, 2337−2347.
(14) Wells. A. *Org. Proc. Res. Dev.* **2006**, *10*, 678−681.
(15) Sylvestre, J.; Chautard, H.; Cedrone, F.; Delcourt, M. *Org. Proc. Res. Dev.* **2006**, *10*, 562−571.
(16) Sharma, S. K.; Husain, M.; Kumar, R.; Samuelson, L. A.; Kumar, J.; Watterson, A. C.; Parmar, V. S. *Pure Appl. Chem.* **2005**, *77*, 209−226.
(17) Thayer, A. M. *Chem. Eng. News* **2006**, *Aug. 14*, 15−27.
(18) Shaw, N. M.; Robins, K. T.; Kiener, A. *Adv. Synth. Catal.* **2003**, *345*, 425−435.
(19) Veit, T. *Eng. Life Sci.* **2004**, *4*, 508−511.
(20) Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z. *Bull. WHO* **1985**, *63*, 965−81.
(21) Cordell, G. A. *Rev. Quim.* **2003**, *17*, 1−15.
(22) Cordell, G. A. *Thai J. Hlth. Res.* **2004**, *18*, 87−105.
(23) Cordell, G. A. *Rev. Quim.* **2004**, *19*, 33−41.
(24) Cordell, G. A.; Colvard, M. D. *Arkivoc* **2006**, *vii*, 97−115.
(25) Reisch, M. S. *Chem. Eng. News* **2001**, *Sept. 3*, 17−22.
(26) Rouhi, A. M. *Chem. Eng. News* **2002**, *April 22*, 30−33.
(27) Anastas, P.; Lankey, R. *Chem. Br.* **2001**, 26−29.
(28) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998; p 30.
(29) Luna, H. *Rev. Soc. Quim. Mex.* **2004**, *48*, 211−219.
(30) Liu, Z.-B.; Weis, R.; Gleider, A. *Food Technol. Biotechnol.* **2004**, *42*, 237−249.
(31) Deussen, H.-J.; Zundel, M.; Valdois, M.; Lehmann, S. V.; Weil, V.; Hjort, C. M.; Oestergaard, P. R.; Marcussen, E.; Ebdrup, S. *Org. Proc. Res. Dev.* **2003**, *7*, 82−88.
(32) Elenkov, M. M.; Hauer, B.; Janssen, D. B. *Adv. Synth. Catal.* **2006**, *348*, 579−585.
(33) Johnson, D. V.; Griengl, H. *Adv. Biochem. Eng./Biotechnol.* **1999**, *63*, 31−55.
(34) Schmidt, M.; Griengl, H. *Top. Curr. Chem.* **1999**, *200*, 193−226.
(35) Griengl, H.; Schwab, H.; Fechter, M. *Trends Biotechnol.* **2000**, *18*, 252−256.
(36) Fechter, M. H.; Greingl, H. *Food Technol. Biotechnol.* **2004**, *42*, 287−294.
(37) Effenberger, F. *Chimia* **1999**, *53*, 3−10.
(38) Geyer, R.; Kartnig, T.; Griengl, H.; Steiner, W. *Food Technol. Biotechnol.* **2001**, *39*, 161−167.
(39) Roda, G.; Riva, S.; Danieli, B.; Griengl, H.; Rinner, U.; Schmidt, M.; Zabelinskaja, A. M. *Tetrahedron* **2002**, *58*, 2979−2983.
(40) Buhler, H.; Bayer, A.; Effenberger, F. *Chemistry (Weinheim)* **2000**, *6*, 2564−2571.
(41) Purkartofer, T.; Pabst, T.; van der Broek, C.; Griengl, H.; Maurer, O.; Skranc, W. *Org. Proc. Res. Dev.* **2006**, *10*, 618−621.
(42) Weis, R.; Poechlauer, P.; Bona, R.; Skranc, W.; Luiten, R.; Wubbolts, M.; Schwab, H.; Glieder, A. *J. Mol. Catal. B: Enzym.* **2004**, *29*, 211−218.
(43) Greenberg, W.; Weiner, D. P.; Adger, B.; Burk, M. WO 2003-US2177 20030123, 2003; *Chem. Abstr.* **2003**, *139*, 129923.
(44) Rudroff, F.; Alphand, V.; Furstoss, R.; Mihovilovic, M. D. *Org. Proc. Res. Dev.* **2006**, *10*, 599−604.
(45) Edegger, K.; Mang, H.; Faber, K.; Gross, J.; Kroutil, W. *J. Mol. Catal. A* **2006**, *251*, 66−70.
(46) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Adv. Synth. Catal.* **2004**, *346*, 125−142.
(47) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Curr. Opin. Chem. Biol.* **2004**, *8*, 120−126.

(48) Jennwein, S.; Schürmann, M.; Wolberg, M.; Hilker, I.; Luiten, R.; Wubbolts, M.; Mink, D. *Biotechnol. J.* **2006**, *1*, 537−548.
(49) Gröger, H. *Chem. Rev.* **2003**, *103*, 2795−2827.
(50) Menzel, A.; Werner, H.; Altenbucher, J.; Gröger, H. *Eng. Life Sci.* **2004**, *4*, 573−576.
(51) Gröger, H.; May, O.; Werner, H.; Menzel, A.; Altenbuchner, J. *Org. Proc. Res. Dev.* **2006**, *10*, 666−669.
(52) Edegger, K.; Stampfer, W.; Seisser, B.; Faber, K.; Mayer, S. F.; Oerhrlein, R.; Hafner, A.; Kroutil, W. *Eur. J. Org. Chem.* **2006**, 1904−1909.
(53) van Duersen, R.; Stampfer, W.; Edegger, K.; Faber, K.; Kroutil, W. *J. Mol. Catal. B: Enzym.* **2004**, *31*, 159−163.
(54) Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2002**, *68*, 402−406.
(55) Stampfer, W.; Eddeger, B.; Kosjek, B.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* **2004**, *346*, 57−62.
(56) Matsuda, T.; Watanabe, K.; Kamitanaka, T.; Harada, T.; Nakamura, K. *Chem. Commun.* **2003**, 1198−1199.
(57) Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 12358−9.
(58) Toneva, K.; Vlahov, S.; Boneva, S.; Vassilev, S.; Vassilev, K. *Dokl. Bulg. Akad. Nauk.* **2002**, *55*, 43−48; *Chem. Abstr.* **2002**, *139*, 116329.
(59) Negi, M.; Subbaraju, G. V.; Manhas, M. S.; Bose, A. K. *Enzyme Microb. Technol.* **1993**, *15*, 483−488.
(60) Zhang, B.-L.; Pionnier, S. *Chem.−Eur. J.* **2003**, *9*, 3604−3610.
(61) Speicher, A.; Roeser, H., Heisel, R. *J. Mol. Catal. B: Enzym.* **2003**, *22*, 71−77.
(62) Hirata, T.; Hamada, H.; Aoki, T.; Suga, T. *Phytochemistry* **1982**, *21*, 2209−2212.
(63) Kergomard, A.; Renard, M. F.; Veschambre, H.; Courtois, D.; Petiard, V. *Phytochemistry* **1988**, *27*, 407−409.
(64) Pollard, D.; Truppo, M.; Pollard, J.; Chen, C.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554−559.
(65) Truppo, M. D.; Kim, J.; Brower, M.; Madin, A.; Sturr, M. G.; Moore, J. C. *J. Miol. Catal. B: Enzym.* **2006**, *38*, 158−162.
(66) Hu, S.; Martinez, C. A.; Kline, B.; Yazbek, D.; Tao, J.; Kucera, D. J. *Org. Process Res. Dev.* **2006**, *10*, 650−654.
(67) Hu, S.; Kelly, S.; Lee, S.; Tao, J.; Flahive, E. *Org. Lett.* **2006**, *8*, 1653−1655.
(68) Thayer, A. M. *Chem. Eng. News* **2006**, *Aug. 14*, 26−27.
(69) Zhu, D.; Rios, B. E.; Rozzell, J. D.; Hua, L. *Tetrahedron: Asymmetry* **2005**, *16*, 1541−1546.
(70) Zhu, D.; Mukherjee, C.; Rozzell, J. D.; Kambourakis, S.; Hua, L. *Tetrahedron* **2006**, *62*, 901−905.
(71) Kamal, A.; Damayanthi, Y.; Reddy, B. S. N.; Lakminarayana, B.; Reddy, B. S. P. *Chem. Commun*. **1997**, 1015−1016.
(72) Cantello, B. C. C.; Eggleston, D. S.; Haigh, D. ; Haltiwanger, R. C.; Heath, C. M.; Hindley, R. M.; Jennings, K. R.; Sime, J. T.; Woroniecki, S. R. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3319−3324.
(73) Vassilev, K.; Toneva, K.; Vlahov, S.; Boneva, S. *Dokl. Bulg. Akad. Nauk.* **2000**, *53*, 129−132.
(74) Bruyn, J. *Koninl. Ned. Acad. Wetenschap. Proc.* **1954**, *57*, 41−45.
(75) Reinhard, E.; Alfermann, A. W. *Adv. Biochem. Eng.* **1980**, *16*, 49−83.
(76) Stohs, S. J.; Staba, E. J. *J. Pharm. Sci.* **1965**, *54*, 56−58.
(77) Graves, J. M. H.; Smith, W. K. *Nature* **1967**, *214*, 1248−1249.
(78) Furuya, T.; Hortani, M.; Kawaguchi, K. *Phytochemistry* **1971**, *10*, 1013−1017.
(79) Hirotani, M.; Furuya, T. *Phytochemistry* **1974**, *13*, 2135−2142.
(80) Furuya, T.; Hirotani, M.; Shinohara, T. *Chem. Pharm. Bull.* **1970**, *18*, 1080−1081.
(81) Nawa, H.; Uchibayashi, M.; Kamiya, T.; Yamano, T.; Arai, H.; Abe, M. *Nature* **1959**, *184*, 469−470.
(82) Alfermann, A. W.; Schuller, I.; Reinhard, E. *Planta Med.* **1980**, *40*, 218−223.
(83) Suga, T.; Hirata, T.; Hirano, Y.; Ito, T. *Chem. Lett.* **1976**, 1245−1248.
(84) Hirata, T.; Aoki, T.; Hirano, Y.; Ito, T.; Suga, T. *Bull. Chem. Soc. Jpn.* **1981**, 3527−3529.
(85) Suga, T.; Aoki, T.; Hirata, T.; Lee, Y. S.; Nishimura, O.; Utsumi, M. *Chem. Lett*. **1980**, 229−230.
(86) Hirata, T.; Lee, Y. M.; Suga, T. *Chem. Lett.* **1982**, 671−674.
(87) Suga, T.; Hamada, T.; Hirata, T. *Plant Cell Rep.* **1983**, *2*, 66−68.
(88) Suga, T.; Hirata, T.; Hamada, H.; Futatsugi, M. *Plant Cell Rep.* **1983**, *2*, 186−188.
(89) Takemoto, M.; Matsuoka, Y.; Achiwa, K.; Kutney, J. P. *Tetrahedron Lett.* **2000**, *41*, 499−502.
(90) Takemoto, M.; Matsuoka, Y.; Tanaka, K.; Achiwa, K.; Stoynov, N.; Kutney, J. P. *Heterocycles* **2002**, *56*, 227−234.
(91) Chai, W.; Hayashida, Y.; Sakamaki, H.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2004**, *27*, 55−60.
(92) Bouwmeester, H. J.; De Kraker, J.-W.; Schurink, M.; Bino, R. J.; De Groot, A.; Franssen, M. C. R. WO 2002-NL591 20020917, 2002; *Chem. Abstr.* **2003**, *138*, 270406.
(93) Lima, A. W. O.; Nascimento, V. B.; Pedrotti, J. J.; Angnes, L. *Anal. Chim. Acta* **1997**, *354*, 325−331.
(94) Signori, C. A.; Fatibello Filho, O. *Quim. Nova* **1994**, *17*, 38−42.
(95) Jaminet, F. *J. Pharm. Berg.* **1950**, *5*, 191−199.
(96) Finkle, B.; Lewis, J. C.; Corse, J. W.; Lundin, R. E. *J. Biol. Chem.* **1962**, *237*, 2926−2931.
(97) Takemoto, M.; Achiwa, K. *Chem. Pharm. Bull.* **2001**, *49*, 639−641.
(98) Takemoto, M.; Achiwa, K. *Tetrahedron: Asymmetry* **1995**, *6*, 2925−2928.
(99) Takemoto, M.; Achiwa, K. *Phytochemistry* **1998**, *49*, 1627−1629.
(100) Takemoto, M.; Achiwa, K. *Chem. Pharm. Bull.* **1998**, *46*, 577−580.
(101) Takemoto, M.; Moriyasu, Y.; Achiwa, K. *Chem. Pharm. Bull.* **1995**, *43*, 1458−1461.
(102) Takemoto, M.; Yamamoto, Y.; Achiwa, K. *Chem. Pharm. Bull.* **1998**, *46*, 419−422.
(103) Naoshima, Y.; Akakabe, Y.; Watanabe, F. *Agric Biol. Chem.* **1989**, *53*, 545−547.
(104) Naoshima, Y.; Akakabe, Y. *J. Org. Chem.* **1989**, *54*, 4237−4239.
(105) Naoshima, Y.; Akakabe, Y. *Phytochemistry* **1991**, *30*, 3595−3597.
(106) Akakabe, Y.; Naoshima, Y. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1993**, *35*, 202−209.
(107) Akakabe, Y.; Takahashi, M.; Kamezawa, M.; Kikuchi, K.; Tachibana, H.; Otani, T.; Naoshima, Y. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1295−1298.
(108) Akakabe, Y.; Naoshima, Y. *Phytochemistry* **1993**, *32*, 1189−1191.
(109) Baskar, B.; Ganesh, S.; Lokeswari, T. S.; Chadha, A. *J. Mol. Catal. B Enzym.* **2004**, *27*, 13−17.
(110) Chadha, A.; Manohar, M.; Soundarajan, T.; Lokeswari, T. S. *Tetrahedron: Asymmetry* **1996**, *7*, 1571−1572.
(111) Hamada, H.; Umeda, N.; Otsuka, N.; Kawabe, S. *Plant Cell Rep.* **1988**, *7*, 493−494.
(112) Galun, E.; Aviv, D.; Dantes, A.; Freeman, A. *Planta Med.* **1985**, *51*, 511−514.
(113) Aviv, D.; Galun, E. *Planta Med.* **1978**, *33*, 70−77.
(114) Aviv, D.; Krochmal, E.; Dantes, A.; Galun, E. *Planta Med.* **1981**, *42*, 236−243.
(115) Aviv, D.; Krochmal, E.; Dantes, A.; Galun, E. *Planta Med.* **1983**, *47*, 7−10.
(116) Galun, E.; Aviv, D.; Dantes, A.; Freeman, A. *Planta Med.* **1983**, *49*, 9−13.
(117) Suga, T.; Hirata, T.; Hamada, H. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2865−2867.
(118) Suga, T.; Hamada, H.; Hirata, T. *Chem. Lett.* **1987**, 471−474.
(119) Suga, T.; Hamada, H.; Hirata, T.; Izumi, S. *Chem. Lett.* **1987**, 903−906.
(120) Hamada, H. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 869−878.
(121) Mukherjee, K. D. *Lipid Biotechnol.* **2002**, 399−415.
(122) Gardner, H. W.; Grechkin, A. N. *Lipid Biotechnol*. **2002**, 157−182.
(123) Giri, A.; Dhingra, V.; Giri, C. C.; Singh, A.; Ward, O. P.; Narasu, M. L. *Biotechnol. Adv.* **2001**, *19*, 175−199.
(124) Robins, R. J.; Walton, N. J. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: San Diego, 1993; Vol. 43, pp 115−187.
(125) Baldassarre, F.; Bertoni, G. Chiappe, C; Marioni, F. *J. Mol. Catal. B: Enzym.* **2000**, *11*, 55−58.
(126) Marioni, F.; Chiappe, C. Ital. Pat. 199907192001; *Chem. Abstr.* **2004**, *140*, 217083.
(127) Yadav, J. S.; Thirupathi Reddy, P.; Nanda, S.; Bhaskar Rao, A. *Tetrahedrom Asymmetry* **2000**, 3381−3385.
(128) Yadav, J. S.; Nanda, S.; Thirupathi, Reddy, P.; Bhaskar Rao, A. *J. Org. Chem.* **2002**, *67*, 3900−3903.
(129) Comasseto, J. V.; Omori, A. T.; Porto, A. L.; Andrade, L. H. *Tetrahedron Lett.* **2004**, *45*, 473−476.
(130) Andrade, L. H.; Utsunomiya, R. S.; Omori, A. T.; Porto, A. L. M., Comasseto, J. V. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 84−90.
(131) Sousa, J. S. N.; Machado, L. L.; de Mattos, M. C.; Solange, S.; Lemos, T. L. G.; Cordell, G. A. *Phytochemistry* **2006**, *67*, 1637−1643.
(132) Nitschke, M.; Haddad, R.; Costa, G. N.; Gilioli, R.; Meurer, E. C.; Gatti, M. S. V.; Eberlin, M. N.; Hoehr, N. F.; Pastore, G. N. *Food Sci. Biotechnol.* **2004**, *13*, 591−596.
(133) Nitschke, M. Pastore, G. M. *Appl. Biochem. Biotechnol.* **2004**, *112*, 163−172.
(134) Suga, T.; Hirata, T.; Izumi, S. *Phytochemistry* **1986**, *25*, 2791−2792.
(135) Mironowicz, A.; Jarosz, B.; Siewinski, A. *Acta Soc. Bot. Pol.* **1995**, *64*, 281−285.
(136) Mironowicz, A. *Phytochemistry* **1998**, *47*, 1531−1534.
(137) Mironowicz, A.; Kromer, K.; Pawlowicz, P.; Siewinski, A. *Acta Soc. Bot. Pol.* **1994**, *63*, 43−48.
(138) Pawlowicz, P.; Siewinski, A. *Phytochemistry* **1987**, *26*, 1001−1004.

(139) Mironowicz, A.; Kukulczanka, K.; Siewinski, A. *Acta Soc. Bot. Pol.* **1993**, *62*, 21−23.
(140) Mironowicz, A. *Acta Soc. Bot. Pol.* **1997**, *66*, 325−328.
(141) Mironowicz, A.; Kromer, K. *Collect. Czech. Chem. Commun.* **1998**, *63*, 1655−1662.
(142) Mączka, W. K.; Mironowicz, A. *Tetrahedron: Asymmetry* **2002**, *13*, 2299−2302.
(143) Mączka, W. K.; Mironowicz, A. *Tetrahedron: Asymmetry* **2004**, *15*, 1965−1967.
(144) Kumaraswarmy, G.; Ramesh, S. *Green Chem.* **2003**, *5*, 306−308.
(145) Nagaoka, H. *Biotechnol. Progr.* **2004**, *20*, 128−133.
(146) Wilson, E. O. *The Diversity of Life*; Penguin Press: London, 1992; p 275.

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