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Carvone: Why and how should one bother to produce this terpene

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Abstract

D-Carvone or (4*S*)-(+)-carvone has a caraway/dill odour and is the main constituent of caraway (*Carum carvi*) and dill (*Anethum graveolens*) seed oils whilst L-Carvone or (4*R*)-(−)-carvone has a sweet spearmint odour and is the main constituent of spearmint (*Mentha spicata*). Carvone is produced by both extraction and purification of essential oils from caraway, dill and spearmint seeds and by chemical and biotechnological synthesis.

The several applications of carvone as fragrance and flavour, potato sprouting inhibitor, antimicrobial agent, building block and biochemical environmental indicator, along with its relevancy in the medical field, justify the interest in this monoterpene.

Keywords: Carvone; Caraway; Dill; Spearmint; Chemical synthesis; Biotechnologycal synthesis

1. Why

1.1. Fragrance and flavour

Carvone has been utilised throughout the centuries as a component of caraway (*Carum carvi* Linnaeus 1753), dill (*Anethum graveolens* Linnaeus 1753) and spearmint (*Mentha spicata* Linnaeus 1753) seeds.

Caraway is naturally found in Northern and Central Europe, Siberia, Turkey, Iran, India and North Africa. This herb was first used by the ancient Arabs and Pliny recommended it for hysterical complaints and pale complexions (Plinius, 77 A.D.). According to the legend, the roots of caraway were mixed with milk to make "Chara", a bread eaten by Julius Caesar and Valerius's soldiers. In Shakespeare's times it was usual to eat apples with caraway at dessert, as mentioned in *Henry IV* (Shakespeare, 1598).

Caraway is one of oldest spices cultivated in Europe. Nowadays, it is cultured from northern temperate to tropical climates, including countries such as Jamaica, India, Canada, United States and Australia. The fruit of caraway is a schizocarp, which at harvest splits into two halves, called "seeds". Caraway seeds are used as a flavouring of bread (e.g., rye bread), cheese, sauerkraut, candies, meat products, sauces and alcoholic liqueurs, such as the German Kümmel and as a source of carvone for cosmetics, toothpaste, chewing gum and pharmaceutical preparations. The seeds have been used in alternative medicine as a laxative, in colic treatment and as a breath freshener, even being safe to add to young children's dishes to help digestion. The seeds have been found to have antipasmodic, carminative, emmenagogue, expectorant, galactagogue, stimulant, stomachic and tonic properties.

There is an annual and a biennial caraway plant. The latter plant is cultivated more widely as it is more productive: biennial caraway seed contains 3–7% oil; annual caraway seed contains 2–3% oil. The main constituents of the seed are (4*S*)-(+)-carvone (50–70%) and (+)-limonene (25–30%) (Hornok, 1992). Myrcene,

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β-caryophyllene, thujone, anethole and pinene are present as minor components. World production of caraway seed oil is estimated to be 10 t/year ("Agriculture, Food and Rural Development" programme, Alberta, Canada).

Another source of carvone is dill (seeds). Dill is a hardy annual plant, native to the Asia Minor and the Mediterranean region. It grows wild in Spain, Portugal and upon the coast of Italy and rarely in Northern Europe. Dill seeds have been applied for thousands of years: they were popular in Ancient Egypt (Wilson, 1988); these seeds, together with cumin and mint, were subject to a tax during I AC (St. Matthews Gospel, New Testament) and Roman gladiators used to friction their limbs with the seed's oil before fights. Dill seeds have been used to flavour cakes and pastries, soups, salads, potatoes, meats, sauerkraut and pickles. The seeds have antispasmodic, carminative, stomachic, emmenagogue and galactogogue properties. Dill seed has an oil content of 2.3–3.5%, of which 40–60% is carvone, whilst dill weed has 0.4–0.8% oil composed of 40% carvone, 32% limonene and 20% phellandrene.

(4*R*)-(−)-Carvone can be extracted from spearmint (*M. spicata*, *M. viridis*), a hardy perennial plant native to the Mediterranean area, known in ancient times as the herb of hospitality. The name *Mentha* derived from that of a nymph, Menthe, loved by Hades, who was metamorphosed into a plant by the jealous Hades' wife Persephone. However, the more Menthe was walked on, the sweeter she smelled.

Mints have been mentioned throughout history. Mint plants were mentioned in the Bible as herbs the Pharisees used for tithing, among anise and cumin. Pliny, in I A.D., suggested that students could improve their minds and scholarship by wrapping a braid of mint around their heads, which should give "delight to the soul" (Plinius, 77 A.D.). Aristotle went as far as to forbid the use of mint by soldiers before battles as the qualities of this herb might diminish their willingness to fight.

Spearmint is mostly used in cuisine. The medical properties of spearmint oil are similar to those of peppermint, being stimulant, carminative and antispasmodic. Its effects are less powerful than those of peppermint and it is thus less used, though it is better adapted for children's maladies. Spearmint oil has a minimum of 51% carvone. Limonene, phellandrene and dihydrocarveol acetate are also present. *M. spicata* is characterised by a high carvone content (60–70%) and a limonene content of 8–15%.

The world market for spearmint oil is approximately 1500 t/year (Peterson & Bienvenu, 1997). Spearmint is mainly produced in the USA. China and South America are starting to increase their production. Even though the production of spearmint oil is smaller than peppermint oil, it is substantially increasing, since many oral care products are using a combination of both spearmint and peppermint oils to "soften" the flavour (in some blends the proportion is already 1:1).

Several efforts have been made to increase the yield of monoterpenes in plants to respond to the increasing world demand for flavourings, fragrances and pharmaceuticals.

1.2. Sprouting inhibitor

Premature sprouting of potatoes usually occurs during storage and is responsible for reducing the number of marketable potatoes and the weight of the remaining potatoes due to high water evaporation from the sprout surfaces. Storage at 2–4 °C and chemical sprout inhibitors are generally used to prevent it. However, temperature causes starch degradation to sugar while synthetic compounds in food products are avoided by consumers. Naturally occurring compounds could be used as antisprouting agents in potatoes, based on the common idea that natural products are less harmful to the environment than chemical products.

(4*S*)-(+)-Carvone was found to be a good potato sprouting inhibitor (Hartmans & Diepenhorst, 1994). When compared to the traditional chemical mixtures of isopropylphenylcarbamate (IPC) and isopropyl-3-chlorophenylcarbamate (CIPC), carvone was as good or even better during long-term storage, also showing antifungal activity against *Fusarium sulphureum*, *Phoma exigua* var. *foveata* and *Helminthosporium solani* (Hartmans, Diepenhorst, Bakker, & Gorris, 1995). Besides, (4*S*)-(+)-carvone is better than those chemicals when compared in terms of human toxicity and ozone depletion (Kerstholt, Ree, & Moll, 1997). Carvone is already commercialised in The Netherlands under the name "Talent" as an effective sprout growth regulator (Hartmans, Lenssen, & de Vries, 1998).

A study carried out by Cizkova, Vacek, Voldrich, Sevcik, and Kratka (2000) during the seasons 1996/97, 1997/98 and 1998/1999, which compared untreated potatoes with those treated with CIPC and "Talent", showed that treating potatoes with vapour of caraway essential oil, in dosage of 0.1 ml/kg tubers, applied regularly over 4 and 6 weeks, successfully prevented bud growth at 10 °C. Weight losses caused by sprouting decreased from 7.5% in untreated potatoes in season 1996/97, to ca. 0.3% in treated potatoes. By keeping a nearly constant carvone head-space concentration, 0% and 2.7% losses by sprouting were achieved in 1997/98 and 1998/99, respectively. This was accomplished using the essential oil on sorbent bentonite, the mixture being dusted as powder once per storage period. Furthermore, the stored tubers treated with (4*S*)-(+)-carvone did not change in taste, odour or colour when cooked.

1.3. Antimicrobial agent

Carvone has both antibacterial and antifungal activity. In a work carried out by Aggarwal et al. (2002), the antimicrobial activity of the essential oils of *M. spicata* (the major components being 27.3% (4*S*)-(−)-limonene and 56.6% (4*R*)-(−)-carvone) and *A. sowa* Roxb (Indian dill, the major components being 21.4% (*R*)-(+)-limonene and 50.4% (4*S*)-(+)-carvone) was assessed. In vitro bioactivity of the isolated oil components showed that both optical isomers of carvone were effective against a wide spectrum of human pathogenic fungi and bacteria and that (*R*)-(+)- and (*S*)-(−)-limonene had comparable bioactivity profiles. Additionally, the antimicrobial properties of these monoterpenes were similar to those of the oil in which they were present.

Essential oil from dill (*A. graveolans*) seeds from Bulgaria, stored for more than 35 years, showed high antimicrobial activity against the fungus *Aspergillus niger* and the yeasts *Saccharomyces cerevisiae* and *Candida albicans* (Jirovetz, Buchbauer, Stoyanova, Georgiev, & Damianova, 2003). (4*R*)-(−)-carvone was also active against *Campylobacter jejuni* whilst (4*S*)-(+)-carvone was effective against *Listeria monocytogenes* (Friedman, Henika, & Mandrell, 2002). In a study by Naigre, Kalck, Rogues, Roux, and Michel (1996), (4*R*)-carvone showed significant activity over *Enterococcus faecium*, *Escherichia coli* and, above a concentration of 10 μg/ml, also against *A. niger*. In a study of growth inhibition of *E. coli* O157:H7, *Salmonella typhimurium* and *Photobacterium leiognathi*, (4*S*)-(+)-carvone was less inhibitory than carvacrol, thymol and *trans*-cinnamaldehyde (Helander et al., 1998). Carvacrol and thymol disintegrated the outer membrane which did not occur by action of (4*S*)-(+)-carvone or *trans*-cinnamaldehyde. The cytotoxicity and genotoxicity of the same compounds, in several short-term microbial and mammalian in vitro assays, showed that they all inhibited viability and proliferation of Hep-2 cells (human epithelial carcinoma cell line) in a dose-dependent manner (Stammati et al., 1999). In the proliferation test, the IC50 of carvone was 0.9 mM (inhibitory concentration for 50% of the population). The morphological analysis suggested an induction of apoptosis by carvone, carvacrol and cinnamaldehyde. None of the tested compounds caused DNA damage at non-toxic doses. A marked dose-dependent differential toxicity was observed in the DNA repair test with carvone.

Pol and Smid (1999) showed that a combination of compounds, such as nisin, carvone and lysozyme, is an effective method for decreasing the number of colonies of food-borne pathogens.

1.4. Medical relevance

Carvone and perillaldehyde were found to inhibit the transformation of *C. albicans* from a coccus to a filamentous form, which is associated with *C. albicans* pathogenicity, and are thus potentially good therapeutic agents against infections caused by this fungus (McGeady, Wansley, & Logan, 2002). *C. albicans* is one of the most common human pathogens, causing a wide variety of infections that may be life-threatening in individuals with impaired immunity, especially in patients with AIDS. Few drugs are effective against candidal infections, the majority having limitations, both in efficacy and side-effects. The results obtained with carvone may lead to better drugs and to an effective treatment. Additionally, administration of terpenoids such as carvone, limonene and perillic acid, was found to increase the total number of leucocytes in Balb/c mice (Raphael & Kuttan, 2003). The total antibody production, antibody-producing cells in spleen, bone marrow cellularity and α-esterase-positive cells increased significantly compared to control mice.

(4*S*)-(+)-Carvone was used in the stereoselective synthesis of the marine antitumor agent eleutherobin (Carter, Hodgetts, McKenna, Magnus, & Wren, 2000). The conversion of (4*S*)-(+)-carvone into the sulfone involves the left-hand-side of this cytotoxic sesquiterpene.

In a study where a series of carvone-related compounds was assessed for their ability to induce increased activity of glutathione *S*-transferase in several tissues of A/J mice, (Zheng, Kenney, & Lam, 1992a) showed that (4*S*)-(+)-carvone exhibited the highest activity as an inducer in all of the tested tissues. Carvone and its related compounds were proved to be a class of potential chemopreventive agents, since the anticarcinogenic activity is correlated with the ability to induce increased activity of detoxifying enzymes. The α,β-unsaturated ketone system in carvone is, apparently, responsible for the high enzyme-inducing activity (Zheng, Kenney, & Lam, 1992b).

(+)-Limonene and (4*S*)-(+)-carvone reduced, in female A/J mice, forestomach tumor formation by about 60% and pulmonary adenoma formation by nearly 35%, due to their capacities to inhibit *N*-nitrosodiethylamine-induced carcinogenesis (Wattenberg, Sparnins, & Barany, 1989). Dietary monoterpenes were found to be helpful, both in prevention and therapy of cancer (Crowell, 1999).

Enteric-coated peppermint oil may improve gastrointestinal function in individuals suffering from irritable bowel syndrome (IBS), a functional disorder of the large intestine (Kline, Kline, Di Palma, & Barbero, 2001). Non-ulcer dyspepsia, gastro-esophageal reflux disorder and intestinal overgrowth of *C. albicans* and *Helicobacter pylori* (a bacteria associated with stomach cancer) may also be treated with enteric-coated peppermint oil. Furthermore, peppermint oil may also be used, especially when combined with caraway oil, to treat esophageal spasm and intestinal colic (Micklefield, Greving, & May, 2000). In fact, clinical studies have suggested that combinations of the two essential oils "produce better results than peppermint oil alone (May, Kohler, & Schneider, 2000). They can even help to dissolve gallstones (Somerville, Ellis, Whitten, Balfour, & Bell, 1985).

Carvone has also been applied in studies to investigate human perception of odours. In a study involving six squirrel monkeys and 10 human subjects, the ability to distinguish between 10 pairs of enantiomers was assessed (Laska, Liesen, & Teubner, 1999). The two species exhibited a similar pattern of odour recognition where some optical isomers were discriminated while others were not, thus indicating that, in both species, chiral recognition of volatile enantiomers is probably restricted to some substances. In both groups, the optical isomers of carvone were discriminated.

The large amounts of carvone enantiomers consumed as food additives and in dental formulations led Jager et al. (2000) to investigate the carvone metabolic pathway in rat and human liver microsomes. When each enantiomer was incubated with liver microsomes, stereoselective biotransformation was observed: (4*R*)-(−)-carvone was converted into (4*R*,6*S*)-(−)-carveol, NADPH-dependently; (4*S*,6*S*)-(+)-carveol was produced from (4*S*)-(+)-carvone.

The health benefits promoted by monoterpenes, diterpenes and tetraterpenes were recently reviewed and discussed by Wagner and Elmadfa (2003). These authors dedicated special attention to the different modes of action of terpenes, in particular the way they influence/prevent oxidative stress, carcinogenesis and cardiovascular diseases.

1.5. Repellent

Carvone can be used as an insecticide against the fruit fly, *Drosophila melanogaster*, although constituents of mint oil show higher insecticidal and genotoxic activities (Franzios et al., 1997). The toxicities of 34 naturally-occurring monoterpenoids, among which was carvone, against the larva of the western corn rootworm, the adult of the twospotted spider mite and the adult house fly, were studied and discussed by Lee, Tsao, Peterson, and Coats (1997).

Essential oils and terpenoids were tested in commercially manufactured mosquito repellent electronic assemblies for 6–7 days against the adult female of *Aedes aegypti* (Vartak & Sharma, 1993). (4*R*)-(−)-Carvone was able to knock down the mosquitoes and to act as a repellent. No mortality caused by either of the tested terpenoids was observed. *A. aegypti* was described as the principal vector in Brazil for diseases such as yellow fever, dengue and haemorrhagic dengue (Consoli & de Oliveira, 1994).

In a project aiming at developing methods of controlling some forest pest insects, Smitt and Hogberg (2002), were interested in the preparation of heavier analogues of carvone. By regioselective transformation of carvone to silyl ether-protected carveol epoxides, and transformation of these to carvone analogues, they were able to prepare prenylbisabolane diterpene (a natural insecticide compound, constituent of the Jamaican plant, *Croton linearis*).

A recent patent application describes spearmint oil as a deer repellent (Mueller, 2002). The spearmint oil, mixed in an oil extender/sticker, added to water and sprayed in foliage, repels deer from eating the sprayed plants. This natural product can be safely used in fruits, vegetables and ornamental plants.

1.6. Building block

The isopropenyl group, at which carvone has one chiral centre, can be oxidised to alcohol or acid functions, while the enone functionality in carvone makes almost all other places in the cyclohexane ring reachable, making this molecule an attractive starting material.

In recent years, carvone has been applied in the synthesis of different compounds, some of high medical relevancy. (4*S*)-(+)-Carvone was used as starting material for the following compounds: terpenoid unsaturated 1,4-dialdehydes (Abad et al., 2000); polyoxygenated atisane-type diterpenoids (Abad, Agullo, Cunat, & Navarro, 2001); new chiral phenols (Pfeiffer, Jauch, & Schurig, 1999); 8,14-secosteroids (Kotyatkina, Zhabinskii, Khripach, & de Groot, 2000). (4*R*)-(−)-Carvone was described as a starting material for the following compounds: oxygenated spongiane-type diterpenoids (Abad, Agullo, Cunat, & Garcia, 2002); taxoids with fully functionalised A-ring (Mehta, Chattopadhyay, & Umarye, 1999); terpenoid unsaturated 1,4-dialdehydes (Abad et al., 2000); enantiomeric 6-isopropenyl-3-methyl-2-cycloheptenones, which may be suitable intermediates for the enantiodivergent synthesis of guaiane sesquiterpenes (de Faria et al., 2000); chiral silatranes (Wagner, Herrmann, Pedersen, & Scherer, 2001); cyclohexane carbocyclic nucleosides (Wang & Herdewijn, 1999a, 1999b); nemorensic acid (Honda & Ishikawa, 1999), which apparently has the ability to cross link strands of DNA at very specific points and is a component of nemorensine, an alkaloid found in plants, toxic to humans and livestock; chiral cyclohexanones (Meulemans, Stork, Jansen, & de Groot, 1998) needed in the syntheses of enantiomerically pure insect antifeedant clerodanes; insect-antifeedant dihydroclerodin (1) and lupulin C (Meulemans, Stork, Macaev, Jansen, & de Groot, 1999). New phosphorated 4,5-dihydroisoxazoles were produced from carvone, limonene and perilaldehyde (de Aguiar & Kover, 1999).

Srikrishna and several co-workers, synthesised several compounds using carvone as reagent, among which were the following compounds: (−)-thaps-8-en-5-ol, in the first enantiospecific synthesis of a thapsane (Srikrishna & Dinesh, 2000); (+)-pinguisenol (Srikrishna & Vijaykumar, 2000); mayurone and thujopsenes, tricyclic sesquiterpenes (Srikrishna & Anebouselvy, 2001); chiral functionalised C-ring derivatives of taxanes (Srikrishna, Kumar, & Reddy, 1998); (−)-9-pupukeanone (Srikrishna & Kumar, 2002); (+)-3-valeranone and (+)-valerane (Srikrishna & Dinesh, 2000).

Ionones and carvone analogues were developed by Anzaldi et al. (2000), some with promising potential as perfume ingredients. (*Z*)-normethyl-carvo-beta-santalol was synthesised from (*R*)-(−)-carvone (Buchbauer, Hofinghoff, & Froese, 1998). This new santalol analogue was described by the authors as having a ''weak woody odour with a dry note of cedrol''. New derivatives of both (+)- and (−)-carvone were synthesised by Cornea, Maior, Baciu, and Elsayed (1987).

As mentioned previously, (4*S*)-(+)-carvone, converted into a sulfone, comprises the left-hand-side of the marine antitumor agent, eleutherobin, which a the cytotoxic sesquiterpene (Carter et al., 2000). The synthesis of the antitumor marine sponge metabolite, puupehedione, was done using (4*R*)-(−)-carvone (Maiti, Sengupta, Giri, Achari, & Banerjee, 2001). Enantiospecific synthesis of both enantiomers of the marine sesquiterpene 2-thiocyanatoneopupukeanane, starting from (4*R*)-(−)-carvone, was achieved by Srikrishna and Gharpure (2002).

(4*R*)-(−)-Carvone is also needed in the production of pentyl and octyl endoperoxide derivatives which demonstrate reasonable antimalarial potency in vitro against *Plasmodium falciparum* HB3 (O'Neill et al., 1998).

1.7. Biochemical environmental indicator

The quantitative accumulation and composition of terpenoids in *Pinus sylvestris* L. depend on the environmental conditions (Berta, Supuka, & Chladna, 1997). Ten terpenoids were identified in the needles of scotch pine trees in a city environment (Nitra, Slovakia) whilst only eight were present in the needles of the same trees in a clean environment (Arboretum Mlynany, Slovakia). In needles of trees from Nitra, accumulation of citral, myrcene, camphene, and especially α-pinene, was lower while the content of β-pinene, *p*-cymene, α-bisabolol, eugenol, carvone and terpineol were higher than in the trees grown on the Arboretum. Proportions between α-pinene and the other terpenes also changed.

Comparison of the terpene composition of the needles of *Pinus strobus* L., grown on both sites, showed that the needles collected in the urban environment had 20 terpenes whilst those from the Arboretum had 22 terpenes (Supuka & Berta, 1998). The production of α-pinene, β-pinene, myrcene, limonene, 1.8-cincole and citral was higher in the cleaner environment and significantly different from that observed in needles from the more polluted site. Besides, urban conditions changed the proportions between α-pinene and α-phellandrene, 1.8-cineole, terpineol, citral and carvone.

Both studies show that secondary metabolites of trees, such as carvone, may be useful as biochemical environmental indicators.

2. How

2.1. By extraction of essential oils

So far, the major sources of carvone have been caraway, dill and spearmint. However, several drawbacks are known to crop production, such as the following: time is needed for plant development; plants depend on weather conditions; soil and fertiliser composition may dramatically influence plant development; crop rotations are needed for soil recovery; there is necessity for both macro and micro-nutrients and occasional necessity for a companion crop; competition of weed occurs; yields may depend on pollinators; plants are subject to attacks by grasshoppers and leaf hoppers; root, stems, leaves and flowers may suffer from several infections; harvesting may be carried out when part of the crop is still not ready. Additionally, a study conducted by Moll and Uiterkamp (1997) comparing (i) carvone derived from caraway seed and a chemically-produced sprouting inhibitor, (ii) onion oil and an equivalent chemical mixture and, (iii) a potato starch derivative for paper coating and other paper-coating agents, showed that, apart from the paper coating case, the technological route was preferable, on environmental grounds, to the agricultural route.

Essential oil contents and carvone ratio are similar in dill, annual and biennial caraway seeds and thus the production of carvone will depend on the harvest yield (Bailer, Aichinger, Hackl, de Hueber, & Dachler, 2001). In a comparative study, dill seed yields were low (400–600 kg/ha in one year and less than 200 kg/ha in an other year, 3.4–4% essential oil) as compared to the annual (900 kg/ha, 2.8–3.3% essential oil) and biennial (1250 kg/ha, 3.9–5% essential oil) caraway. In dill seeds, the carvone content was approximately 11 mg/g seeds and independent of the essential oil content. Bouwmeester, Davies, Smid, and Welten (1995a) demonstrated that, in the two varieties of caraway, formation of both limonene and carvone occurs during the early stages of seed development, rapidly reaching a steady state. The quantity and quality of the essential oil of caraway thus seem to depend mainly on assimilate availability during the early stages of seed development. Both enantiomers of limonene, carvone and *cis*- and *trans*-carveol, were detected in the two caraway varieties and, apart from (−)-*cis*-carveol, also in dill (Bouwmeester, Davies, & Toxopeus, 1995b).

Using near infra-red spectrometry in a recurrent, divergent mass selection programme to increase the carvone content in winter-caraway, populations with ca. 15% higher content of carvone were produced (Toxopeus, Lubberts, Neervoort, Folkers, & Huisjes, 1995). Crossing winter cv. Bleija and the annual variety Karzo caraway, resulted in an annual population with ca. 20% higher carvone productivity than the standard Karzo.

In the fruit of caraway, the biosynthesis of limonene and carvone is a multi-step pathway that starts from geranyl diphosphate (Bouwmeester, Gershenzon, Konings, & Croteau, 1998). This compound is firstly cyclised to (+)-limonene. This intermediate is stored in the essential oil ducts without further metabolism or is converted, in the next step, to (+)-*trans*-carveol by limonene-6-hydrolase. In the last step, (+)-*trans* -carveol is oxidised by a dehydrogenase to (4*S*)-(+)-carvone.

In *M. cardiaca*, (−)- and (+)-limonene are the precursors of (−)-and (+)-carvone, respectively (Rani & Akhila, 1998). Labelled (4*R*)-(−)-carvone was detected in *M. spicata* cuttings fed, in vivo, with C14-labelled sucrose, glucose and especially fructose, but not with C14-labelled palmitate or acetate (Maffei, Camusso, & Caramellino, 2001). The incorporation of sugars into (4*R*)-(−)-carvone and the lack of incorporation of palmitate or acetate, confirm the existence of an alternative, non-mevalonate biosynthetic pathway for monoterpene synthesis, in *M. spicata*.

2.2. Chemical synthesis

In the late fifties and during the sixties, several works were published describing carvone chemical synthesis. Linder and Greenspan (1957) produced this terpenoid from limonene monoxide. Waida and Terauchi (1961) synthesised L-carvone whilst Vig, Sharma, Chander, and Raj (1966) described the production of racemic (+)-carvone (naturally found in gingergrass oil). (Sharma, Sethi, Bedi, & Aggarwal, 1980), successfully synthesised 5,5-ethylenedioxy-2-methylhept-2-en-1-ol, which is a key intermediate in the synthesis of this form of carvone. In 1975, Shono, Nishiguchi, Yokoyama, and Nitta (1975) described a novel synthesis of carvone from α-pinene.

Few papers concerning the chemical synthesis of carvone were published after 1990. Oxidation of (+)-limonene in sodium citrate-water buffers, with and without low density polyethylene, was reported by Kutty, Braddock, and Sadler (1994). The result were limonene oxidation end-products, including (4*S*)-(+)-carvone, carveol, limonene oxide, perilaldehyde and linalool and a hydrolysis product, α-terpineol. Wacker oxidation of limonene, using $PdCl_2/CuCl_2/O_2$, which resulted in the formation of *cis*-carvyl acetate, *trans*-carvyl acetate, *trans*-carveol, carvone and α-terpinyl acetate, was carried out by Silva, Patitucci, Bizzo, D'Elia, and Antunes (2002). Oxidation of limonene in *t*-butanol using $PdCl_2/CuCl_2/t$-butyl hydroperoxide was also done, higher yields of oxidation products being obtained in the presence of added chloride ion.

The reaction of 6-substituted derivatives of (5*R*)-(*tert*-butyldimethylsiloxy)-2-cyclohexenone with (2-propenyl)$_2$Cu(CN)Li$_2$, resulting in 1,4-addition products, which upon treatment with DBU (1,8-diazabicyclo-[5.4.0]undec-7-ene) gave (4*S*)-(+)-carvone in 93.0% yield, was carried out by Hareau, Koiwa, Hikichi, and Sato (1999). Similarly, (4*R*)-(−)-carvone, in 96.2% yield was also synthesised by the same authors.

2.3. Biotechnological synthesis

Krings and Berger (1998) showed the advantages of producing biotechnologically flavours and fragrances, among which are: (i) the ability to label microbiologically produced compounds "natural product", making them attractive to increasingly health- and nutrition-conscious consumers; (ii) the potential of using industrially designed biochemical pathways for up-regulating metabolism; (iii) the possibility of producing chiral compounds responsible for odour perception; (iv) independence of agricultural and local conditions and (v) the usually straightforward product recovery. Furthermore, biocatalysis allows the production of enantiomericaly pure compounds, the modification of a molecule at chemically inert carbons and the selective modification of a specific functional group in multifunctional molecules. The disadvantages are related to the common low water solubility, chemical instability, cytotoxicity and high volatility of terpenes. Some works have been published describing successful production of carvone with whole cells or purified enzymes.

The genes encoding a thermostable limonene hydratase were placed on a clone fragment in *E. coli*, conferring the ability of growth on limonene and production of perillyl alcohol and α-terpineol (Savithiry, Cheong, & Oriel, 1997). Whole cell conversions at high temperature, in both aqueous phase and limonene phase, produced α-terpineol and carvone.

Immobilised plant cells of *Solanum aviculare* and *Dioscorea deltoidea* were able to biotransform (*S*)-(−)- and (*R*)-(+)-limonene into *cis*- and *trans*-carveol and carvone (Vanek, Valterova, Vankova, & Vaisar, 1999a; Vanek, Valterova, & Vaisar, 1999b). Both carveols and carvone were racemic mixtures. The specific activities of these plant enzymes were, however, low for industrial applications. Low rates were also obtained with the basidiomycete *Pleurotus sapidus*, which converts (+)-limonene into *cis*- and *trans*-carveol and carvone (Onken & Berger, 1999). The metabolism of (−)- and (+)-carveol in *Streptomyces* was reported by Noma and Nishimura (1987) while the biotransformation of carveol by the alga, *Euglena gracilis* Z., was studied by Noma and Asakawa (1992).

Rhodococcus globerulus PWD8, a toluene degrader strain, is able to convert (+)-limonene into *trans*-carveol and carvone (Duetz, Fjallman, Ren, Jourdat, & Witholt, 2001). After 2 h of reaction, 90% of limonene had been converted into *trans*-carveol at a rate of 0.73 mM/h and into carvone, which reached a concentration of 0.08 mM. The concentration of carvone increased in the following few hours, reaching a maximum of 0.29 mM after 27 h, as *trans*-carveol concentration decreased, indicating that this compound was being slowly converted into carvone.

These cells were found to produce *trans* and *cis*-carveol and/or carvone as result of limonene metabolism, depending on the type and concentration of the carbon source used for cell growth (de Carvalho & da Fonseca, 2003). In aqueous systems, cells grown on ethanol and toluene only produced *trans*-carveol, while cells grown on limonene and on toluene, with more air available, produced both *trans*-carveol and carvone. In biphasic systems, limonene was converted to *trans* and *cis*-carveol as well as to carvone, independently of the carbon source used, although carveol and carvone production rates were higher in toluene and limonene grown cells, respectively.

R. erythropolis DCL14, a strain able to grow on limonene as sole carbon source, was found to have a novel degradation pathway for limonene, starting with an epoxidation at the 1,2 double bond (van der Werf, Swarts, & de Bont, 1999a). This strain contains several NAD, dichlorophenolindophenol (DCPIP)- and NADP-dependent carveol dehydrogenases, that convert carveol into carvone. When incubated with a distereomeric mixture of (4*R*)- or (4*S*)-carveol, the DCPIP-dependent CDH, which is a novel nicotinoprotein, only converted the (6*S*)-carveol stereoisomers (van der Werf et al., 1999b). However, when the DCPIP-dependent CDH was incubated with the pure carveol stereoisomers, the (6*R*)-stereoisomers of carveol were also converted, at 35% of the (6*S*)-carveol (van der Werf & Boot, 2000).

The relatively low water solubilities of both carveol and carvone were overcome with an organic:aqueous system (de Carvalho & da Fonseca, 2002a). The biotransformation was carried out with whole cells of *R. erythropolis* DCL14. *n*-Dodecane was the solvent that allowed the highest retention of cell activity and viability. With this system, two products are obtained at the end, (4*R*)-(−)-carvone and isomerically resolved (−)-*cis*-carveol. A distereomeric excess, higher than 98%, was achieved at a carveol conversion of 59% (de Carvalho, van Keulen, & da Fonseca, 2002). Relatively high productivity (0.164 mg carvone/h ml org phase) and yield (0.68 g carvone/g carveol) were attained with an air-driven column reactor, after adapting the cells to the presence of solvent, substrate and product (de Carvalho & da Fonseca, 2002b).

3. Conclusions

The various applications of carvone, as fragrance and flavour, potato sprouting inhibitor, antimicrobial agent, building block, biochemical environmental indicator and also its applications in the medical field, justify the research aimed to increase the world production of this monoterpene. Agricultural development and production of caraway, dill and spearmint seeds with higher carvone contents, also using cleaner chemical processes of purification could be a logical approach to producing carvone. However, the mounting human demand for the active components of these plants and the environmental awareness of consumers suggest that the production of carvone with specialised micro-organisms, using biotechnological techniques, is a more efficient way to satisfy such demand and requirements.

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References

Abad, A., Agullo, C., Castelblanque, L., Cunat, A. C., Navarro, I., & de Arellano, M. C. R. (2000). Synthesis of terpenoid unsaturated 1,4-dialdehydes. pi-facial selectivity in the Diels–Alder reaction of the 1-vinyl-2- methylcyclohexene moiety of polycyclic systems with DMAD. *Journal of Organic Chemistry, 65*, 4189–4192.

Abad, A., Agullo, C., Cunat, A. C., & Navarro, I. (2001). Stereoselective synthesis of polyoxygenated atisane-type diterpenoids. *Tetrahedron Letters, 42*, 8965–8968.

Abad, A., Agullo, C., Cunat, A. C., & Garcia, A. B. (2002). Synthesis of oxygenated spongiane-type diterpenoids from carvone. *Tetrahedron Letters, 43*, 7933–7936.

Aggarwal, K. K., Khanuja, S. P. S., Ahmad, A., Kumar, T. R. S., Gupta, V. K., & Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flavour and Fragrance Journal, 17*, 59–63.

de Aguiar, A. P., & Kover, W. B. (1999). Chemo- and regioselective synthesis of new phosphorated 4,5-dihydroisoxazoles from different monoterpenes. *Heterocyclic Communications, 5*, 227–230.

Anzaldi, M., Sottofattori, E., Dusatti, F., Ferro, M., Pani, M., & Balbi, A. (2000). Synthesis of ionones and carvone analogues: olfactory properties and preliminary toxicity assays. *European Journal of Medicinal Chemistry, 35*, 797–803.

Bailer, J., Aichinger, T., Hackl, G., de Hueber, K., & Dachler, M. (2001). Essential oil content and composition in commercially available dill cultivars in comparison to caraway. *Industrial Crops and Products, 14*, 229–239.

Berta, F., Supuka, J., & Chladna, A. (1997). The composition of terpenes in needles of Pinus sylvestris in a relatively clear and in a city environment. *Biologia, 52*, 71–78.

Bouwmeester, H. J., Davies, J. A. R., Smid, H. G., & Welten, R. S. A. (1995a). Physiological Limitations to Carvone Yield in Caraway (*Carum Carvi* L). *Industrial Crops and Products, 4*, 39–51.

Bouwmeester, H. J., Davies, J. A. R., & Toxopeus, H. (1995b). Enantiomeric composition of carvone, limonene, and carveols in seeds of dill and annual and biennial caraway varieties. *Journal of Agricultural and Food Chemistry, 43*, 3057–3064.

Bouwmeester, H. J., Gershenzon, J., Konings, M. C. J. M., & Croteau, R. (1998). Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway – I. Demonstration of enzyme activities and their changes with development. *Plant Physiology, 117*, 901–912.

Buchbauer, G., Hofinghoff, J., & Froese, A. (1998). On the synthesis and odour impression of (*Z*)-normethyl-carvo-beta-santalol. *Monatshefte fur Chemie, 129*, 705–710.

Carter, R., Hodgetts, K., McKenna, J., Magnus, P., & Wren, S. (2000). Studies on the stereoselective synthesis of the marine antitumor agent eleutherobin. *Tetrahedron, 56*, 4367–4382.

de Carvalho, C. C. C. R., & da Fonseca, M. M. R. (2002a). Maintenance of cell viability in the biotransformation of (−)-carveol with whole cells of *Rhodococcus erythropolis*. *Journal of Molecular Catalysis B-Enzymatic, 19*, 389–398.

de Carvalho, C. C. C. R., & da Fonseca, M. M. R. (2002b). Influence of reactor configuration on the production of carvone from carveol by whole cells of *Rhodococcus erythropolis* DCL14. *Journal of Molecular Catalysis B-Enzymatic, 19*, 377–387.

de Carvalho, C. C. C. R., & da Fonseca, M. M. R. (2003). Towards the bio-production of *trans*-carveol and carvone from limonene: induction after cell growth on limonene and toluene. *Tetrahedron: Asymmetry, 14*, 3925–3931.

de Carvalho, C. C. C. R., van Keulen, F., & da Fonseca, M. M. R. (2002). Modelling the bio-kinetic resolution of diastereomers present in unequal initial amounts. *Tetrahedron: Asymmetry, 13*(15), 1637–1643.

Cizkova, H., Vacek, J., Voldrich, M., Sevcik, R., & Kratka, J. (2000). Caraway essential oil as potential inhibitor of potato sprouting. *Rostlinna Vyroba, 46*, 501–507.

Consoli, G.B., de Oliveira, R.L., (1994). *Principais Mosquitos de Importância Sanitária no Brasil*. Rio de Janeiro: Fiocruz (in Portuguese).

Cornea, F., Maior, O., Baciu, I., & Elsayed, N. H. (1987). Synthesis and characterization of some new derivatives of (+)-carvone and (−)-carvone. *Revista de Chimie, 38*, 570–573.

Crowell, P. L. (1999). Prevention and therapy of cancer by dietary monoterpenes. *Journal of Nutrition, 129*(3), 775–778.

Duetz, W. A., Fjallman, A. H. M., Ren, S. Y., Jourdat, C., & Witholt, B. (2001). Biotransformation of D-limonene to (+) *trans*-carveol by toluene-grown *Rhodococcus opacus* PWD4 cells. *Applied and Environmental Microbiology, 67*, 2829–2832.

de Faria, M. L., Magalhães, R. D., Silva, F. C., Matias, L. G. D., Ceschi, M. A., Brocksom, U., et al. (2000). Enantiodivergent syntheses of cycloheptenone intermediates for guaiane sesquiterpenes. *Tetrahedron-Asymmetry, 11*, 4093–4103.

Franzios, G., Mirotsou, M., Hatziapostolou, E., Kral, J., Scouras, Z. G., & Mavragani-Tsipidou, P. (1997). Insecticidal and genotoxic activities of mint essential oils. *Journal of Agricultural and Food Chemistry, 45*, 2690–2694.

Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection, 65*, 1545–1560.

Hareau, G. P. J., Koiwa, M., Hikichi, S., & Sato, F. (1999). Synthesis of optically active 5-(*tert*-butyldimethylsiloxy)-2-cyclohexenone and its 6-substituted derivatives as useful chiral building blocks for the synthesis of cyclohexane rings. Syntheses of carvone, penienone, and penihydrone. *Journal of the American Chemical Society, 121*, 3640–3650.

Hartmans, K. J., & Diepenhorst, P. (1994). The use of carvone as a sprout inhibitor for potatoes. *Potato Research, 37*, 445–446.

Hartmans, K. J., Diepenhorst, P., Bakker, W., & Gorris, L. G. M. (1995). The use of carvone in agriculture – sprout suppression of potatoes and antifungal activity against potato-tuber and other plant-diseases. *Industrial Crops and Products, 4*, 3–13.

Hartmans, K. J., Lenssen, J. M., & de Vries, R. G. (1998). Use of talent (carvone) as a sprout growth regulator of seed potatoes and the effect on stem and tuber number. *Potato Research, 41*, 190–191.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry, 46*, 3590–3595.

Honda, T., & Ishikawa, F. (1999). Enantiospecific synthesis of (+)-nemorensic acid, a necic acid component of the macropyrrolizidine alkaloid, nemorensine. *Journal of Organic Chemistry, 64*, 5542–5546.

Hornok, L. (1992). *Cultivation and processing of medicinal plants*. Chichester: John Wiley and Sons.

Jager, W., Mayer, M., Platzer, P., Reznicek, G., Dietrich, H., & Buchbauer, G. (2000). Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. *Journal of Pharmacy and Pharmacology, 52*, 191–197.

Jirovetz, L., Buchbauer, G., Stoyanova, A. S., Georgiev, E. V., & Damianova, S. T. (2003). Composition, quality control, and antimicrobial activity of the essential oil of long-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. *Journal of Agricultural and Food Chemistry, 51*, 3854–3857.

Kerstholt, R. P. V., Ree, C. M., & Moll, H. C. (1997). Environmental life cycle analysis of potato sprout inhibitors. *Industrial Crops and Products, 6*, 187–194.

Kline, R. M., Kline, J. J., Di Palma, J., & Barbero, G. J. (2001). Enteric-coated, pH-dependent peppermint oil capsules for the treatment of irritable bowel syndrome in children. *The Journal of Pediatrics, 138*, 125–128.

Kotyatkina, A. I., Zhabinskii, V. N., Khripach, V. A., & de Groot, A. (2000). Synthesis of 8,14-secosteroids from (S)-(+)-carvone. *Collection of Czechoslovak Chemical Communications, 65*, 1173–1182.

Krings, U., & Berger, R. G. (1998). Biotechnological production of flavours and fragrances. *Applied Microbiology and Biotechnology, 49*, 1–8.

Kutty, V., Braddock, R. J., & Sadler, G. D. (1994). Oxidation of D-limonene in presence of low-density polyethylene. *Journal of Food Science, 59*, 402–405.

Laska, M., Liesen, A., & Teubner, P. (1999). Enantioselectivity of odor perception in squirrel monkeys and humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 277*, R1098–R1103.

Lee, S., Tsao, R., Peterson, C., & Coats, J. R. (1997). Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: *Chrysomelidae*), twospotted spider mite (Acari:*Tetranychidae*), and house fly (Diptera:*Muscidae*). *Journal of Economic Entomology, 90*, 883–892.

Linder, S. M., & Greenspan, F. P. (1957). Reactions of limonene monoxide – the synthesis of carvone. *Journal of Organic Chemistry, 22*, 949–951.

Maffei, M., Camusso, W., & Caramellino, D. (2001). Fructose is the most uptaken sugar for (−)-carvone biosynthesis in *Mentha spicata* L. *Journal of Plant Physiology, 158*, 811–813.

Maiti, S., Sengupta, S., Giri, C., Achari, B., & Banerjee, A. K. (2001). Enantiospecific synthesis of 8-epipuupehedione from (*R*)-(−)-carvone. *Tetrahedron Letters, 42*, 3667.

May, B., Kohler, S., & Schneider, B. (2000). Efficacy and tolerability of a fixed combination of peppermint oil and caraway oil in patients suffering from functional dyspepsia. *Alimentary Pharmacology and Therapeutics, 14*, 1671–1677.

McGeady, P., Wansley, D. L., & Logan, D. A. (2002). Carvone and perillaldehyde interfere with the serum-induced formation of filamentous structures in *Candida albicans* at substantially lower concentrations than those causing significant inhibition of growth. *Journal of Natural Products, 65*, 953–955.

Mehta, G., Chattopadhyay, S. K., & Umarye, J. D. (1999). A new carvone based construction of the ring-A of taxoids. *Tetrahedron Letters, 40*, 4881–4884.

Meulemans, T. M., Stork, G. A., Jansen, B. J. M., & de Groot, A. (1998). Enantioselective synthesis of highly functionalised cyclohexanones starting from R-(−)-carvone. *Tetrahedron Letters, 39*, 6565–6568.

Meulemans, T. M., Stork, G. A., Macaev, F. Z., Jansen, B. J. M., & de Groot, A. (1999). Total synthesis of dihydroclerodin from (R)-(−)-carvone. *Journal of Organic Chemistry, 64*, 9178–9188.

Micklefield, G. H., Greving, I., & May, B. (2000). Effects of peppermint oil and caraway oil on gastroduodenal motility. *Phytotherapy Research, 14*, 20–23.

Moll, H. C., & Uiterkamp, A. J. M. S. (1997). Comparative evaluation by lifecycle and risk assessment of agrobiological and technological routes of production. *Industrial Crops and Products, 6*, 333–341.

Mueller, N.J. (2002). United States Patent Application 20020071877.

Naigre, R., Kalck, P., Rogues, C., Roux, I., & Michel, G. (1996). Comparison of antimicrobial properties of monoterpenes and their carbonylated products. *Planta Medica, 62*, 275–277.

Noma, Y., & Nishimura, H. (1987). Bottrospicatols, novel monoterpenes produced on conversion of (−)- and (+)-*cis*-carveol by *Streptomyces*. *Agricultural and Biological Chemistry, 51*, 1845–1849.

Noma, Y., & Asakawa, Y. (1992). Enantio- and diastereoselectivity in the biotransformation of carveols by *Euglena gracilis* Z. *Phytochemistry, 31*, 2009–2011.

O'Neill, P. M., Searle, N. L., Raynes, K. J., Maggs, J. L., Ward, S. A., Storr, R. C., et al. (1998). A carbonyl oxide route to antimalarial yingzhaosu A analogues: synthesis and antimalarial activity. *Tetrahedron Letters, 39*, 6065–6068.

Onken, J., & Berger, R. G. (1999). Effects of R-(1)-limonene on submerged cultures of the terpene transforming basidiomycete *Pleurotus sapidus*. *Journal of Biotechnology, 69*, 163–168.

Peterson, L., & Bienvenu, F. (1997). Spearmint. In K. Hyde (Ed.), *The New Rural Industries, a handbook for farmers and investors*. Australia: RIRDC.

Pfeiffer, J., Jauch, J., & Schurig, V. (1999). Synthesis of new terpene-substituted phenols stereochemistry of 6-(4′-hydroxyphenyl)-1,6-dihydro-carvone. *Enantiomer, 4*, 363–368.

Plinius Secundus G., (77 A.D.). *Naturalis Historiae* (Natural History (1952) Pliny the Elder, Trans.). Cambridge, USA: Harvard University Press, The Loeb Classical Library.

Pol, I. E., & Smid, E. J. (1999). Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes*. *Letters in Applied Microbiology, 29*, 166–170.

Rani, K., & Akhila, A. (1998). Biosynthesis of (+)- and (−)-carvone and limonene and their biogenetic relationship with other oxygenated monoterpenes in *Mentha cardiaca*. *Fitoterapia, 69*, 337–348.

Raphael, T. J., & Kuttan, G. (2003). Immunomodulatory activity of naturally occurring monoterpenes carvone, limonene, and perillic acid. *Immunopharmacology and Immunotoxicology, 25*, 285–294.

Savithiry, N., Cheong, T. K., & Oriel, P. (1997). Production of alpha-terpineol from *Escherichia coli* cells expressing thermostable limonene hydratase. *Applied Biochemistry and Biotechnology, 63-5*, 213–220.

Shakespeare, W., (1598). Henry IV, part II, act V, scene III.

Sharma, S. D., Sethi, A. S., Bedi, A. L., & Aggarwal, R. C. (1980). Synthesis of 5,5-ethylenedioxy-2-methylhept-2-en-1-ol, a key intermediate for synthesis of Dl-carvone and a new synthesis of isolimonene. *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry, 19*, 811–812.

Shono, T., Nishiguchi, I., Yokoyama, T., & Nitta, M. (1975). Novel synthesis of *E*-carvone from *E*-alpha-pinene. *Chemistry Letters*, 433–436.

Silva, A. D., Patitucci, M. L., Bizzo, H. R., D'Elia, E., & Antunes, O. A. C. (2002). Wacker PdCl2–CuCl2 catalytic oxidation process: oxidation of limonene. *Catalysis Communications, 3*, 435–440.

Smitt, O., & Hogberg, H. E. (2002). Syntheses of a prenylbisabolane diterpene, a natural insecticide from *Croton linearis*, and of the bisabolane sesquiterpenes (−)-delobanone and (−)-epi-delobanone. *Tetrahedron, 58*, 7691–7700.

Somerville, K. W., Ellis, W. R., Whitten, B. H., Balfour, T. W., & Bell, G. D. (1985). Stones in the common bile duct: experience with medical dissolution therapy. *Postgraduate Medical Journal, 61*, 313–316.

Srikrishna, A., Kumar, P. P., & Reddy, T. J. (1998). Carvone based approaches to chiral functionalised C-ring derivatives of taxanes. *Tetrahedron Letters, 39*, 5815–5818.

Srikrishna, A., & Dinesh, C. (2000). Chiral synthons from carvone: Part 41. Enantiospecific synthesis of (+)-3-valeranone and (+)-valerane. *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry, 39*, 323–325.

Srikrishna, A., & Vijaykumar, D. (2000). An enantiospecific approach to pinguisanes from (R)-carvone. Total synthesis of (+)-pinguisenol. *Journal of the Chemical Society-Perkin Transactions, 1*, 2583–2589.

Srikrishna, A., & Anebouselvy, K. (2001). An enantiospecific approach to tricyclic sesquiterpenes mayurone and thujopsenes. *Journal of Organic Chemistry, 66*, 7102–7106.

Srikrishna, A., & Gharpure, S. J. (2002). Chiral synthons from carvone, Part 56. Enantiospecific synthesis of (−)-4-thiocyanatone-opupukeanane. *Arkivoc*, 52–62.

Srikrishna, A., & Kumar, P. R. (2002). Chiral synthons from carvone. Part 53. First enantiospecific synthesis of (−)-9-pupukeanone. *Tetrahedron Letters, 43*, 1109–1111.

Stammati, A., Bonsi, P., Zucco, F., Moezelaar, R., Alakomi, H. L., & von Wright, A. (1999). Toxicity of selected plant volatiles in microbial and mammalian short-term assays. *Food and Chemical Toxicology, 37*, 813–823.

Supuka, J., & Berta, F. (1998). The composition of terpenes in needles of white pine (*Pinus strobus* L.) growing in urban environment. *Ekologia-Bratislava, 17*, 419–433.

Toxopeus, H., Lubberts, J. H., Neervoort, W., Folkers, W., & Huisjes, G. (1995). Breeding research and in vitro propagation to improve carvone production of caraway (*Carum carvi* L). *Industrial Crops and Products, 4*, 33–38.

van der Werf, M. J., Swarts, H. J., & de Bont, J. A. M. (1999a). *Rhodococcus erythropolis* DCL14 contains a novel degradation pathway for limonene. *Applied and Environmental Microbiology, 65*, 2092–2102.

van der Werf, M. J., van der Ven, C., Barbirato, F., Eppink, M. H. M., de Bont, J. A. M., & vanBerkel, W. J. H. (1999b). Stereoselective carveol dehydrogenase from *Rhodococcus erythropolis* DCL14 – a novel nicotinoprotein belonging to the short chain dehydrogenase/reductase superfamily. *Journal of Biological Chemistry, 274*, 26296–26304.

van der Werf, M. J., & Boot, A. M. (2000). Metabolism of carveol and dihydrocarveol in *Rhodococcus erythropolis* DCL14. *Microbiology-Uk, 146*, 1129–1141.

Vanek, T., Valterova, I., Vankova, R., & Vaisar, T. (1999a). Biotransformation of (−)-limonene using *Solanum aviculare* and *Dioscorea deltoidea* immobilized plant cells. *Biotechnology Letters, 21*, 625–628.

Vanek, T., Valterova, I., & Vaisar, T. (1999b). Biotransformation of (S)-(−)- and (R)-(+)-limonene using *Solanum aviculare* and *Dioscorea deltoidea* plant cells. *Phytochemistry, 50*, 1347–1351.

Vartak, P. H., & Sharma, R. N. (1993). Vapor toxicity and repellence of some essential oils and terpenoids to adults of *Aedes aegypti* (L) (Diptera, Culicidae). *Indian Journal of Medical Research Section A-Infectious Diseases, 97*, 122–127.

Vig, O. P., Sharma, S. D., Chander, S., & Raj, I. (1966). Terpenoids. 13. Synthesis of Dl-Carvone. *Indian Journal of Chemistry, 4*, 275–283.

Wagner, G., Herrmann, R., Pedersen, B., & Scherer, W. (2001). Synthesis and structure of chiral silatranes derived from terpenes. *Zeitschrift fur Naturforschung Section B-A Journal of Chemical Sciences, 56*, 25–38.

Wagner, K. H., & Elmadfa, I. (2003). Biological relevance of terpenoids – overview focusing on mono-, di- and tetraterpenes. *Annals of Nutrition and Metabolism, 47*, 95–106.

Waida, T., & Terauchi, M. (1961). Synthesis of L-Carvone. *Kogyo Kagaku Zasshi, 64*, 2080–2086.

Wang, J., & Herdewijn, P. (1999a). A novel and enantioselective approach to the synthesis of cyclohexane carbocyclic nucleosides starting from (−)-carvone. *Nucleosides & Nucleotides, 18*, 591–592.

Wang, J., & Herdewijn, P. (1999b). Enantioselective synthesis and conformational analysis of cyclohexene carbocyclic nucleosides. *Nucleosides & Nucleotides, 18*, 593–594.

Wilson, H. (1988). *Egyptian food and drink*. Aylesbury, UK: Shire Publishers.

Wattenberg, L. W., Sparnins, V. L., & Barany, G. (1989). Inhibition of N-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Research, 49*, 2689–2694.

Zheng, G., Kenney, P. M., & Lam, L. K. T. (1992a). Effects of Carvone compounds on glutathione-*S*-transferase activity in A/J Mice. *Journal of Agricultural and Food Chemistry, 40*, 751–755.

Zheng, G. Q., Kenney, P. M., & Lam, L. K. T. (1992b). Anethofuran, carvone, and limonene – potential cancer chemopreventive agents from dill weed oil and caraway oil. *Planta Medica, 58*, 338–341.