ChemTexts (2024) 10:11
https://doi.org/10.1007/s40828-024-00191-2

LECTURE TEXT

(+)-Nootkatone, the flavor of grapefruit

Raghavendra Ramachanderan[1] · **Bernd Schaefer**[2]

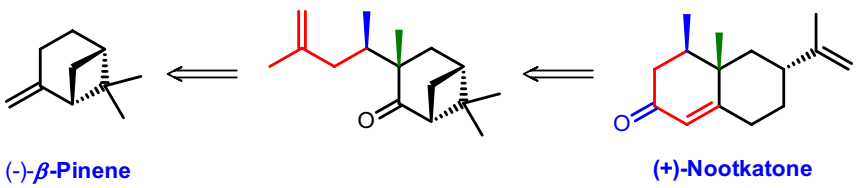
Received: 3 May 2024 / Accepted: 27 May 2024 / Published online: 29 November 2024

Abstract

Nootkatone is one of the most valuable citrus flavors. Originally isolated from the heartwood of the Nootka cypress (*Callitropsis nootkatensis*), it was probably the identification of nootkatone as a minor component of grapefruit oil that catapulted this sesquiterpene into the focus of flavor research and made it a highly sought-after citrus aroma in recent decades. The odors of (+)-nootkatone and (−)-nootkatone are perceptible in the air at threshold concentrations of 30 ppm and 66,000 ppm of saturated vapor, respectively. While (+)-nootkatone embodies a strong grapefruit scent and has a bitter taste, (−)-nootkatone has a faint woody vetiver note and is virtually tasteless. In 2020, nootkatone was registered by the US Environmental Protection Agency as an insect repellent and natural insecticide that may repel and kill mosquitoes, fleas, ticks, mites, lice, and termites. Moreover, numerous pharmacological activities of nootkatone have been thoroughly investigated. As expected, a wealth of synthetic approaches, which are essentially based on the oxidation of valencene and on total syntheses, have been published in the last 60 years since its discovery. However, in addition to extraction from natural sources (e.g., grapefruit peel), the enzymatic oxidation of valencene and fermentative processes are the preferred production technologies today. The market is expected to grow considerably over the next 10 years, not least owing to the wide range of potential applications in the pharmaceutical, personal, and home care sector.

How the reader may benefit: learn more about the total synthesis of natural products, allylic oxidation, and Robinson annulation, gain deeper insights into the biosynthesis of sesquiterpenes and finally discover how enantiopure compounds, such as (+)-nootkatone, are produced at an industrial scale.

Graphical Abstract



Keywords Nootkatone · Valencene · Sesquiterpene · Citrus · Grapefruit · Flavor · Insecticide · Repellent · Robinson annulation · Total synthesis · Biosynthesis

✉ Bernd Schaefer
bernd.g.schaefer@basf.com

[1] Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

[2] Ruprecht-Karls-Universität Heidelberg; BASF SE, RGD/CN–B009, 67056 Ludwigshafen am Rhein, Germany

Infobox: Benefits and recommendations for the reader

This lecture text invites you to an exciting but also entertaining journey that takes you from the discovery to the industrial production of one of the most important citrus aromas. After a detailed introduction, the known total syntheses are benchmarked against each other, and selected examples are discussed in more detail. Advanced students can benefit by learning more about the orchestration of synthesis methods in total synthesis and deepening their knowledge of allylic oxidation and Robinson annulation. The reader will also learn how nature is synthesizing a variety of sesquiterpenes and how nootkatone is produced by bioconversion in the flavor and fragrance industry. This lecture text is not intended to be read in a linear fashion. If you are interested in a particular topic, please feel free to jump directly to the section of interest. The content ranges from

1. Introduction
2. Syntheses of Nootkatone
3. Biosynthesis
4. Production
5. Market, Supply, Demand, Prize

As you browse through this paper, you will find numerous infoboxes and additional information that contain interesting and sometimes entertaining aspects, but they are not essential to the story and can be skipped. To simplify retracing the numerous building blocks, the drawings of most chemical syntheses are in color. The color code is consistent within a total synthesis but not between two different syntheses.

Introduction

Nootka

The story of nootkatone commences with a misunderstanding at the end of March 1778 on the west coast of North America. On 12 July 1776, James Cook set sail from Plymouth on his third voyage with HMS Resolution and HMS Discovery. On behalf of the British Admiralty, he was to follow a secret plan to explore the Northwest Passage from the Pacific. The voyage took him around the Cape of Good Hope, south of Australia and New Zealand to the Society Islands, and then northeast across the Hawaiian Islands until he finally sighted the west coast of Oregon on 7 March 1778 [1]. Then, 3 weeks later, he cruised west of the largest North American island in the Pacific, later named Vancouver Island in honor of George Vancouver (1757–1798), a midshipman on Cook's expedition. There he decided to disembark to rest the crews, replenish the ships' water supplies, make essential ship repairs, and trade with the natives before continuing north to the Bering Strait.

On Sunday, 29 March 1778, he entered "King George's Sound," as he first named the strait, where he anchored for a month close to the settlement of Yuquot (Friendly Cove), south of Bligh Island. The island is situated in the middle of the sound and was later named after William Bligh (1757–1798), the then sailing master of the HMS Resolution (Fig. 1) [2–4]. When James Cook and his crew encountered the Mowachaht and Muchalaht First Nation people they advised him to "come around" (*Nuu-chah-nulth*: *nuutkaa* is "to circle around") into the port with his ships [5]. Cook however, mistook "Nootka" for the indigenous name for the area and renamed the sound. Cook's misunderstanding also led to the indigenous people of the area being referred to as "Nootka." It was not until 1979 that the tribes of western Vancouver Island began calling themselves *Nuu-chah-nulth*.

After James Cook explored the Pacific Northwest coast of America on behalf of the British Royal Navy, several traders including James Hanna (†1787) and John Meares (c. 1756–1809), came to Nootka Sound to do business in the sea otter fur trade with China. In 1788, Meares erected a trading post on land he allegedly purchased from Chief Maquinna near the native village of Yuquot at the entrance to Nootka Sound.

Similar ambitions and claims arose on the Spanish side as well, especially since Spanish explorer Juan José Pérez Hernández (c. 1725–1775) had already visited Nootka Sound in 1774. In 1789, the escalation of hostilities garnered the area worldwide attention with the Nootka crisis, in which the young USA and not least the Nootka people led by Chief Maquinna were also involved, as war between Great Britain and the Spanish Empire threatened to break out. (Fig. 1).

It took five years and considerable diplomatic efforts to restore peace to the area. The endeavor also included a British expedition to Nootka Sound led by George Vancouver, who by then had been promoted to lieutenant, to negotiate the right to colonize the Pacific Northwest coast with the Spanish commissioner Juan Francisco de la Bodega y Quadra (1743–1794; Fig. 1). Finally, after the settlement of a total of three Nootka Conventions, among other provisions, Great Britain and Spain agreed to abandon Nootka Sound and refrain from establishing permanent bases in the area [12].

Nootka cypress (*Callitropsis nootkatensis*)

Misunderstandings and confusion also prevailed about the tree from which nootkatone was first extracted. The Nootka-cedar, also called Yellow-cedar, Alaskan-cedar, or Alaskan

yellow-cedar, is not a true cedar (*Cedrus*), but a cypress in the Cupressaceae family, which has a checkered taxonomic and nomenclatural history [13]. *Callitropsis nootkatensis*, native to the moist areas of coastal mountains of the Pacific Northwest, including those of the Cascades, from the Kenai Peninsula in Alaska to the Klamath Mountains in northernmost California, was first recognized in 1792 or probably 1793 on the shores of Nootka Island by Archibald Menzies (Fig. 2) [14, 15].

On the basis of the specimens collected by Archibald Menzies, the first formal description of the Nootka cypress (*Cupressus nootkatensis* D. Don) was provided by David Don (1799–1841), a librarian at the Linnean Society of London [15]. However, in 1842, the French botanist Édouard Spach (1801–1879) established the genus *Chamaecyaris* and renamed the Nootka cypress *Chamaecyparis nootkatensis* (D. Don) Spach [20]. Again some 20 years later, the Danish botanist Anders Sandøe Ørsted (1816–1872) considered the cones of Nootka cypress sufficiently different from those of *Chamaecyparis* to place the taxon in its own genus, which he named *Callitropsis* [21]. However, this was by no means the end of the considerations about the correct nomenclature of *Callitropsis nootkatensis* (D. Don) Oersted. In 1999, the Dutch and Vietnamese botanists Aljos Farjon and Tiên Hiêp Nguyên discovered a new cypress species, which was first thought to be endemic to the karst limestone mountains of Hà Giang Province in northern Vietnam but was later also found in southeast China [22, 23]. It has become commonly known as Vietnamese golden cypress (*Xanthocyparis vietnamensis* Farjon and T. H. Nguyên) belonging to the newly described genus *Xanthocyparis* [24]. Farjon and coauthors felt the cone morphology of *Xanthocyparis vietnamensis* to be so similar to that of Nootka cypress that they proposed accommodating the latter in the same genus by the name *Xanthocyparis nootkatensis* (D. Don) Farjon and Harder. However, this proposal is not yet generally accepted because of both nomenclatural priority and genetic evidence, so the debate about the correct taxonomy is likely to continue [25–29].

Discovery of nootkatone

Nootkatone in *Callitropsis nootkatensis*

The timber of the Nootka cypress, especially the heartwood, is highly valued for its hardness and durability. The latter is attributed to its extractable constituents, which prevent damage from insects and most fungal diseases [30–34]. As early as 1926, R.H. Clark and C.C. Lucas examined the foliage of the Nootka cypress and, as it turned out later, found (−)-α-pinene, (+)-3-carene, and (+)-limonene as the main components of the leaf oil [35–37]. More detailed studies on the heartwood of the Nootka cypress, however, are owing to Gunhild Maria Elisabeth Aulin-Erdtman (1916–2011) and her husband Holger Erdtman (1902–1989), both researchers at the Royal Institute of Technology in Stockholm,

Fig. 1 James Cook (1728–1779) gained historical merit as a conscientious cartographer through his discoveries in the Pacific and as a pioneer in the prevention of scurvy by supplying sailors with citrus fruits. But his legacy is also burdened by his involvement in colonialism and violence against indigenous people, to which he himself ultimately fell victim (left; © public domain) [6, 7]. After Cook's death on Hawaii, William Bligh became navigator of the expedition and contributed much to leading the ships back to England. Then 10 years later, he rose to fame for the mutiny on the three-masted Bounty led by master's mate Fletcher Christian (1764–1793) and for his subsequent 6701 km open-boat voyage from the waters around Tonga to the island of Timor in 1789 (2nd left; © public domain). It is not certain whether James Cook ever encountered Chief Maquinna (2nd right; © public domain), but Maquinna became a powerful leader during the heyday of the maritime fur trade in the Nootka Sound area in the 1780s and 1790s and was deeply affected by the Nootka crisis, in which his younger brother Callicum (†1789) was killed by a Spanish sailor which led him to murder ship crews and take European slaves on several occasions, including the English armorer John Rodgers Jewitt (1783–1821) [8, 9]. George Vancouver (right; © public domain), commissioned to settle the Nootka crisis, is best known as an explorer and cartographer of the Pacific Northwest coast of America, specifically the present-day coasts of the US states of Washington and Oregon and the Canadian province of British Columbia, as far north as Cook Inlet and the Prince William Sound in the area of Anchorage, Alaska. [10, 11]

Fig. 2 Mature Nootka cypress (*Callitropsis nootkatensis*, Mount Higgins, Cascade Range, Washington State) grows up to 40 m tall and bears its foliage in flat sprays of dark green, 3–5 mm long-scale leaves hanging from the branches (© Walter Siegmund). The valuable heartwood, which is well known for its strength and durability, has been used for shipbuilding, fencing, interior finishing work, flooring and as firewood [16, 17]. The oldest specimens, up to more than 1800 years old, are found in the Caren Range on the west coast of British Columbia, opposite Vancouver Island. The first Nootka cypresses were introduced into Europe by the Imperial Botanical Garden of St. Peterburg [18]. Archibald Menzies (1754–1842) was a Scottish surgeon and botanist who was appointed as naturalist by George Vancouver to describe fauna and flora on his voyage 1791–1795 (© public domain) [19]

Sweden [38]. In the 1950s, they started extensive research in this field. The steam distillation of finely divided heartwood led to the discovery of compounds, such as carvacrol, methyl carvacrol, nootkatin, and chamic and chaminic acids [39–43]. In 1962, Holger Erdtman and Yoshiyuki Hirose discovered two other acetone-soluble heartwood constituents, which turned out to be the eremophilanoid [44] sesquiterpenes nootkatene and nootkatone [30, 45, 46]. The correct chemical structure elucidation of (+)-nootkatone [(4R,5S,7R)-7-isopropenyl-4,5-dimethyl-3,4,6,7,8,9-hexahydronaphthalene-2-one], however, is owing to William D. MacLeod of the Fruit and Vegetable Chemistry Laboratory in Pasadena, who at the time was working on the peel oil of *Citrus paradisi* and published his results 3 years later [47]. In recent years, other researchers have succeeded in adding some more compounds, such as valencene-13-ol and nootkatol, to this list, so that a comprehensive profile of the most important ingredients is now available (Fig. 3, Table 1) [48, 49].

Nootkatone in grapefruit (*Citrus paradisi*)

Interest in nootkatone was also sparked in the field of flavor research at approximately the same time. In 1949, Ernest Guenther, Vice President and Technical Director of the fragrance house Fritzsche Brothers Inc. in New York (now part of Givaudan)[50] listed a variety of constituents of distilled Florida grapefruit oil, such as α-pinene, (+)-limonene, linalool, citral, and probably geraniol and geranyl acetate, identified by classical methods [51]. Four years later, Justus G. Kirchner (1911–1987) and John M. Miller from the Fruit and Vegetable Chemistry Laboratory of the US Department of Agriculture in Pasadena, California, investigated the volatile components in grapefruit juice[52, 53] using the then new method of thin-layer chromatography[54, 55] without any major progress. Even the first attempts to decode the olfactory principle of grapefruits with the help of gas chromatography did not result in the hoped-for breakthrough [56–59]. However, this changed in 1963 when C.G. Beisel, V.E. Johnson, and P.A. Kustel from the American citrus growers' nonstock membership cooperative Sunkist Growers Inc.[60], now based in Valencia, California, observed a distinctly separated, unidentified peak in gas chromatograms of cold-pressed grapefruit oil, the area of which correlated with the intensity of the flavor. This observation ultimately led William D. MacLeod to identify this peak as nootkatone, which turned out to be the major component in determining the flavor of grapefruit (Fig. 4) [61]. Furthermore, nootkatone was also found in cold-pressed domestic lemon, lime, orange, and tangerine oils and imported bergamot oil but at

much lower concentrations. In grapefruit peel oil, the concentration of nootkatone ranges as high as 0.3%, while in other citrus peel oils, it is less than 0.01%.

Infobox: Grapefruit

The grapefruit tree is a member of citrus plants (*Citrus*), a genus of plants in the Rue family (Rutaceae) indigenous to tropical and subtropical Southeast Asia. Genetic, phylogenetic, and biogeographic analyses of citrus plants indicate that they originated from the southeastern foothills of the Himalayas, a region of present-day eastern Assam, northern Myanmar, and western China's Yunnan Province and that they split into distinct species as early as the Late Miocene epoch (11.6–5.3 million years ago) [62, 63]. Very early on, ancient civilizations used and domesticated various citrus species. The cultivation of citrus plants spread from there with the Austronesian expansion (c. 3000–1500 BCE) across Micronesia and Polynesia, as well as via the incense route to the Middle East and the Mediterranean region (c. 1200 BCE) and finally throughout Europe and the Americas [64, 65]. Citrus plants cultivated today can be traced back to only a few species, namely, the mandarin orange (*Citrus reticulata*), the pomelo (*Citrus maxima*), and the citron (*Citrus medica*), although these are also thought to have been bred by humans (Fig. 5) [66].

The history of grapefruits commences about 370 years ago on Barbados, an island in the Lesser Antilles of the West Indies [67]. The grapefruit is an accidental hybrid between a male sweet orange (*Citrus sinensis*) and a female pomelo (known as shaddock in the West Indies) (*Citrus maxima*). Legend has it that a certain Captain Philip Chaddock (sic) brought pomelo plants to Barbados between 1642 and 1650. According to a paper from David Watts [68] investigating *Man's influence on the vegetation of Barbados* from 1966, the first records of sweet oranges on Barbados date back to 1676, which seems reasonable because oranges have been known in Portugal since approximately 1550 and were dispersed over other European countries in 1646. Further evidence for both sweet oranges and pomelos being present on Barbados in 1687 come from a treatise *A Voyage to the islands of Madeira, Barbados, Nieves, St. Christophers, and Jamaica* authored by the Irish physician and naturalist Sir Hans Sloane (1660–1753) [69, 70]. In 1750, Reverend Griffith Hughes published *The natural history of Barbados* in 10 volumes [71]. In Book V, he describes a

Table 1 Constituents of the Nootka cypress heartwood essential oil obtained by steam distillation

Constituents of the oil	Percentage in the oil (% w/w)
Methyl carvacrol	1.33
Valencene	1.50
Terpinen-4-ol	2.08
Nootkatin	3.50
Nootkatol	5.20
Valencene-13-ol	6.35
Nootkatone	17.39
Nootkatene	20.08
Carvacrol	35.37
Unspecified	7.20

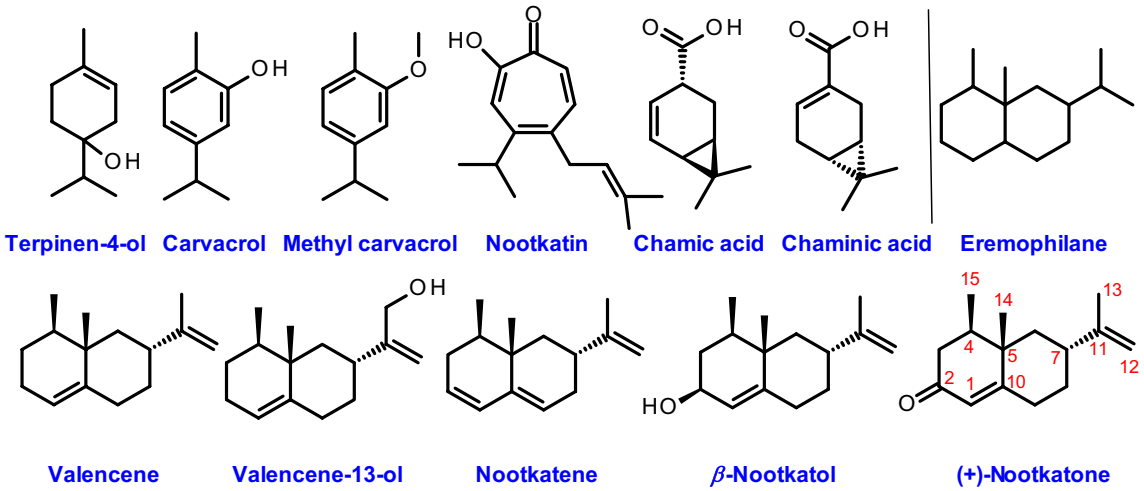


Fig. 3 Extractable constituents of the heartwood of *Callitropsis nootkatensis*

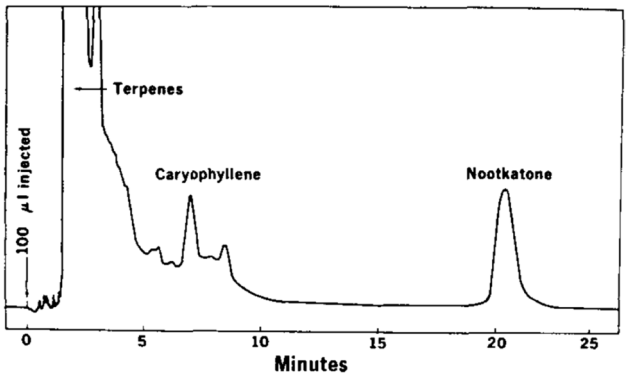


Fig. 4 Gas chromatogram of grapefruit oil on a 1/4-inch column at 215 °C (chromatogram taken from lit. ref. [61])

pyriform (i.e., pear-shaped) "forbidden fruit," which may have become extinct on the island. However, in November 1751 George Washington (1732–1799), in his *Barbadoes Diary* (1751–1752), mentioned a maliform (i.e., apple-shaped) "forbidden fruit" to be served at a dinner party he attended [72, 73]. Obviously, the fruits were grown on a larger scale and were readily available on the island at the time. The name Grape-fruit first appears in *Hortus Jamaicenensis* (1814) by John Lunan (1771–1839), a botanist, planter, and magistrate in Jamaica. He chose this name because of the similarity of both the taste and the way the maliform fruits grow on the tree (Fig. 6) [74]. He also realized how to breed grapefruits: "The best way is to take a stem or a twig and ingraft or inoculate it on a good China Orange stock."

Finally, the first comprehensive scientific description of grapefruit (*Citrus paradisi*) was published in 1830. Therefore, priority is attributed to James MacFadyen (1799–1850), a Scottish physician and botanist who made significant contributions to the systematic exploration of plants in the Caribbean in general (Fig. 7) [75, 76].

It was a mysterious French count, Odet Philippe (1785–1869), who brought the first grapefruit seedlings from the Caribbean to Florida. After his first attempts to settle on the east coast of Florida failed owing to resistance from the indigenous people, he moved to the west coast, to an area now known as Old Tampa Bay [77]. He acquired some land there and started growing Duncan grapefruits.

From these modest beginnings, it took some time for grapefruit to grow into a real large business. One of the first pioneers in the American grapefruit industry was Kimball C. Atwood (1853–1934). Originally active in the insurance business, at the age of 39 years, he recognized a new opportunity in grapefruit production and with a 265-acre grapefruit grove, he realized his American dream with The Atwood Grapefruit Co. at Manavista, Florida. In the best years, approximately 1927, the company produced 160,000 boxes of grapefruit, which were sold in the US and overseas.

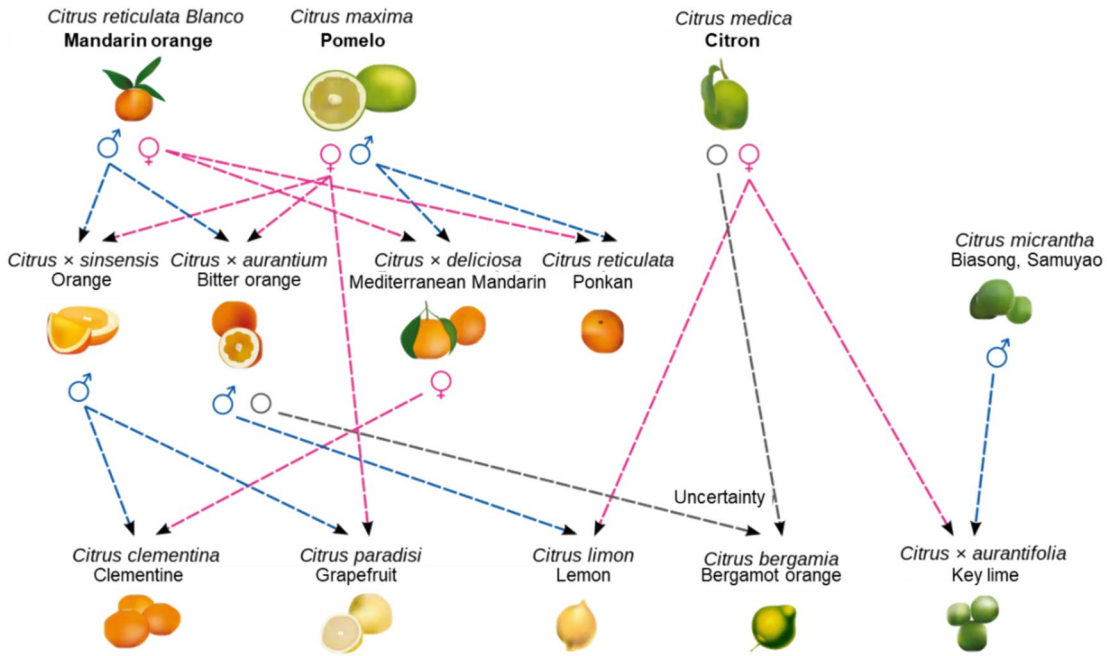


Fig. 5 Genetic relationships of several important species of citrus fruits (© Furfur)

Fig. 6 A bunch of Duncan grapefruits (© Jpbrigand2)

In 1907, an Atwood Co. foreman, R.B. Foster, picked a grapefruit from a Walters grapefruit tree to have it with his lunch. Upon cutting open the fruit, Foster discovered that the flesh of the grapefruit was not yellow but rather pink. He informed Atwood of this finding. However, the latter considered the discovery to be an anomaly and showed little interest. Foster thereupon sold his discovery to a local grapefruit farm, Royal Palm Nurseries at Oneco, Manatee County, Florida, which used it to breed new and improved grapefruit varieties with pink and red flesh (Figs. 8 and 9) [78].

In 1913, Sam A. Collins discovered a second type of grapefruit with red pulp, originating as a limb sport of a Marsh grapefruit tree in an orchard owned by W.B. Thompson at Oneco, Florida, and once again, the new variety was transferred to Royal Palms Nurseries for further development [79]. Thompson grapefruits became a great commercial success. A second bud sport of Marsh was discovered in 1936. This led to Shambar, which is now grown mainly in Argentina. Over the decades, further new breeding led to numerous improved and commercially successful cultivars of seedless, red grapefruits, highly valued for their sweet, pleasant taste [80, 81]. It may come as a surprise, but to date, virtually all grapefruit varieties grown in the US can be traced back to Odet Philippe's first Duncan grapefruit orchards in Florida (Fig. 10) [82].

Today, the USA is only the fourth largest producer of grapefruit and pomelos in terms of production volume. The market is clearly dominated by China, followed by Vietnam and Mexico [83]. In 2021, 9,556,999 tons of grapefruit and pomelos were produced worldwide. The top ten producing countries together generated 88.6% of the total harvest (Table 2).

Last but not least, it should be mentioned that the mysterious pyriform "forbidden fruit" of Reverend Hughes

Fig. 7 James MacFadyen (1799–1850) gave grapefruit its Linnean name, *Citrus paradisi*, borrowing from Hughes' "forbidden fruit" (portrait by Emilio Piani, © public domain)

was rediscovered in 1990 not on Barbados but on the neighboring island of Saint Lucia [84]. It is called Shaddette. Remarkably, Kim Bowman and Frederick Gmitter Jr. of the Department of Fruit Crops at the University of Florida found a distinctly different maliform pomelo variety on the island as well, eventually also called "forbidden fruit," which bears some resemblance to the fruit George Washington had for dinner.

Infobox: Chemical aspects of grapefruit

Grapefruits are also exciting from a chemical perspective. They contain several compounds with extraordinary properties.

Nootkatone Grapefruits are traded internationally, which implies storage for long periods at low temperature. This may be detrimental to the quality of the fruits and has serious economic implications. Consequently, monitoring quality deterioration during storage, transportation and marketing is an important objective of the citrus industry. The concentration of volatile components, such as nootkatone, can be used as a senescence indicator to determine the best postharvest treatment conditions and ensure optimum quality on the market [85].

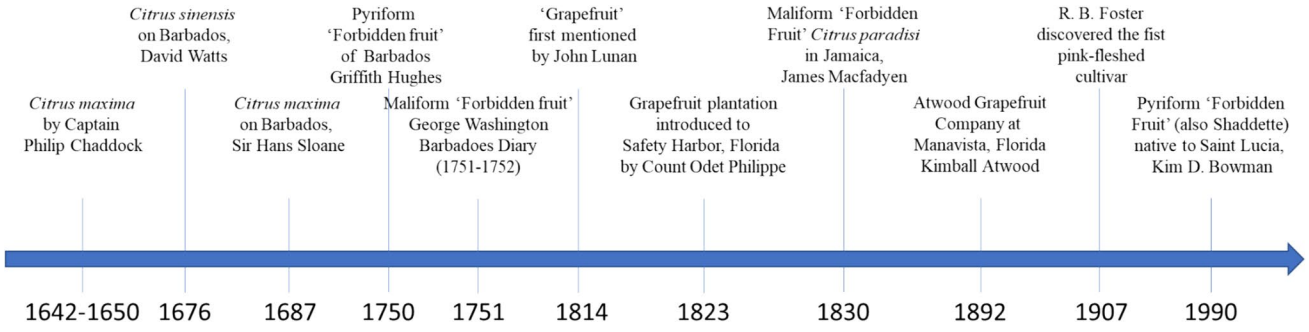


Fig. 8 The eventful history of the grapefruit began 350 years ago on Barbados

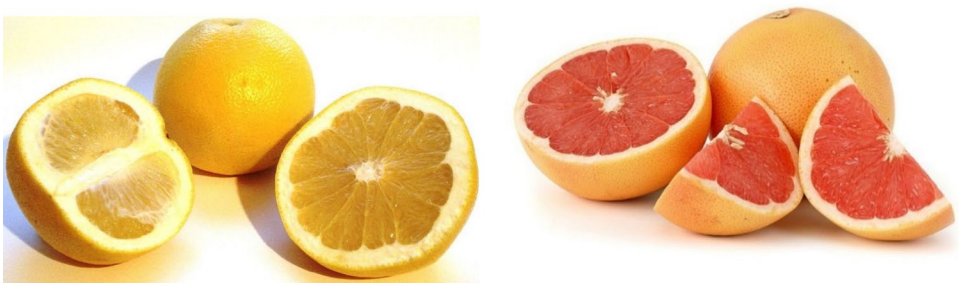


Fig. 9 Grapefruits (*Citrus paradisi*) with yellow and red pulp (© Genet, raeky)

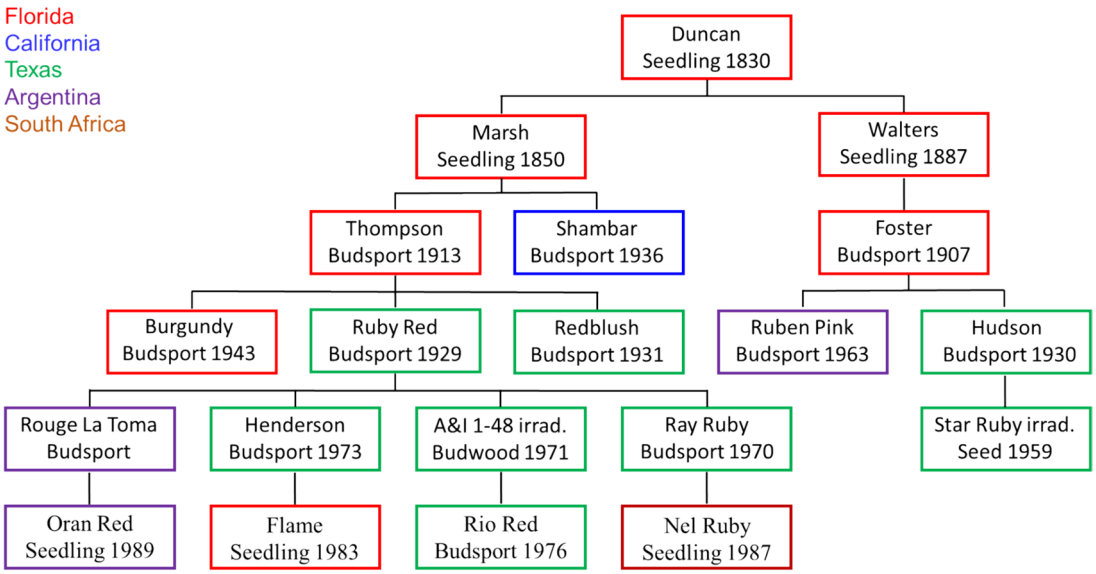


Fig. 10 The family tree of the American grapefruit, tracing the ancestry of some important commercial cultivars, including those from abroad [80]. Of these, the Flame, Rio Red, and Star Ruby varieties have the highest market shares

Carotenoids The serendipitous discovery of pink grapefruits naturally also aroused the interest of some chemists. In 1928, M.B. Matlack of the US Department of Agriculture in Washington reported that the colorant is lycopene [86]. A more detailed analysis then showed that in both Foster and Thompson grapefruits, β,β′-carotene also contributes to the shade of red color (Fig. 11)[87]. In fact, it turned out that practically all red grapefruit cultivars differ in the ratio of lycopene to β,β′-carotene (Table 3) [88].

Terpenes The processing of grapefruit yields peels on a large scale, from which the essential oil is obtained. This oil is widely supplied to the pharmaceutical, food, and cosmetic industries. A significant part is used for flavor-

Table 2 The largest producers of grapefruit and pomelos in 2021

Position	Country	Quantity (in tons)
1	China	5,200,000
2	Vietnam	1,034,680
3	Mexico	453,208
4	USA	386,460
5	South Africa	352,541
6	Sudan	277,875
7	Thailand	266,645
8	Turkey	249,000
9	Israel	128,974
10	Argentina	116,151
	Rest of the World	1,091,465
	Total	9,556,999

ing foods and to produce cosmetics, perfumes, soaps, and detergents.

The main component of grapefruit peel oil is (+)-limonene, some α-terpinene and a multitude of mono- and sesquiterpenes and some esters and aldehydes at low concentrations (Table 4, Fig. 12) [89, 90].

Grapefruit mercaptan In 1982, Edouard Demole and Günther Ohloff (1924–2005) at Firmenich also started investigating the flavoring compounds of grapefruit juice and oil [91]. They found that aside from nootkatone, (*R*)-1-*p*-menthene-8-thiol, later called grapefruit mercaptan, contributes significantly to the flavor of grapefruits [92]. Not surprisingly, the odors of the two enantiomers differ significantly. The taste detection threshold of the (*R*)-enantiomer in water is in the range of 2×10^{-5} ppb, which means that a miniscule amount of 2×10^{-5} mg of (*R*)-1-*p*-menthene-8-thiol in one metric ton of water can still be detected by taste. This ranks grapefruit mercaptan among the flavors with the lowest detection thresholds ever recorded (Fig. 13, Table 5).

Flavonoids The bitter taste of grapefruit is largely determined by flavanone glycosides, especially naringin. Its aglycone naringenin is bound to the disaccharide neohesperidose, consisting of one rhamnose and one glucose unit. Unlike naringin, naringenin has no bitter taste. The concentration of naringin in both, the fresh pink and white grapefruit peel extracts ranges from 140–160 mg/g grapefruit peel dry weight (Fig. 14) [95–97].

Interestingly, naringin can be converted into a sugar-sweet compound in a two-step one-pot reaction simply by low-pressure hydrogenation under alkaline conditions. The yield is virtually 100% over both steps (Scheme 1) [99]. The resulting naringin dihydrochalcone is 300 times sweeter than sucrose at the threshold level [100].

In the human body, metabolism occurs in the liver by stepwise glycolytic cleavage of naringin catalyzed by naringinase. The intermediate after removal of rhamnose is called prunin, which provides the flavorless aglycone naringenin after a second glycolytic step [102]. The enzyme naringinase is widespread in nature. It is not only found in mammals but also in plants, and fungi. It is also used for fruit juice debittering in industrial food processing [103].

In 1991, David G. Bailey from Victoria Hospital, University of Western Ontario, Canada, published a seminal paper in The Lancet on the interaction of citrus juices with felodipine and nifedipine. He and his collaborators reported that grapefruit juice, in contrast to orange juice, can have a dramatic impact on the bioavailability

Table 3 Descriptive lycopene/β,β′-carotene ratios in the juices of various red grapefruit cultivars

Cultivar	Lycopene (ppm)	β,β′-Carotene (ppm)	Ratio	Standard deviation
Ruby Red	1.51	1.19	1.27	0.6
Marsh Red	3.82	1.60	2.39	1.5
Flame	9.55	3.26	2.93	2.3
Star Ruby	13.62	2.76	4.93	1.9
Ray Ruby	7.84	1.39	5.64	1.7
Rio Red	11.27	1.80	6.26	1.8

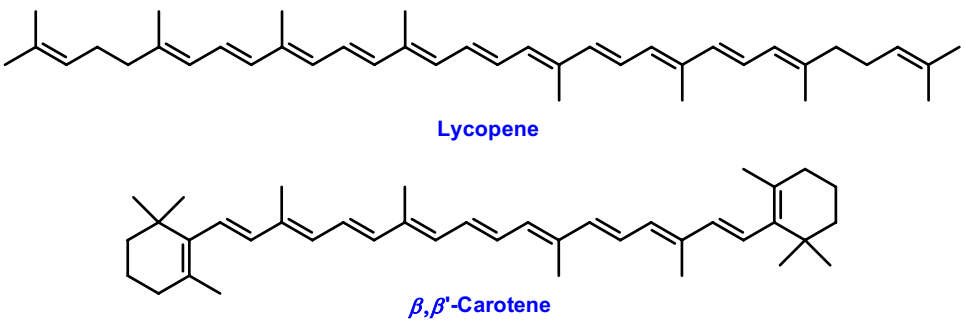


Fig. 11 Lycopene and β,β′-carotene are the main colorants of grapefruit juice

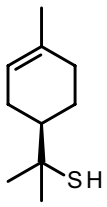
Table 4 Volatile constituents of Redblush grapefruit peel essential oil from Kenya

Compound	Concentration (%)
(+)-Limonene	91.1
α-Terpinene	1.3
α-Pinene	0.5
Heptyl acetate	0.5
(*Z*)-Carvone	0.4
Sabinene	0.4
cis-Carveol	0.3
Decanal	0.3
Octanal	0.3
trans-Carveol	0.2
(*E*)-*p*-Mentha-2,8-dien-1-ol	0.2
Citronellal	0.2
Nootkatone	0.2
Sabina ketone	0.2

of some drugs [104, 105]. Today, more than 85 active pharmaceutical ingredients (APIs) are known, which are impaired by grapefruit constituents. This is caused by different components of the grapefruit and afflict different pharmacological pathways.

After oral administration, some classes of drugs require so-called organic anion-transporting polypeptides (OATPs) to be absorbed in the gut. The attenuation of the transporter decreases the bioavailability of the drug. In 2002, David Bailey reported that naringin can dramatically reduce the bioavailability of the antihistamine pharmaceutical drug fexofenadine by inhibiting the human enteric organic anion-transporting polypeptide (OATP)1A2 [106, 107]. To date, a significant reduction in systemic exposure has been demonstrated for some β-blockers, quinolone antibiotics, and a direct renin inhibitor when administered concomitantly with fruit juice [108].

Fig. 13 Only the (*R*)-enantiomer of grapefruit mercaptan contributes significantly to the genuine, unmistakable flavor of fresh grapefruit juice [93]



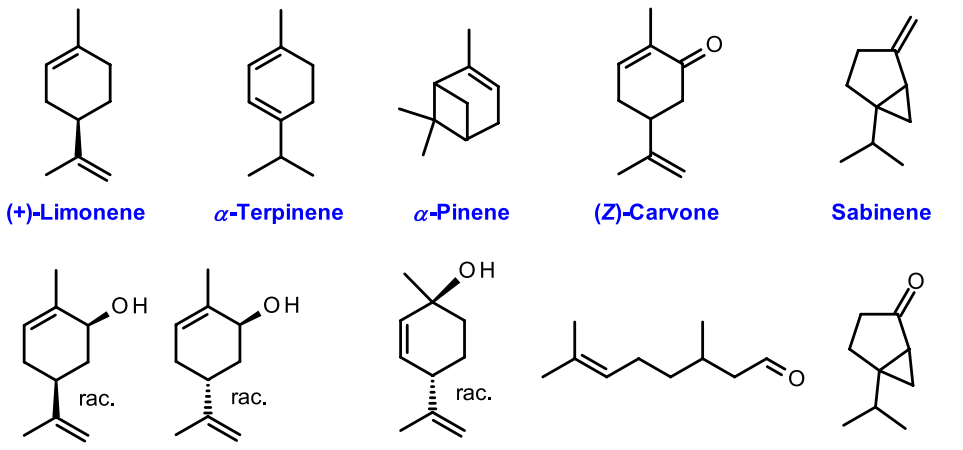
Grapefruit mercaptan
(*R*)-1-*p*-menthene-8-thiol

Furanocoumarins Well investigated is also the interaction of furanocoumarins, such as bergamottin and 6',7'dihydroxybergamottin, with the cytochrome P450 enzyme 3A4 (CYP3A4). Furanocoumarins, which are found in grapefruits and to a lesser extent in bitter oranges, pomelos, bergamots [109], and some limes but not in sweet oranges, are metabolized by CYP3A4 (Fig. 15). These metabolites bind covalently to the enzyme, which leads to irreversible inhibition. CYP3A4, which is expressed in the epithelial cells of the small and large intestine and in the hepatocytes of the liver, is a very important enzyme as it is involved in the metabolism of around 50% of all drugs. The drug dosing assumes normal CYP3A4 function. Accordingly, unintended inhibition of the enzyme leads to an overdose of the active substance [110].

Nootkatone from other plant sources

It is now known that (+)-nootkatone is formed by several other plant species and genera in addition to the Nootka cypress and various citrus species. As early as 1970, Niels H. Andersen from the University of Washington, Seattle, Washington discovered that vetiver oil, which is obtained by steam distillation from the roots of *Vetiveria zizanioides*, contains traces of (+)-nootkatone. More recently,

Fig. 12 Selection of compounds that constitute the essential oil of grapefruit peel



(+)-Limonene **α-Terpinene** **α-Pinene** **(*Z*)-Carvone** **Sabinene**

***cis*-Carveol** ***trans*-Carveol** **(*E*)-*p*-Mentha-2,8-dien-1-ol** **Citronellal** **Sabina ketone**

Table 5 Odor characteristics [93]

Compound	Taste detection threshold* (ppb)	Odor
(*R*)-limonene	35 (lit. [94])	Orange note
(*S*)-limonene	–	Turpentine note
(*R*)-1-*p*-menthene-8-thiol	2×10^{-5}	Grapefruit-like, strong impact
(*S*)-1-*p*-menthene-8-thiol	8×10^{-5}	Weak, nonspecific, nearly odorless

*In water

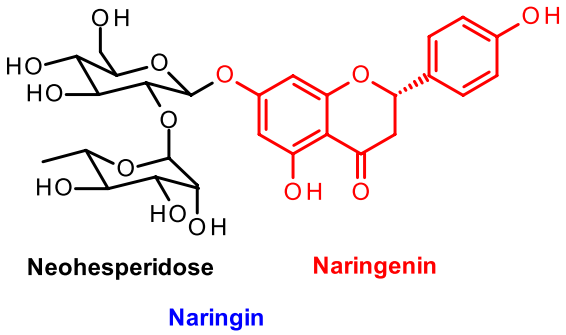
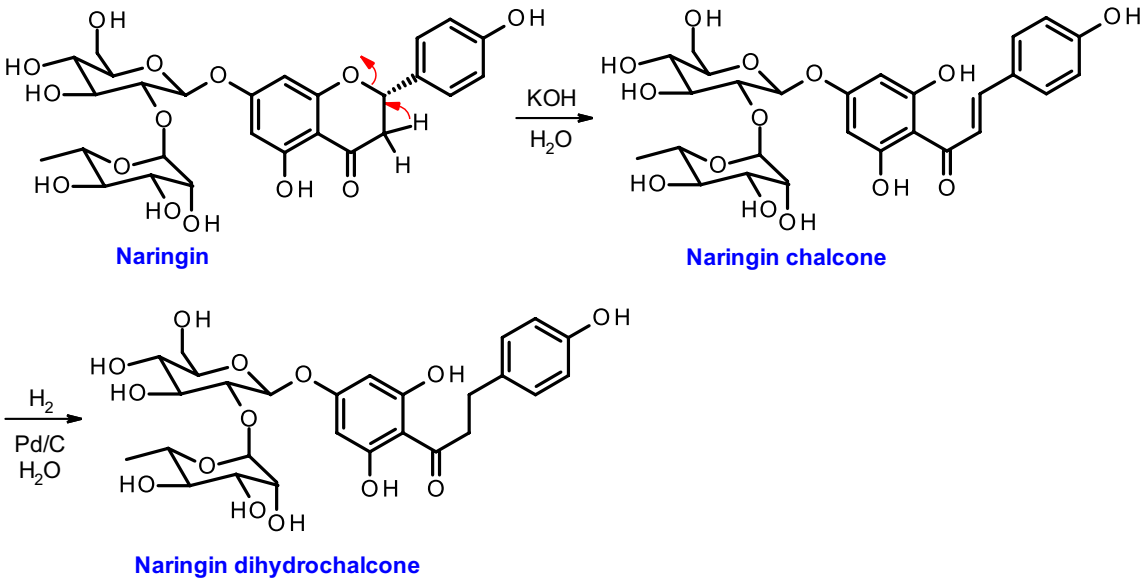


Fig. 14 The typical concentration of naringin in grapefruit juice concentrate is approximately 200 mg/100 g [98]

(+)-nootkatone was found in the fruits of sharp-leaf Galangal (*Alpinia oxyphylla*), a species of ginger native to East Asia, and in the rhizomes of species of the *Cyperus* genus, such as Nut grass, also known as Purple nut sedge (*Cyperus rotundus* L.), and Jointed flatsedge, also known as Prip-rioca (*Cyperus articulates* L.). In 2016, Félix Tomi from the Université de Corse-CNRS examined the wood oil of *Xanthocyparis vietnamensis* and found nootcatene (20.7%) and nootkatone (4.7%) among other eremophilane-sesquiterpenes. Furthermore, nootkatone is also a constituent of the heartwood of the Leyland cypress (×*Hesperotropsis leylandii*) and the Chinese juniper (*Juniperus chinensis* L.; Table 6, Fig. 16) [111].



Scheme 1 The flavanone-chalcone rearrangement is an equilibrium reaction that strongly depends on pH [101]

Fig. 15 Bergamottin is a typical furanocoumarin in grapefruit juice that inhibits cytochrome P450 enzymes

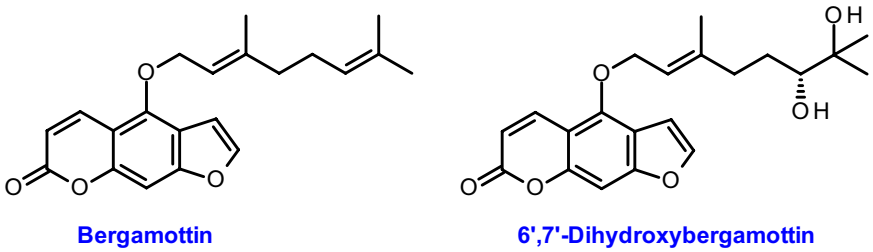


Table 6 (+)-Nootkatone is produced by various plant genera

Family	Genus	Species	Main source	Nootkatone in essential oil (%) [112]	Lit
Cupressaceae	*Chamaecyparis*	*Callitropsis nootkatensis* (D. Don) Spach	Heartwood	17.4	[45, 49]
	Leyland cyperus	×*Hesperotropsis leylandii* (A. B. Jacks. & Dallim.) Garland & Gerry Moore	Heartwood	8.2	[113]
	Xanthocyparis	*Xanthocyparis vietnamensis* (D. Don) Farjon & Harder	Wood oil		[114]
	Sabina chinensis	*Juniperus chinensis* L	Heartwood		[115]
Rutaceae	*Citrus*	*Citrus paradisi* Macf	Peel oil	0.2	[60]
		Citrus limon (L.) Burm	Peel oil		[60]
		Citrus sinensis (L.) Osbeck	Peel oil		[60]
		Citrus reticulata Blanco	Peel oil		[60]
Gramineae	*Vetiveria*	*Vetiveria zizanioides* (L.) Nash	Root oil		[116]
Zingiberaceae	*Alpinia*	*Alpinia oxyphylla* Miq	Fruits	9.0	[117, 118]
Cyperaceae	*Cyperus*	*Cyperus rotundus* L	Rhizome		[119, 120]
		Cyperus articulates L	Rhizome essential oil		[121]
Thymelaeaceae	*Daphne*	*Daphne oleoides* subsp. *oleoides*	Essential oil of aerial parts	18.5	[112]
Lamiaceae	*Anisomeles*	*Anisomeles indica* Kuntze	Leaf essential oil		[122]

Fig. 16 *Juniperus chinensis* L. Bonsai (© Peter coxhead), Vetiveria zizanioides (© treesftf), *Alpinia oxyphylla* Miq. (© Meneerke bloem), *Cyperus rotundus* (© public domain, Javier martin), *Daphne oleoides* (© gailhampshire from Cradley, Malvern, U.K.), and *Anisomeles indica* (© J.M.Garg) (from left to right)

Flavor of nootkatone

The olfactory properties of nootkatone were investigated by Herman G. Haring et al. from the Naarden Research Department in 1972 [123]. They found a significant difference between the taste threshold concentrations of natural (+)-(4*R*,4a*S*,6*R*)-nootkatone and artificial (−)-(4*S*,4a*R*,6*S*)-nootkatone in water, namely, 0.8 ppm versus 600 ppm, respectively. The odors of (+)-nootkatone and (−)-nootkatone are perceptible in air at threshold concentrations of 30 ppm and 66,000 ppm of saturated vapor, respectively. Likewise, the flavors of the enantiomers also differ. Although (+)-nootkatone embodies a strong grapefruit scent and has a bitter taste, (-)-nootkatone has a faint woody vetiver note and is virtually tasteless (Table 7) [124, 125].

By 1970, nootkatone became generally recognized as safe (GRAS) by the Flavor and Extract Manufacturers' Association (FEMA, FEMA No. 3166) and is frequently used in grapefruit beverages at concentrations of 2–6 ppm [126–128]. In combination with other citrus oils, such as bitter orange oil or bergamot oil, it imparts a pleasant citrus note.

Infobox: Fresca® from Coca-Cola

As early as in 1962, the *Coca-Cola Company* registered Fresca® as a "frozen concentrated orange juice and frozen concentrated tangerine juice" [129]. The tradename is borrowed from the Italian and Spanish word *fresca* (meaning "fresh") [130]. However, 5 years later, when put on the market, Fresca® became one of the first sparkling, sugar-free, grapefruit-flavored beverages (Fig. 17) [131]. Originally formulated with grapefruit oil, owing to supply fluctuations the main flavoring ingredient was apparently soon partially or completely replaced by nootkatone, without any negative impact on sales [132–134]. Meanwhile, Fresca® has been rebranded as "Fresca Sparkling Soda Water." It is also available with flavors other than grapefruit and is described as "the original no sugar, no calorie sparkling beverage" [135].

Fig. 17 The original Fresca bottle was designed by industrial designers, Hodgman-Bourke of New York, New York, around 1966. The groove under the logo panel was designed to catch condensation from the top half of the bottle; the dimples on the lower half are intended to represent carbon dioxide bubbles and improve grip (© WmArbaugh)

Insecticidal properties of nootkatone

However, nootkatone binds not only to olfactory receptors, such as the human olfactory receptors 10 and 52 (hOR10 and hOR52) [136], but also to receptors of the nervous system, which makes it an insect repellent and natural insecticide [137]. In 2020, nootkatone was registered by the US Environmental Protection Agency (EPA) for these applications on the basis of studies showing that nootkatone formulations can control mosquitoes [e.g., the Yellow fever mosquito (*Aedes aegypti*) and the Asian tiger mosquito (*Aedes albopictus*)], fleas [e.g., the Oriental rat flea (*Xenopsylla cheopis*)], ticks [e.g., the Deer tick (*Ixodes scapularis*), the Lone star tick (*Amblyomma americanum*), and the Blacklegged tick (*Ixodes scapularis*)], and a wide variety of other health-threatening vectors (e.g., mites, bed bugs, and lice) and pests [e.g., the Formosan subterranean termite (*Coptotermes formosanus*)] [138–140]. These findings have also made nootkatone a lead structure in the discovery of even more effective repellents and insecticides [141–143].

Nevertheless, the mode of action of nootkatone is still under investigation. According to the Bio-Pesticides Database of the University of Hertfordshire, it is thought that nootkatone may bind synergistically to the α-adrenergic-like type 1 octopamine receptor (Octα_1R) of invertebrates, causing fatal spasms [144, 145].

Infobox: Octopamine receptors

Octopamine was discovered by Vittorio Erspamer (1909–1999) in the salivary glands of the octopus at the University of Rome in 1948 [146, 147]. It is a widely distributed neurotransmitter in invertebrates, including arthropods, mollusks, annelids, echinoderms, and cnidarians. Chemically and pharmacologically, octopamine is

Table 7 Nootkatone is a white to yellowish crystalline solid with a melting point of 33 °C. The optical rotation is in the range of 184° to 196° [126]. The flavors of the enantiomers are very different

Enantiomer	Threshold concentration (in water; ppm)	Threshold concentration (sat. vapor phase in air; ppm)	Description
(+)-Nootkatone	0.8	30	Strong grapefruit odor Bitter in taste *Odor rating scale:* strongly fresh, green, sour; dusty-woody; animalerogenic, slightly herbal, fruity
(−)-Nootkatone	600	66,000	Weak woody (vetiver note) No grapefruit character Virtually no taste *Odor rating scale:* dusty-woody, spicy, slightly fresh, green, sour, herbal

related to norepinephrine (noradrenaline), the corresponding neurotransmitter in vertebrates [148]. In insects and crustaceans, octopamine affects complex behavior patterns (reproduction [149], social behavior [150], fight and flight responses [151], and food intake [152]) that can be as diverse in detail as, for instance, the glow of fireflies (Lampyridae) [153, 154], the excitability of muscle cells [155], and metabolic activity [156]. Octopamine is also found in vertebrates [157], but surprisingly also in numerous plants, e.g., grasses, such as the Annual Rye Grass (*Lolium multiflorum*) [158] and many species of the *Aesculus*, *Geranium*, *Pelargonium*, *Eucalyptus*, and *Citrus* genera [159–162]. As a component of nectar, octopamine, together with its metabolic precursor tyramine, influences the behavior of pollinators by altering their perception of rewards and their memory of floral signals, thus controlling the metabolic, sensory and cognitive basis of foraging (Fig. 18) [163].

The first octopamine receptors were discovered in the early 1970s [164, 165], and suggestions that octopamine might be a neurotransmitter followed soon [166, 167], but clinching proof was provided by Peter D. Evans, a young zoologist at Cambridge University [168, 169]. Over several decades, he has focused his research career on invertebrate neurochemistry and made numerous seminal contributions to the pharmacology of octopamine as well as other biogenic amines in the insect nervous system. As in the adrenergic system, a variety of subtypes of octopamine receptors are now known, which can be divided into four distinct classes (Table 8) [170–173]. No octopamine-specific receptors are known in vertebrates. Although octopamine does bind weakly to adrenergic receptors, it likely has no functional relevance [174, 175].

On the basis of phylogenetic studies, it is thought that all three types of monoamine signaling via the norepinephrine, tyramine, and octopamine receptors coexisted in ancient bilaterians 610 Mya [176]. Studying living fossils from the Protostomia and Deuterostomia clades, Philipp Bauknecht and Gáspár Jékely at the Max Planck Institute for Developmental Biology in Tübingen, Germany could identify and pharmacologically characterize *Platynereis dumerilii* and *Priapulus caudatus* (both Protostomes) adrenergic α_1 and α_2 receptors as well as octopamine α, octopamine β, tyramine type 1, and tyramine type 2 receptors. On the other hand, in *Saccoglossus kowalevskii* (Deuterostome), they found octopamine α, octopamine β, tyramine type 1, and tyramine type 2 receptors, coexisting with α_1 and α_2 receptors [148]. In the course of the evolution of species, of the six ancestral receptor families, the octopamine and tyramine receptors have been lost in most deuterostomes (including the phylum Cordata), and the adrenergic receptors have been lost in most ecdysozoans (a group of protostomes, including the phylum Arthropoda) [175].

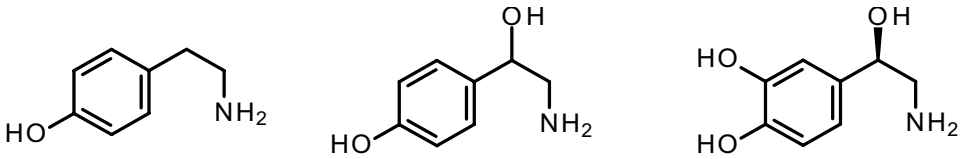
More recent studies suggest that nootkatone (also) acts antagonistically at the γ-aminobutyric acid (GABA) receptor. On the basis of molecular modeling, the structural similarity of nootkatone to picrotoxinin, a GABA antagonist known since the 1980s [177], suggested a comparable effect from the outset of the study (Fig. 19). Indeed, combined toxicological and neurophysiological investigations on *Aedes aegypti* and susceptible wild-type and insecticide-resistant *Drosophila melanogaster* strains demonstrated the efficacy at the GABA receptor [178]. Nevertheless, much more work needs to be done to explore the full toxicological profile of nootkatone.

Infobox: Indian berry (*Anamirta cocculus*) and picrotoxinin

Indian berry or Fish berry [*Anamirta cocculus* (L.) Wight & Arn., syn.: *Menispermum cocculus* L.] is the only plant species of the genus *Anamirta* in the "moonseed family" (Menispermaceae). It is native to South and Southeast Asia: India, Sri Lanka, Thailand, Indonesia, Papua New Guinea, and the Philippines. The stems and roots of these psychoactive plants contain various alkaloids, such as berberine, palmatine, magnoflorine, and columbamine. The poisonous picrotoxin can be extracted from the seeds, which was traditionally used in pharmacy, e.g., as an effective pediculicide (Figs. 20 and 21).

Pharmacological properties of nootkatone

In addition to its insecticidal properties, the pharmacological activities of nootkatone have been thoroughly studied since about 2010. Many studies have demonstrated its antimicrobial [188–190], antiviral [191, 192], anti-inflammatory [193, 194], and anticancer [195, 196] properties. Moreover, nootkatone appears to have cardiovascular [197, 198], neuro-, hepato-, and nephroprotective [199–202], as well as pulmonary [203] and testicular [204] protective effects. Finally, it may be an interesting lead structure in antiobesity [205], antidiabetic [206], anti-allergy [207], and immunomodulation[208] research [209, 210]. However, only the future will show whether these positive initial results can be turned into effective and safe drugs.



Tyramine **(+/-)-Octopamine** **(*R*)-(-)-Norepinephrine**

Fig. 18 Octopamine and related neurotransmitters

Table 8 Classes of octopamine receptors. Activation of a specific receptor subtype either by octopamine or tyramine leads to unique changes in cAMP and/or Ca^{2+} (OA: octopamine, TA: tyramine, cAMP: cyclic adenosine monophosphate)

Classes	Description	Subtype	Changes	Sensitivity to
OctαR	α-adrenergic-like	Octα$_1$R	↑ Ca^{2+} ↑ cAMP	OA > TA OA > TA
		Octα$_2$R	↑ Ca^{2+} ↓ cAMP	OA ≥ TA OA > TA
OctβR	β-adrenergic-like	Octβ$_1$R	↑cAMP	OA > TA
		Octβ$_2$R	↑cAMP	OA > TA
		Octβ$_3$R	↑cAMP	OA > TA
OAMB	Mushroom body octopamine receptor	OAMB-AS	↑ Ca^{2+}	OA
		OAMB-K3	↑ Ca^{2+} ↑cAMP	OA
TyrR	Mixed octopamine/tyramine receptors	Tyr1R	↑ Ca^{2+} ↓ cAMP	OA ≥ TA TA > OA
		Tyr2R	↑ Ca^{2+}	TA
		Tyr3R	↑ Ca^{2+} ↓ cAMP	TA > OA TA > OA

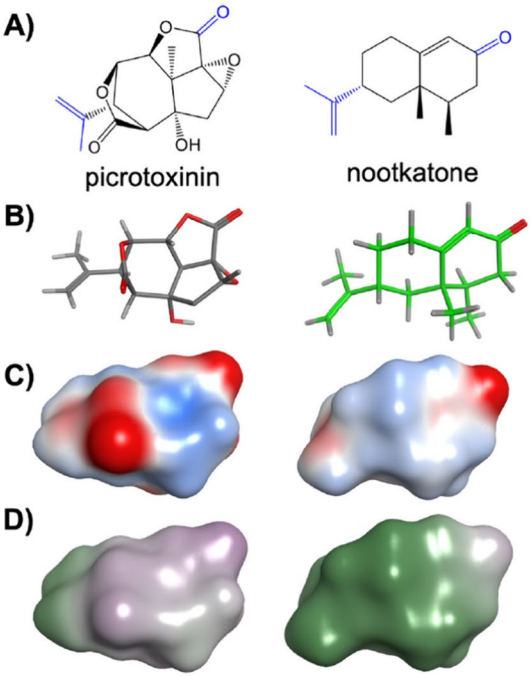


Fig. 19 Molecular modeling overlay of picrotoxinin and nootkatone showing structural similarities. **A** Chemical depiction of the structures with common moieties shown in blue; **B** three-dimensional (3D) optimized structures showing picrotoxinin (gray) and nootkatone (green) and the overlay between the two molecules. A hydrophobic isopropenyl moiety and ketone moiety are shared between the two compounds; **C** electrostatic surface of the two compounds with blue showing positive areas and red showing negative areas; **D** lipophilic surface of the two compounds with the hydrophilic areas shown with purple and lipophilic areas shown with green (© Elsevier)

Fig. 20 The fruits were also used for the preparation of pesticides and for fishing to stun and catch fish (hence "fish berries"). Moreover, cases have also been reported in which picrotoxin has been misused for performance enhancement in equestrian sports ([179] © Vinayaraj)

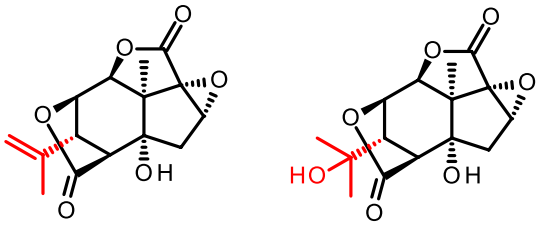


Fig. 21 Picrotoxin refers to an equimolar mixture of the sesquiterpene lactones picrotoxinin and picrotin from *Anamirta cocculus*. Picrotoxin was first isolated from the fruits of the plant in 1812 by the French chemist and pharmacist Pierre-François-Guillaume Boullay (1777–1869). Unlike the nontoxic picrotin, picrotoxinin is highly toxic to humans, with doses as low as 10 mg [180] being as toxic as strychnine [181, 182]. Picrotoxinin acts as a noncompetitive $GABA_A$ receptor antagonist, probably not at the GABA recognition site but perhaps within the ion channel [183, 184]. Since GABA (γ-aminobutyric acid) is an inhibitory neurotransmitter, picrotoxinin has a stimulatory effect. Picrotoxin was previously used as an antidote to barbiturate poisoning [185]. A new treatment option for Meniere's disease is picrotoxin [186]. Poisoning with picrotoxinin is treated with diazepam and, if necessary, barbiturates such as phenobarbital [187]

Syntheses of nootkatone

Since the discovery of nootkatone by Gunhild and Holger Erdtman in 1962, this sesquiterpene has attracted the interest of many synthetic chemists. Accordingly, a plethora of approaches have been published over the last 60 years, which can generally be divided into two groups. One involves methods for the oxidation of valencene (Table 9), the other total syntheses (Table 10).

Infobox: Valencene

(+)-Valencene [210] is a characteristic aromatic compound in some citrus fruits, such as sweet oranges (e.g., Valencia oranges) and grapefruits, and to some extent in mandarins (*Citrus reticulata* Blanco). It is also found in the heartwood of the Nootka cypress (*Callitropsis nootkatensis*), Java grass (*Cyperus rotundus*), *Xanthocyparis vietnamensis*, *Lantana montevidensis*, *Lantana camara*, Baboon wood (*Virola surinamensis*), Yangmei (*Myrica rubra*), *Stachys macrostachya*, *Salvia bracteate*, and in notable concentrations in dried Lemon balm (*Melissa officinalis*) from Anatolia (> 20%). Its organoleptic properties are described as sweet, fresh, citrus, grapefruit, woody, orange, dry, green, and oily (odor) and orange, citrus, fruity, juicy, citrus peel, peely, and woody (taste) [211]. Valencene is mainly obtained by extraction and steam distillation from citrus fruits, e.g., from the peel of Florida oranges. The production volumes are in the order of 15 t/a, with approximately 5 t/a being converted into nootkatone [210, 212].

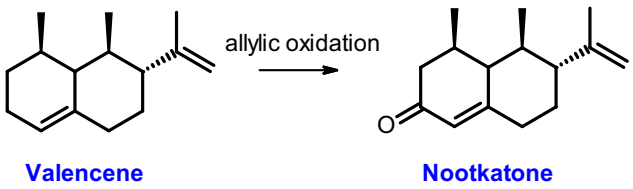
Oxidation of valencene

Valencene is a privileged starting material that has been frequently used to obtain nootkatone by allylic oxidation. The first report on this transformation originates from G.L.K. Hunter and W.B. Brogden Jr. of the Fruit and Vegetable Chemistry Laboratory of the US Department of Agriculture in Winter Haven, Florida. They used di-*t*-butyl chromate to obtain nootkatone in 67% yield (Scheme 2, Table 9, entry 1). Since then, more than ten different methods have been published to achieve this goal in mediocre to good yields. Only a selection of these approaches is considered in more detail here.

In 2002, Jorge A.R. Salvador and James H. Clark at the University of York used Co(II) and Cu(II) complexes on mesoporous silica to oxidize valencene with *t*-butyl hydroperoxide to obtain nootkatone in 75% yield under optimized conditions (Table 9, entry 5).

In a much-noticed paper, Véronique Nardello-Rataj and coworkers from the Université Lille, together with Paul L. Alsters from DSM, reported a one-pot synthesis of (+)-nootkatone via dark singlet oxygenation of valencene. Thereby the amphiphilic bis(dimethyldioctyl)ammonium molybdate catalyst has three functions: (1) generating singlet oxygen from hydrogen peroxide, (2) dehydrating the resulting hydroperoxide, and (3) acting as a "balanced catalytic surfactant" facilitating a three-phase-microemulsion system. On a 510 mg scale, the authors obtained nootkatone in 47% yield (Table 9, entry 9).

In the same year, Phil S. Baran and coworkers at the Scripps Research Institute at La Jolla, California, succeeded



Scheme 2 Allylic oxidation of valencene leads to nootkatone

Table 9 A selection of syntheses of nootkatone from valencene

Entry	First/Corr. Author (Com.)	Publ. Year	Key steps/Strategy	Stereo-chemis-try	Number of Steps	Overall yield (%)	Refs
1	G.L.K. Hunter	1965	By oxidation with di-*t*-butyl chromate	(+)	1	67	[213]
2	William G. Dauben	1969	By oxidation with CrO_3, pyridine	(+)	1	81	[214]
3	Günther Ohloff (Firmenich)	1971	$^1O_2$ oxidation	(+)	1	Not provided	[215]
4	Charles W. Wilson, III	1978	By oxidation with t-butyl peracetate and potassium dichromate	(+)	3	47	[216]
5	James H. Clark	2002	By oxidation with *t*BuOOH and a hetero-geneous Cobalt-catalyst	(+)	1	75	[217]
6	Jorge A.R. Salvador	2007	By allylic oxidation with $NaClO_2$, *t*BuOOH	(+)	1	38	[218]
7	Francisco M. Guerra	2014	By allylic oxidation with *t*BuOOH and a copper – aluminum mixed oxide catalyst	(+)	1	60	[219]
8	Yuanhua Wang	2015	By allylic oxidation with *t*BuOOH and a Rh catalyst	(+)	1	71	[220]
9	Véronique Nardello-Rataj	2016	By dark$^1O_2$ oxidation (H_2O_2, molybdate catalyst)	(+)	1	47	[221]
10	Phil S. Baran	2016	Electrochemical oxidation, mediator: tetrachloro-*N*-hydroxyphthalimide	(+)	1	77	[222, 223]
11	Yuanhua Wang	2019	By allylic oxidation with air and a Rh catalyst, *N*-hydroxyphthalimide	(+)	1	70	[224]
12	Soufiane El Houssame	2020	By allylic oxidation with *t*-BuOOH and Co-Ag/SiO_2	(+)	1	87	[225]
13	Gilson DeFreitas-Silva	2021	By allylic oxidation with oxygen and a Mn porphyrin catalyst	(+)	1	64	[226]
14	Kang Zhou, Jie Wu	2022	By 4CzIPN-catalyzed photooxidation	(+)	1	65	[227]

in synthesizing nootkatone by the anodic oxidation of valencene in 77% yield by applying tetrachloro-*N*-hydroxyphthalimide as a mediator and *t*-butyl hydroperoxide as a terminal oxidant (Table 9, entry 10).

The highest yield of all known oxidation methods was reported by Soufiane El Houssame from the Université Sultan Moulay Slimane at Khouribga, Morocco, in 2020. Using *t*-butyl hydroperoxide and a Co-Ag catalyst supported on SiO_2, they obtained nootkatone in 87% yield (Table 9, entry 12).

Nootkatone can also be obtained by photoredox catalysis, as reported by Kang Zhou and Jie Wu from the National University of Singapore. Aerobic oxidation of valencene in the presence of 4CzIPN and *N*-hydroxy phthalimide as cocatalysts, and irradiation with 456 nm LEDs provided a mixture of nootkatone and *cis*-nootkatol in 65% and 15% yield, respectively (Table 9, entry 14).

Infobox: Allylic oxidation

The allylic oxidation of olefins is a strategic transformation in the total synthesis of natural products [228]. Accordingly, since the first chromium- and selenium-based oxidation procedures [229, 230], a variety of methods have been developed, which now also include Cu-, Rh-, Co-, Ru-, Pd-, and Fe-agents, as well as organo- and biocatalytic processes [231].

Total syntheses of nootkatone

On the other hand, nootkatone has also proven to be an attractive target for total synthesis. The first was published by Peter Schudel et al. from Givaudan in 1968. Key step of his approach is a Robinson-type annulation to establish the hexahydronaphthalene-2-one framework which has subsequently featured in almost half of all known syntheses of nootkatone (Table 10). A few of these are presented below.

Infobox: Robinson annulation

The Robinson annulation, a one-pot cascade reaction involving a Michael addition and an Aldol condensation[256], was first described in 1935 by William Sage Rapson (1912–1999), a student from New Zealand working with Robert Robinson, a professor of chemistry at Oxford University, and has been widely used in natural product synthesis ever since (Fig. 22) [257]. Prominent examples include the total synthesis of cortisone by Robert Robinson himself [258] and, of course, the enantioselective, proline-catalyzed syntheses of the Wieland-Miescher and Hajos-Wiechert ketones by chemists at Hoffmann-La-Roche and Schering (Hajos-Parrish-Eder-Sauer-Wiechert Robinson annulation) [259–261], which paved the way for the contemporary field of organocatalysis [262, 263]. More recent applications of the Robinson annulation can be found in the total synthesis of abscisic acid [264], artemisinin [265], platensimycin [266], morphine [267], galantamine [268], and in a multitude of syntheses of nootkatone.

Fig. 22 In 1947, the British organic chemist Sir Robert Robinson (1886–1975) was awarded the Nobel prize in chemistry for his investigations on plant products of biological importance, especially the alkaloids. He is known for the Robinson annulation [256], the Robinson-Gabriel synthesis [269, 270], and the Allan-Robinson reaction [271]. Robinson earned fame for his contributions to total synthesis, e.g., of tropinone (arguably the first cascade reaction) [272], cholesterol [273], and diethylstilbestrol [274]; the elucidation of the molecular structures of codeine, thebaine [275], and penicillin [276], but also for his concept of arrow pushing (bent arrow to represent electron movement) [277] and biomimetic synthesis [272]. In 1957, he cofounded *Tetrahedron* for Pergamon Press

The total synthesis of racemic nootkatone from Mario Pesaro, Giuliano Bozzato, and Peter Schudel commences with 4-acetyl-1-ethoxycyclohexene (Table 10, entry 1). After a Wittig reaction with methyltriphenylphosphonium iodide in THF and hydrolysis of the enolether, 4-isopropenylcyclohexanone is obtained. Condensation with ethyl formate and methylation provide a mixture of stereoisomers of aldehydes and enolethers, which were subjected to a Robinson-type annulation with methyl acetoacetate. Re-esterification with diazomethane and purification by chromatography affords a racemic mixture of dienones, which are reacted with the Gilman reagent lithium dimethylcuprate [278] to provide methyl *epi*-nootkatone carboxylate. The final steps include desaturation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), stereoselective reduction with sodium boranate, and finally saponification and decarboxylation. The authors did not report any yields but found that the properties of their product were identical to those of natural nootkatone (Scheme 3).

A 1971 paper in Recueil des Travaux Chimiques des Pays-Bas of A. van der Gen, M. van der Linde, J.G. Witteveen and H. Boelens from the famous Naarden Research Department in Naarden, the Netherlands, features the first and only total synthesis of enantiopure (−)-nootkatone (Table 10, entry 6). Starting from (+)-sabinene, ozonolysis and condensation with (piperidin-1-yl)methanol leads to an enone that is hydrogenated in the presence of a Pt/C catalyst. Equilibration of the stereocenter at position 3 with KOH in methanol at 20 °C provides a mixture of diastereomers in a ratio of 3*R*:3*S* = 9:1. This is followed by a diastereoselective Robinson annulation with *trans*-3-penten-2-one. The authors attribute the stereoselectivity of the reaction to the steric hindrance by the cyclopropane ring and the additional stabilization of the transition state by the interaction of the enolate oxygen with the pentenone carbonyl group, which eventually leads to the desired *syn*-dimethyl configuration. The subsequent acidic cleavage of the cyclopropane ring proceeds without significant impairment of the stereochemical integrity at position 7. Finally, (−)-nootkatone is obtained by treatment with sodium acetate in glacial acetic acid in an overall yield of 19.4% (Scheme 4).

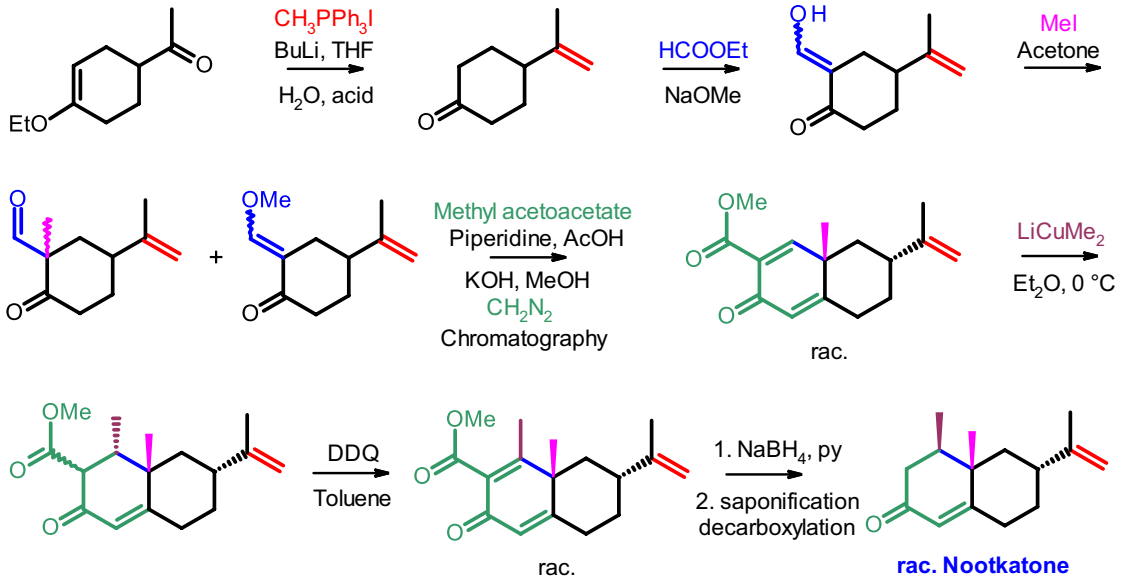
A landmark synthesis of enantiomerically pure (+)-nootkatone, which also influenced other work, was published in 1980 by Tetsuji Yanami, Masaaki Miyashita, and Akira Yoshikoshi of Tohoku University in Sendai, Japan (Table 10, entry 11). Their strategy avoided the Robinson annulation as a key step but targeted a central intermediate **A**, which offered some new retrosynthetic opportunities but also posed new challenges. This involved the establishment of a quaternary and a second adjacent stereocenter with two methyl groups in the syn configuration, an intramolecular aldol condensation and finally the cleavage of the cyclobutane ring (Fig. 23).

Table 10 Selection of total syntheses of nootkatone

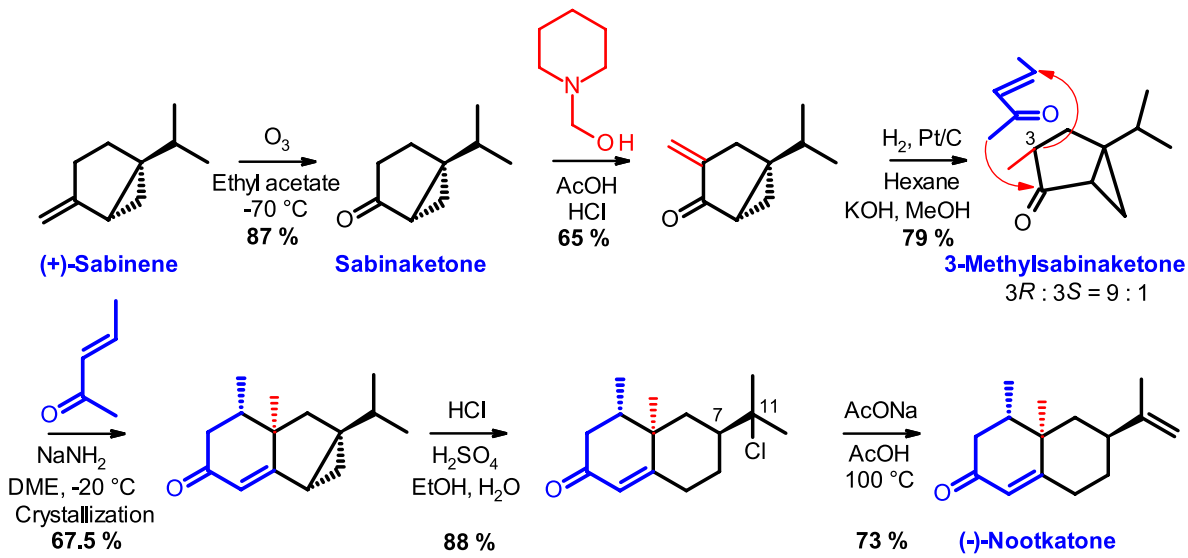
Entry	First/Corr. Author (Com.)	Publ. Year	Key steps/Strategy	Stereo-chemis-try	Number of Steps[#]	Overall yield (%)	Refs
1	Peter Schudel (Givaudan)	1968	From 4-acetyl-1-ethoxy-cyclohexene by *Robinson*-type annulation	rac	8	Not provided	[232]
2	Hiroshi Hikino	1968	From kusunol by *allylic oxidation*	(+)	3	Not provided	[233]
3	Robert M. Coats	1970	By *Robinson* annulation from 2-methylcyclo-hexane-1,3-di-one	rac	9 to valencene	1.5	[234, 235]
4	James A. Marshall	1970	From dimethyl γ-ketopimelate by *Robinson* annulation	rac	10	4.0	[236, 237]
5	A. van der Gen	1971	From (−)-β-pinene by *Robinson* annulation	rac	7	4.0	[238]
6	A. van der Gen	1971	From (+)-sabinene by *Robinson* annulation	(−)	6	19.4	[239]
7	K.P. Dastur, Arthur J. Birch	1973	From 3,4,5-trimethylanisole by *Birch and Diels–Alder* reaction	rac	8	6.1	[240, 241]
8	A. Reginald Pinder	1974	*Robinson* annulation of 4,4-eth-ylenedioxy-2-methylcyclohex-anone	rac	9	Not provided	[242]* [243]
9	Yoshikazu Takagi	1978	From (+)-2-methyl-4-isoprope-nylcyclohexanone by *Robinson* annulation	(+)	5	0.4	[244]
10	Tamejiro Hiyama	1979	From 4-methoxycarbonyl-2-methylcyclohexanone by *cyclopentenone annulation*	rac	8	27.1	[245]
11	Akira Yoshikoshi	1980	From (1*R*)-(+)-nopinone by *Hosomi–Sakurai reaction and aldol condensation*	(+)	6	14.1	[246]
12	Tamejiro Hiyama	1981	By *cyclopentenone annulation*	rac	8	20.8	[247]
13	Sigeru Torii	1982	By *functionalization* of an octalin-1-one derivative	rac	14	15.3	[248]
14	Sigeru Torii	1982	From (1*R*)-(+)-nopinone by *aldol* condensation	(+)	16	17.7	[249]
15	George Majetich	1985	By intramolecular *Sakurai* reac-tion	rac	5	5.2	[250]
16	Roger A. Laine ref. to Akira Yoshikoshi (entry 11)	2009	From (−)-β-pinene by *Grignard/anionic oxy-Cope* reactions	(+)	8	33.7	[251, 252]
17	D. Srinivasa Reddy	2013	From (*Z*)-nona-6,8-dien-2-one by sequential *Diels–Alder/aldol* reactions, protecting group-free synthesis	rac	4	14.0	[253, 254]
18	Roger A. Laine	2019	From (−)-β-pinene by ozonolysis, *Grignard/anionic oxy-Cope* reactions	(+)	8	15.3	[255]

[#]Starting from commercially available raw materials

*The publication was withdrawn by the authors at the 158th A.C.S. National Meeting. New York, September 1969, until clarification of the non-reproducible stereochemical result of the key step in their synthesis [237]



Scheme 3 Total synthesis of racemic nootkatone by Mario Pesaro, Giuliano Bozzato, and Peter Schudel, 1968



Scheme 4 Total synthesis of (−)-nootkatone by A. van der Gen, M. van der Linde, J.G. Witteveen and H. Boelens, 1971

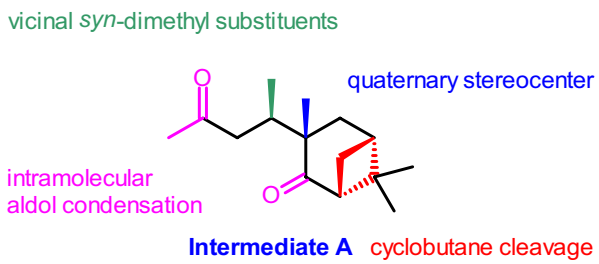


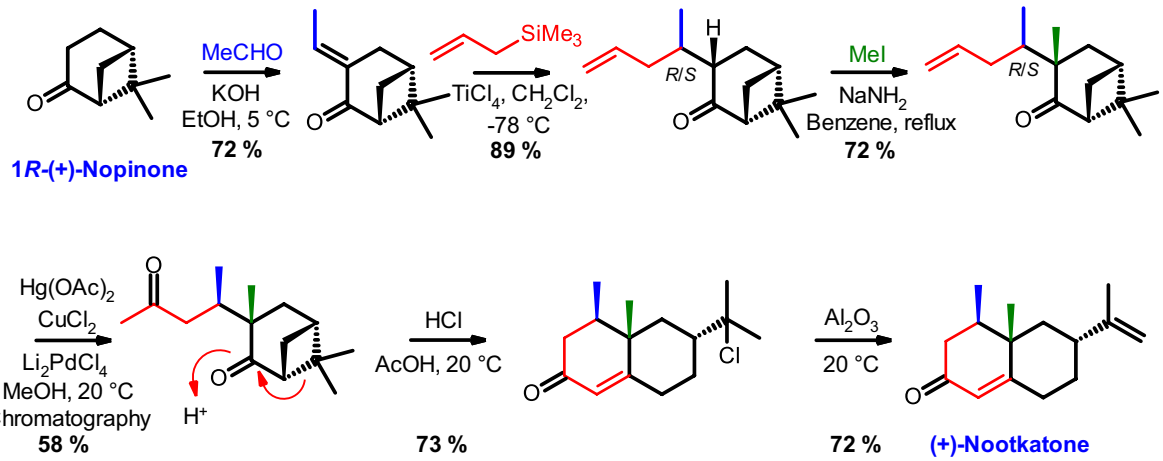
Fig. 23 Challenges of intermediate **A** of the synthesis by Akira Yoshikoshi et al.

The crossed aldol condensation of 1*R*-(+)-nopinone with acetaldehyde leads to ethylidene nopinone, which is subjected to a stereoselective Hosomi–Sakurai reaction [279], and subsequently to stereoselective methylation, introducing a methyl group at position 5 with the desired configuration. Oxidative oxymercuration/demercuration provides an 81:19 mixture of diones, which can be separated by chromatography. Cascade acid-catalyzed cyclobutane ring cleavage/intramolecular aldol condensation and finally elimination of

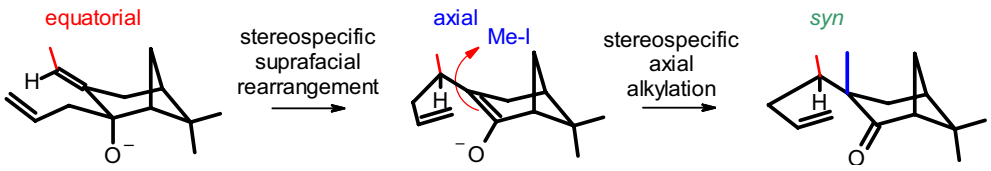
hydrogen chloride afforded (+)-nootkatone in 14.1% overall yield (Scheme 5).

The best synthesis of enantiomerically pure (+)-nootkatone, both in terms of average step yield and overall yield, was reported in 2009 by Anne M. Sauer, William E. Crowe, Gregg Henderson, and Roger A. Laine from Louisiana State University, on the basis of the route of Yoshikoshi et al. (Table 10, entry 16). Conceptually, their approach differs from that of Yoshikoshi et al. mainly in that they wanted to establish the stereocenters at positions 4 and 5 by a cascade Cope rearrangement/methylation reaction (Scheme 6).

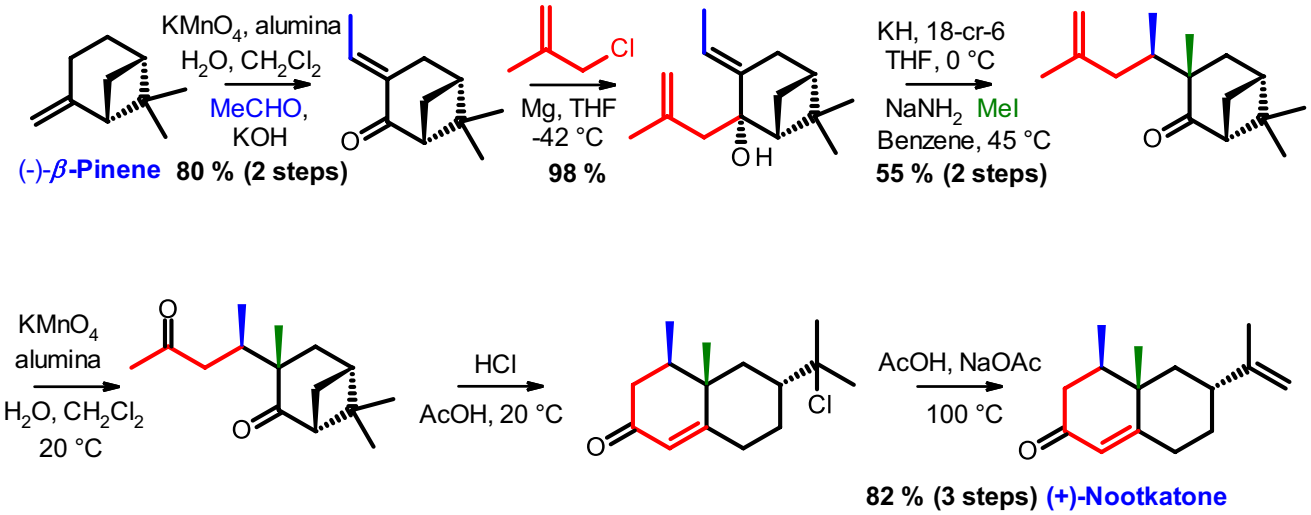
Ethylidene nopinone, the first intermediate in Laine's synthesis, is available in two steps from (−)-β-pinene by permanganate oxidation and crossed aldol condensation. At low temperature, the Grignard reaction with methallyl magnesium chloride delivered the tertiary alcohol with remarkable stereoselectivity (> 20:1) and yield. However, the anionic Cope rearrangement required some optimization. When performed at ambient temperature a retro-ene reaction was observed, leading to considerable fragmentation (i.e., loss of the methallyl moiety). By decreasing the temperature, this problem could be solved to some extent. The original plan to combine the sigmatropic rearrangement reaction with methylation failed owing to the unexpected O-alkylation and was ultimately abandoned. However, employing Yoshikoshi's protocol worked as expected. The following steps are also very similar to the synthesis described previously. The use of methallyl chloride in the Grignard reaction made it possible to avoid the use of mercury reagents. The conditions of the last step are very similar to those of the synthesis by H. Boelens et al. Overall, the yield over eight steps amounts to a remarkable 33.7%, which makes this route outstanding despite all the borrowings (Scheme 7).



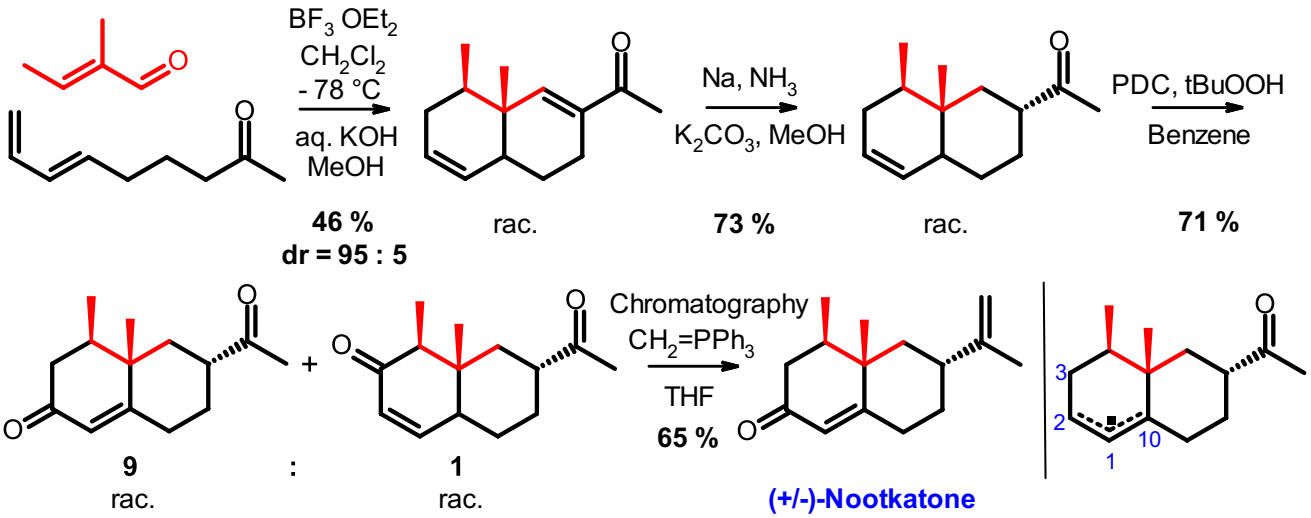
Scheme 5 Total synthesis of (+)-nootkatone by Tetsuji Yanami, Masaaki Miyashita, and Akira Yoshikoshi, 1980



Scheme 6 Key step in the synthesis of (+)-nootkatone by Roger A. Laine et al.



Scheme 7 Total synthesis of (+)-nootkatone by Anne M. Sauer, William E. Crowe, Gregg Henderson, and Roger A. Laine, 2009



Scheme 8 Total synthesis of racemic nootkatone by Kishor L. Handore, B. Seetharamsingh, and D. Srinivasa Reddy, 2013

Considering that nona-6*E*,8-dien-2-one [280] and tiglic aldehyde [281] are commercially available, the four-step total synthesis of racemic nootkatone by Kishor L. Handore, B. Seetharamsingh and D. Srinivasa Reddy of the CSIR-National Chemical Laboratory in Pune, India, represents the shortest total synthesis published to date (Table 10, entry 17). A cascade Diels–Alder reaction/Aldol condensation in the first step directly afforded the main nootkatone framework with a *syn*-dimethyl configuration (diastereomer ratio 95:5). Chemoselective reduction of the enone with sodium in liquid ammonia delivered a 1:1 mixture of diastereomers, which, upon exposure to potassium carbonate in methanol, provided the pure racemate. Interestingly, oxidation with pyridinium dichromate/tBuOOH yielded a mixture of two regioisomers, the main product of which is presumably formed by a preceding double bond migration via an allyl radical at position 10-1-2 [282]. It could be purified by chromatography and finally converted to racemic nootkatone by a Wittig reaction (Scheme 8).

Biosynthesis

Another approach to (+)-nootkatone is by extraction from natural sources (e.g., grapefruit peel) [210], fermentation, and enzymatic oxidation. The basis for all these methods is biosynthesis, which branches off from the central terpene biosynthesis pathway at the stage of the sesquiterpene farnesyl diphosphates. The main intermediates in this three-step downstream synthesis are valencene and nootkatol.

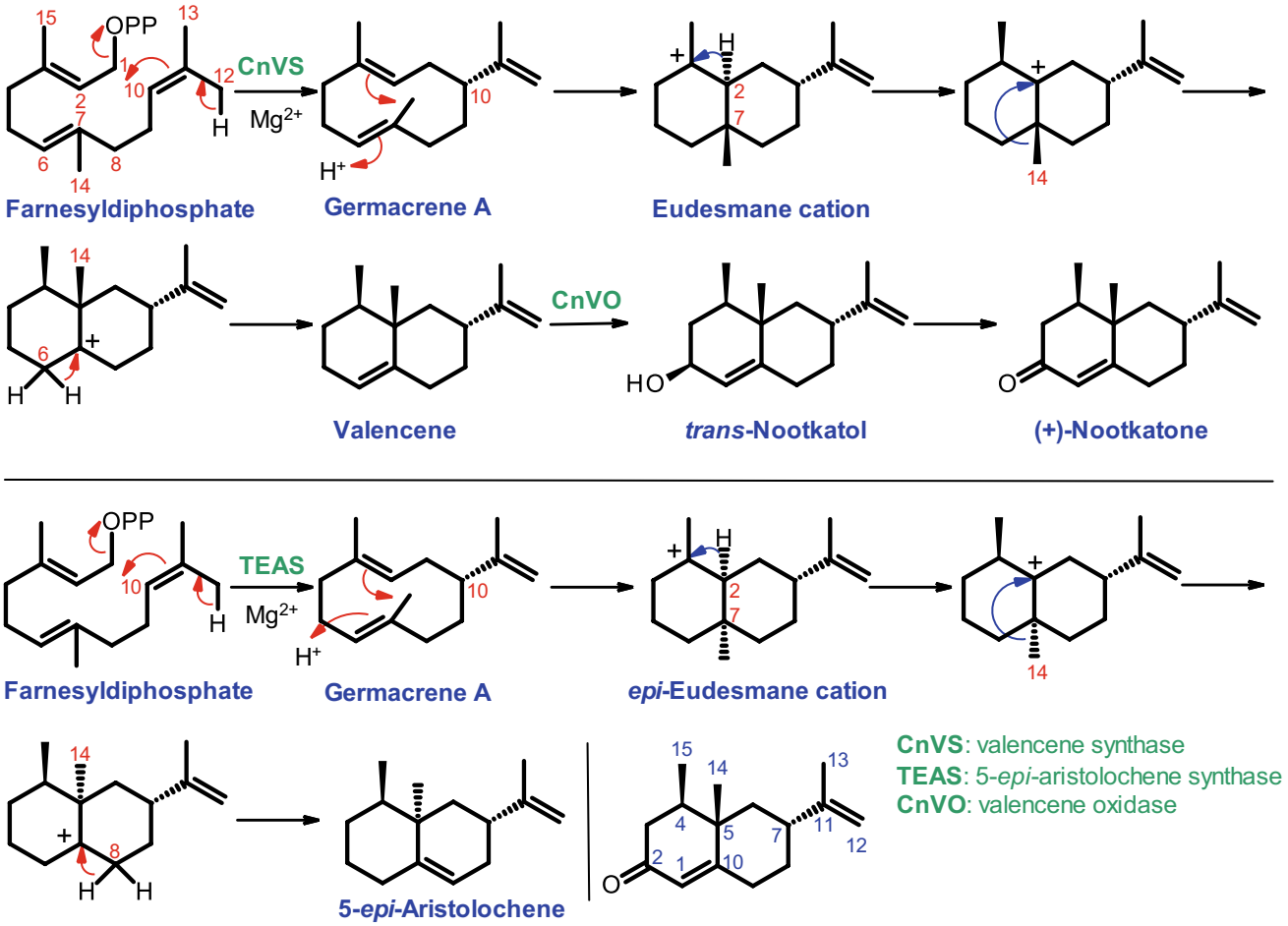
Infobox: Biosynthetic pathway to farnesyl diphosphate

Farnesyl diphosphate results from three equivalents of isopentenyl diphosphate, the basic building block of all natural terpenes. Plants have two biosynthetic pathways that they use to synthesize isopentenyl diphosphate (Table 11). Interestingly, depending on the downstream products, they prefer one biosynthesis pathway or another [283].

Over the last two decades, several valencene synthases have been described, comprising those from oranges (*Citrus sinensis*, ***C**itrus **s**inensis **t**er**p**ene **s**ynthase **1*** (Cstps1)) [287], grapevine (*Vitis vinifera*) [288], grapefruits (*Citrus paradisi*) [289], *Eryngium glaciale* [290], and Nootka cypress (*Callitropsis nootkatensis*, ***C**allitropsis **n**ootkatensis* **v**alencene **s**ynthase (CnVS)) [212]. In the first Mg^{2+}-dependent step, they initiate the formation of the farnesyl cation, which cyclizes and leads to germacrene A after deprotonation at position 12. Subsequently, cyclization is initiated by a transient protonation at C-6, which leads to valencene after two

Table 11 Terpene biosynthetic pathways in plants

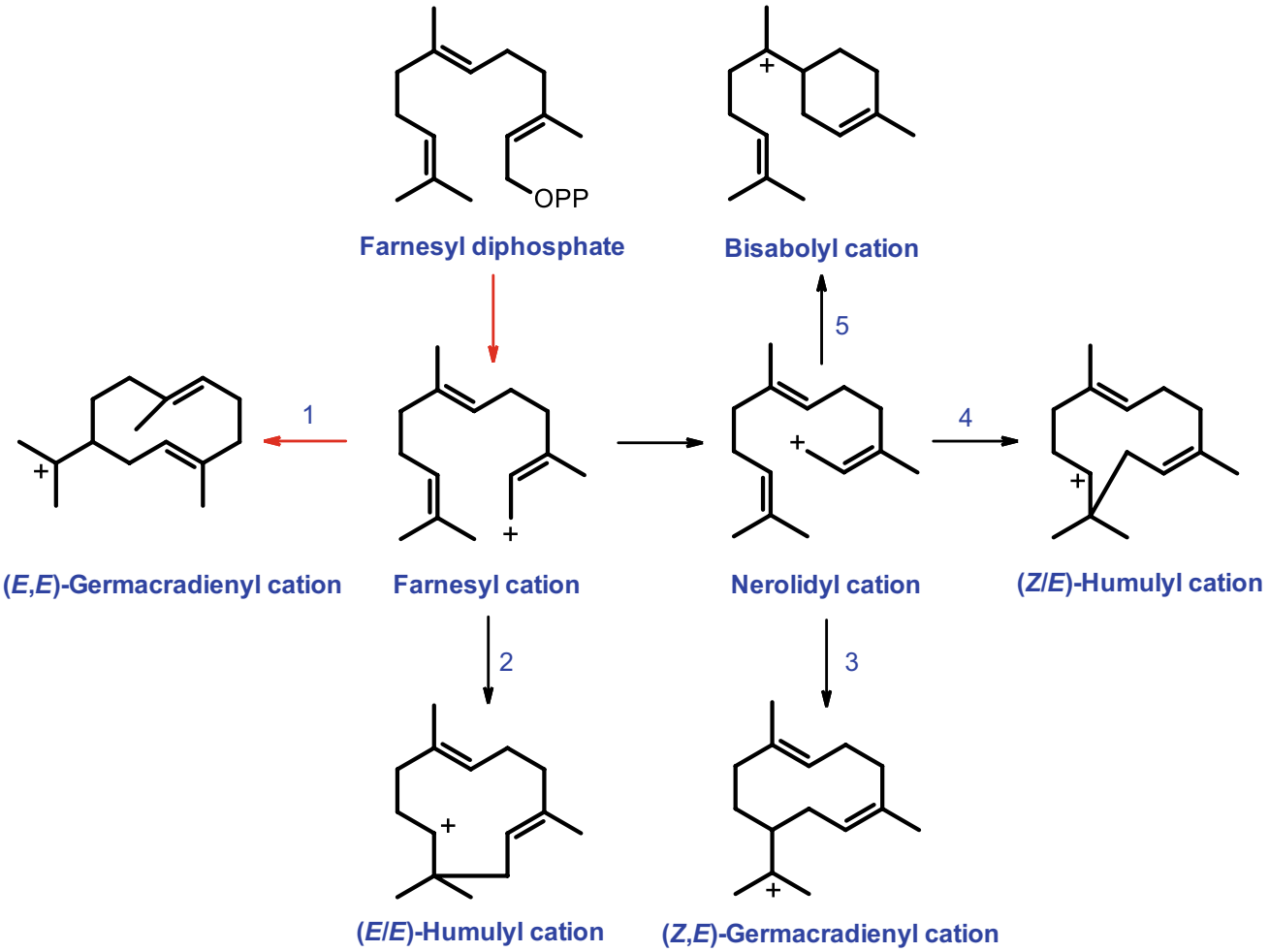
Pathway	Seminal paper by Year	Intracellular location	Starting material	Downstream products
Mevalonic acid	Feodor Leynen [284] 1967	Cytoplasm	Acetate	Sesquiterpenes, triterpenes (e.g., sterols), and polyprenols
Methyl-*D*-erythritol phosphate [285]	Michel Rohmer [286] 1999	Chloroplast	Glyceraldehyde, pyruvate	Monoterpenes, diterpenes (e.g., phytols), and tetraterpenes (e.g., carotenoids)



Scheme 9 Biosynthesis of (+)-nootkatone and 5-*epi*-aristolochene in Nootka cypress (*Callitropsis nootkatensis*) and Tobacco (*Nicotiana tabacum*), respectively

Table 12 Sequence alignment of amino acids forming the active site of 5-*epi*-aristolochene synthase with the corresponding positions of valencene synthase [287]

Synthase	Active site residue									
Position	264	273	403	404	440	444	520	525	527	528
Valencene	R	W	G	Y	C	D	Y	D	Y	T
5-*epi*-aristolochene	R	W	**T**	Y	C	D	Y	D	Y	T



Scheme 10 Different types of cyclization of farnesyl diphosphate lead to a remarkable series of downstream products

successive Wagner-Meerwein rearrangements [291]. These cascade reactions are tightly controlled by the steric and electronic properties of the enzyme's active pocket.

Surprisingly, the amino acids of the active pocket of valencene synthase differ from those of **T**obacco 5-***epi***-**a**ristolochene **s**ynthase (TEAS) [292] only at position 403, where glycine is replaced by threonine, which ultimately leads to 5-*epi*-aristolochene owing to the different geometries of the intermediates (Scheme 9, Table 12) [283]. Many more cyclic sesquiterpenes resulting from similar cyclization reactions are known (see Infobox).

The first direct proof that valencene is oxidized via nootkatol to nootkatone is owing to studies by Bouwmeester et al. at the University of Wageningen, in the Netherlands. In 2003, they serendipitously found that a microsomal preparation from the chicory root can convert valencene into nootkatone, although nootkatone itself is not found in chicory [293]. As it turned out, in chicory this conversion is catalyzed by the monooxygenase CYP71AV8 [294]. In 2014, Katarina Cankar in the group of Jules Beekwilder discovered that the oxidation of valencene in Nootka cypress is also carried out by a single, but distinct cytochrome P450 enzyme, which they named *Callitropsis nootkatensis* valencene oxidase (CnVO), belonging to a previously uncharacterized family of cytochrome P450s, the CYP706 subfamily. The intermediate product of the CnVO-catalyzed oxidation proved to be *trans*-nootkatol, which is oxidized directly to (+)-nootkatone (Scheme 9) [295].

Meanwhile, similar transformations are known to be performed by several citrus species, as well as in *Alpinia oxyphylla*, however, in which a number of enzymes are involved, such as cytochrome P450 monooxidases coupled to cytochrome P450 reductases, or alcohol dehydrogenases. [210, 296–299]

Infobox: Cyclic sesquiterpenes from farnesyl diphosphate

A large variety of sesquiterpenes, such as germacrene A (via 1), avermitolol (via 1), α-humulene (via 2), β-caryophylene (via 2), pentalenene (via 2), (+)-τ-muurolol (via 3), (−)-δ-cadinene (via 3), (+)-epi-cubenol (via 3), longifolene (via 4), amorpha-4,11-diene (via 5), (+)-epi-isozizaene (via 5), and (−)-α-bisabolol (via 5), result from different types of cyclization of farnesyl diphosphate (Scheme 10, Fig. 24) [300–302].

Production

Extraction

Until now, the commercial production of nootkatone is based to a certain extent on extraction from grapefruit peel [210]. A variety of methods are used, ranging from steam distillation of pulverized grapefruit peel to extraction with petroleum ether and adsorption of essential nootkatone oil on polymers [303, 304]. However, these methods are very complex owing to the low nootkatone content in the raw material, which makes the processes expensive [305]. Therefore, other manufacturing processes have been developed.

Chemical synthesis from valencene

To the best of our knowledge, none of the total syntheses have ever been used to produce large quantities of nootkatone. Industrial chemical synthesis relies on allyl oxidation, particularly of valencene. Over the past 55 years, approximately

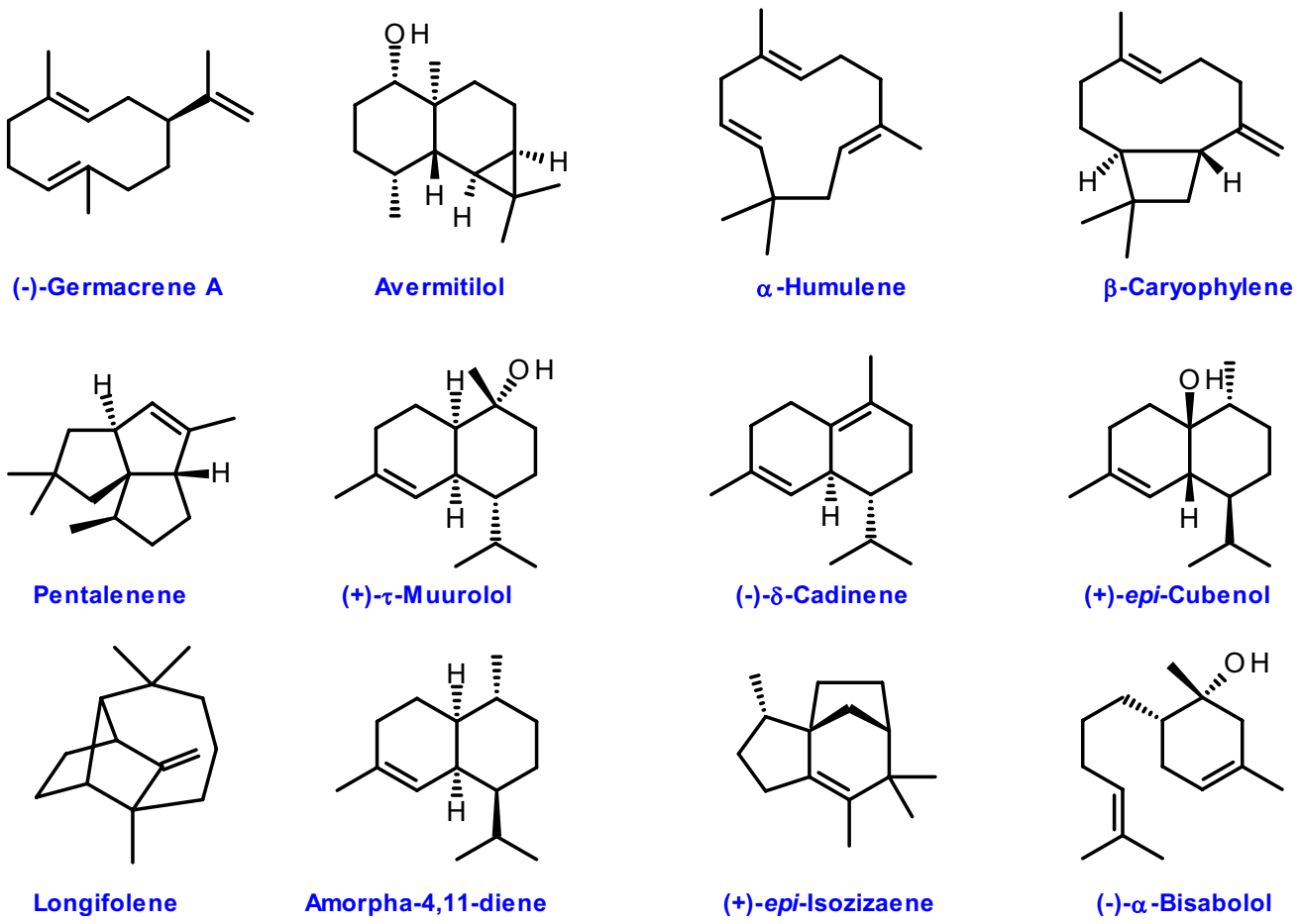
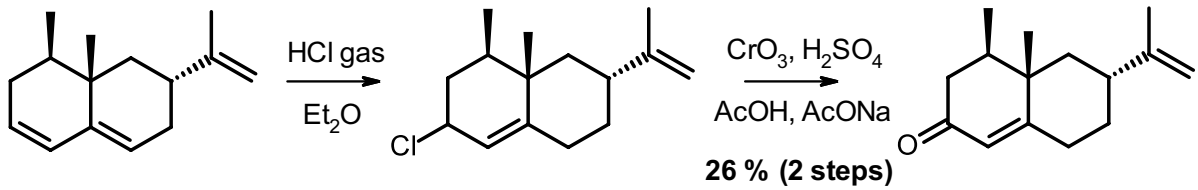


Fig. 24 Cyclic sesquiterpenes from farnesyl diphosphate

Table 13 Selection of patents claiming the synthesis of nootkatone

Entry	Company	Priority	Starting material	Method	Steps	Yield (%)	Refs
1	Firmenich	25–09-1968	Nootkatene	Addition of HCl followed by Jones oxidation	2	26	[306]
2	Firmenich	22–07-1969	Valencene	$^1O_2$ photo oxidation, $Pb(OAc)_4$ oxidation	2	25	[307]
3	Firmenich	18–12-1995	Valencene	O_2, lipoxygenase	1	40	[308]
4	Givaudan	08–09-1999	Valencene	Air, Laccase	1	29	[309]
5	Givaudan	04–06-2019	Valencene	O_2, Iron(III) porphyrin complex	1	67	[310]
6	Mianyang Simaiergu Biotechnology	30–08-2022	Valencene	Air, Iron porphyrin catalyst	1	41	[311]
7	Hoffmann-La Roche	17–04-1985	Valencene	Air, *N*-hydroxy phthalimide, Ac_2O, $CuCl_2$, pyridine	2	/	[312]
8	Daicel	13–06-2001	Valencene	O_2, *N*-hydroxy phthalimide, $Co(OAc)_2$, $Co(acac)_3$, acetonitrile	1	62	[313]
9	Daicel	12–03-2001	Valencene	O_2, *N*-Hydroxy phthalimide, $Co(OAc)_2$, $Co(acac)_3$, limonene, acetonitrile	1	67	[314]
10	Daicel	26–04-2002	Valencene	O_2, *N*-hydroxy phthalimide, $Co(OAc)_2$, $Co(acac)_3$, $Co(NO_3)_2$, acetonitrile	1	62	[315]
11	Sensient Fragrances	22–04-2020	Valencene	Air, *N*-hydroxy phthalimide, *N*-hydroxy phthalimide, MIBK	1	65	[316]
12	Hasegawa	17–08-1982	Valencene	O_2, $Co(acac)_3$, AcOEt	1	58	[317]
13	Vertellus Specialties	26–02-2010	Valencene	O_2, (Au + Pd)@TiO_2, water	1	/	[318]
14	Allylix (ref. to [219])	20–03-2007	Valencene	*t*BuOOH, sodium chlorite, acetonitrile, water	1	44	[319]
15	INT Trading	11–06-2013	Valencene	*t*BuOOH, MnO_2, CH_2Cl_2	1	56	[320]



Scheme 11 Synthesis of nootkatone from nootkatene

ten companies have developed various processes for the production of nootkatone, listed in Table 13. The first patent application in this field was filed by Firmenich in 1968. It claims the addition of hydrogen chloride to nootkatene and a subsequent Jones oxidation, to obtain nootkatone in 26% yield (Scheme 11, Table 13, entry 1). Other patents from Firmenich deal with photooxidation and oxidation with oxygen in the presence of a lipoxygenase (Table 13, entries 2 and 3; see also Sect. Microbial and enzymatic oxidation of valencene). Givaudan approached the field of enzymatic and chemical valencene oxidation significantly later, submitting two patent applications on oxidations catalyzed by laccases and iron(III) porphyrin complexes, respectively (Table 13, entries 4 and 5). The latter method was also claimed a couple of years later by the Chinese company Mianyang Simaiergu Biotechnology (Table 13, entry 6). Hoffmann La-Roche and some other companies, Daicel in particular, developed methods for the oxidation of valencene in the presence of *N*-hydroxyphthalimide (Table 13, entries 7–11). Hasegawa and Vertellus Specialties oxidized valencene with oxygen, catalyzed by cobalt acetylacetonate and a titanium dioxide catalyst, respectively (Table 13, entries 12 and 13). Finally, *t*BuOOH oxidations of valencene are known from patent applications from Allylix and INT Trading (Table 13, entries 14 and 15). All in all, the best methods in terms of yield appear to be oxidations with oxygen involving iron(III) porphyrin complexes or N-hydroxyphthalimide (Table 13, entries 5 and 9).

Bioconversion

Biocatalysis offers a completely different approach to nootkatone that is much better suited to today's customer requirements. There is a clear trend towards natural raw materials for both flavorings and fragrances. According to Regulation No. 1334/2008 of the European Parliament and of the Council on Flavorings, a "natural flavoring substance" shall mean a

Table 14 Selection of heterologous high-performance strains producing valencene

Entry	Host	Gene Manipulation	Max. titer (mg/l)	Refs
1	*Rhodobacter sphaeroides*	Expressing valencene synthase from *Callitropsis nootkatensis* (CnVS)	352 (after 72 h)	[212]
2	*Saccharomyces cerevisiae*	Expressing CnVS, downregulating of squalene synthesis, and overexpressing of farnesyl diphosphate pathway	539 (after 136 h)	[325]
3	*Saccharomyces cerevisiae*	Expressing CnVS, optimization of central metabolism, MVA pathway, and sesquiterpenoid synthase	1200 (after 150 h)	[326]
4	*Saccharomyces cerevisiae*	Expressing CnVS, *Hyocymus muticus* hydroperoxide lyase (HPO), *Arabidopsis thaliana* cyctochrome P450 reductase (AtCPR), and *Komagataella phaffii* alcohol dehydrogenase; overexpressing of truncated hydroxyl-methylglutaryl-CoA reductase (HMGR) and type III membrane protein; downregulating squalene synthase; deleting transcription factor ROX1	3730 (after 198 h)	[327]
5	*Saccharomyces cerevisiae*	Expressing CnVS, precursor supply enhancement, mannitol uptake facilitation, and cofactor regeneration acceleration	5610 (after ~ 180 h)	[328]
6	*Saccharomyces cerevisiae*	Gene screening, protein engineering based on ***E****ryngium* ***g****laciale* **v**alencene **s**ynthase (EgVS), and biosynthetic pathway optimization	16,600 (after 120 h)	[290]

flavoring substance obtained by appropriate physical, enzymatic, or microbiological processes from materials of vegetable, animal, or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes' [321]. Chemical syntheses cannot fulfill these requirements, but enzymatic and fermentative processes can, provided certain provisions are taken [188, 210, 322]. Consequently, biotechnology companies devote a lot of resources to two routes: the fermentative production of valencene, followed by its enzymatic oxidation, and the fermentative manufacture of nootkatone.

Production of valencene by fermentation

Several methods have been described to produce valencene by fermentation, but the productivity of the strains used differs considerably. Relevant valencene synthases used for production are those from *Callitropsis nootkatensis* [323] and from *Eryngium glaciale* [324]. Table 14 summarizes some of the currently most efficient valencene fermentation processes in terms of the maximum concentration achieved. Typical hosts are *Rhodobacter sphaeroides* and *Saccharomyces cerevisiae*. The valencene synthase from *Callitropsis nootkatensis* is frequently used, and the biosynthetic pathway as well as the fermentation process are optimized by all genetic and biotechnological means (Table 14, entries 1 and 2). For instance, in 2022, Beiwei Zhu from Dalian Polytechnic University and Yongjin J. Zhou from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences in Dalian, China, described a roughly two-fold increase in the productivity of (+)-valencene in *Saccharomyces cerevisiae* by engineering the central metabolism, the mevalonate pathway, and the sesquiterpenoid synthase (Table 14, entry 3). Independently, in the same year, Bo Chen from Beijing Evolyzer Co., Ltd., and Shuang Li from South China University of Technology in Guangzhou, China achieved even higher productivity by a series of metabolic engineering strategies including enhancing precursor supply, facilitated uptake of mannitol and accelerating cofactor regeneration (Table 14, entries 4 and 5). However, to the best of our knowledge, the highest titer of 16,600 mg after 120 h was reported by Tiangang Liu from the Hubei Engineering Laboratory for Synthetic Microbiology of the Wuhan Institute of Biotechnology, China, in 2022 (Table 14, entry 6).

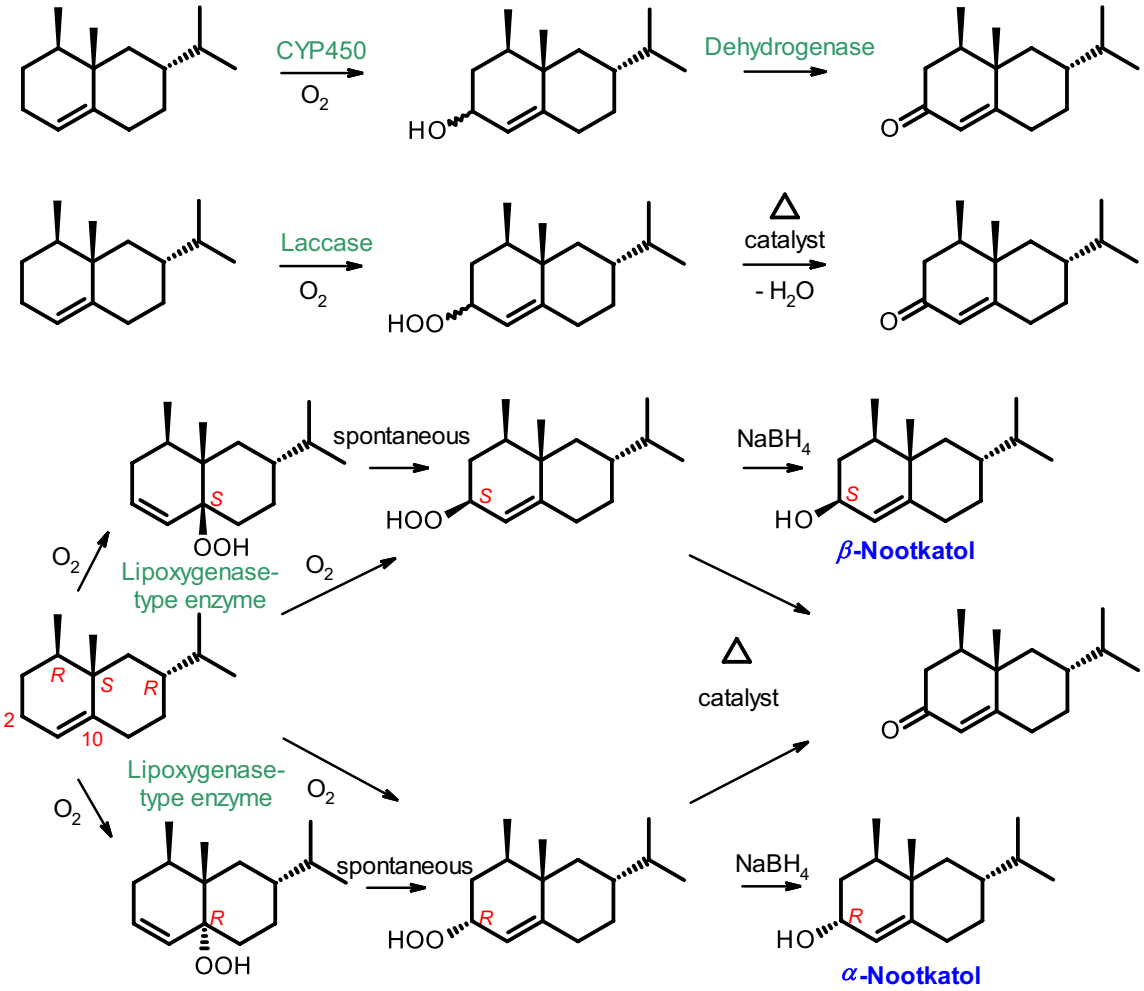
Microbial and enzymatic oxidation of valencene

The first paper on the microbial oxidation of valencene was published in 1973 by R.S. Dhavlikar and G. Albroscheit of Dragoco in Holzminden, Germany [329]. They used enterobacterial strains from Dutch soil and infected local German beer to produce nootkatone with 12% yield under optimized conditions. Later, it was found that other bacterial strains, comprising wild-type and mutant strains of *Rhodococcus* species, *Pseudomonas putida*, and *Bacillus megaterium* can also be used for valencene oxidation [309, 330, 331]. Already in the early 1980s, F. Drawert, R.G. Berger and R. Godelmann, at the Technische Universität München, Germany, could oxidize valencene with callus cells of *Citrus paradisi* cv. White Marsh to achieve nootkatone concentrations of 1.11 mg/l after 6 h of the log phase [332, 333].

Over the following decades, a multitude of biotransformations have been developed for the synthesis of nootkatone from valencene. Table 15 displays a selection of these highly efficient whole-cell but also enzymatic approaches [210, 334]. For example, Yoshinori Asakawa et al. at Tokushima Bunri University, Japan, used microalgae, such as *Chlorella vulgaris*, for the photooxidation of valencene, achieving concentrations of up to 360 mg/l in the fermentation broth after 14 days (Table 15, entry 1). Even higher titers were obtained with callus cells of Jiaogulan (*Gynostemma pentaphyllum*)

Table 15 Nootkatone produced by the enzymatic oxidation of valencene

Entry	Kingdom	Strain	Max. titer (mg/l)	Refs
1	Microalgae	*Chlorella vulgaris*	360 (after 336 h)	[335]
2	Plants	Callus of *Gynostemma pentaphyllum*	650 (after 480 h)	[336]
3	Fungi	*Yarrowia lipolytica* 2.2ab	852 (after 120 h)	[337, 338]
4	Fungi	Cell-free one-pot oxidation with a dye-decolorizing peroxidase (Ftr-DyP) and a laccase from *Funalia trogii*	1100 (after 48 h)	[339]
5	Fungi	Laccase from *Botrytis cinerea*	1296 (after 48 h)	[309]



Scheme 12 Various enzymatic and chemical routes from (+)-valencene to (+)-nootkatone. Both α- and β-nootkatol are also interesting flavorings, albeit the threshold values are significantly greater than that of (+)-nootkatone. While the β-isomer imparts sour and grapefruit-like flavors, the β-isomer tastes slightly sweet [349]

after 20 days (Table 15, entry 2). However, fungi, e.g., *Yarrowia lipolytica*, are superior because they grow much faster, which considerably improves the space–time yield (Table 15, entry 3). Ultimately, oxidation can also be performed with cell extracts and purified enzymes, i.e., peroxidases and laccases from various fungal sources and considerable titers are achieved within 2 days (Table 15, entries 4 and 5).

Table 16 Selection of heterologous high-performance strains producing nootkatone

Entry	Host	Gene manipulation	Max. titer (mg/l)	Ref
1	*Pichia pastoris*	Coexpressing valencene synthase from *Callitropsis nootkatensis* (CnVS), *Hyocymus muticus* hydroperoxide lyase (HPO), and *Arabidopsis thaliana* cyctochrome P450 reductase (AtCPR); overexpressing alcohol dehydrogenase from *Pichia pastoris* and truncated HMGR	208 (after 108 h)	[343]
2	*Saccharomyces cerevisiae*	Expressing CnVS, HPO, AtCPR, and *Komagataella phaffii* alcohol dehydrogenase; overexpressing of truncated hydroxy-methylglutaryl-CoA reductase (HMGR) and type III membrane protein; downregulating squalene synthase; deleting transcription factor ROX1	1020 (after 198 h)	[327]
3	*Escherichia coli*	Expressing a bifunctional construct of cytochrome P450 BM3 from *Bacillus megaterium* and an alcohol dehydrogenase from *Sphingomonas yanoikuyae*	1080 (after 24 h)	[350]

As manifold as the enzymes are that can be used to oxidize valencene, just as multifaceted are the technical routes. For example, heme-containing cytochrome P450 monooxygenases (CYP450s), which originate from *Pseudomonas putida*, *Bacillus megaterium* [331], *Bacillus subtilis* [340], Egyptian henbane (*Hyoscyamus muticus*) [341], Chicory (*Cichorium intybus*) [294], Tobacco (*Nicotiana tabacum*) [341], Sugarleaf (*Stevia rebaudiana*) [342], and Nootka cypress (*Callitropsis nootkatensis*) [295], can be used for the selective hydroxylation of valencene at position 2. Subsequently, the oxidation of nootkatol to nootkatone can be performed by dehydrogenases, such as alcohol dehydrogenases from yeasts, such as *Pichia pastoris* [343] and *Saccharomyces cerevisiae* [344], as well as by short-chain dehydrogenases from *Bacillus megaterium* [345].

On the other hand, laccases, which are copper-containing oxidases, from *Botrytis cinerea*[309] and *Funalia trogii* [339] convert valencene to valencene-2-hydroperoxide. In the second step, water is cleaved off to obtain nootkatone simply by heating or using iron, cobalt, and copper catalysts or ascorbic acid [309].

Finally, lipoxygenase-type enzymes from *Pleurotus sapidus* [346] and *Pleurotus florida* [347] can also be used for hydroperoxidation. As is to be expected from the lipoxygenase reactivity, valencene-10*R*/*S*-hydroperoxides are also formed; however, they rapidly isomerize to valencene-2*R*/*S*-hydroperoxides [348]. Reduction with sodium borohydride leads to α- and *β*-nootkatol. Catalytic dehydration, however, directly provides nootkatone (Scheme 12) [334].

Production of nootkatone by fermentation

Finally, nootkatone can also be produced by heterologous synthesis in various hosts. Typically, the endogenous farnesyl diphosphate pathway, based on either mevalonate or methyl-*D*-erythritol phosphate, is combined with a heterologous valencene synthase gene, e.g., from *Citrus sinensis* or *Callitropsis nootkatensis*, and a (+)-valencene oxidase gene from numerous sources.

In 2014, at the Technical University of Graz, Austria, Harald Pichler developed a strain of the yeast *Pichia pastoris* that coexpressed valencene synthase from *Callitropsis nootkatensis* (CnVS), *Hyocymus muticus* hydroperoxide lyase (HPO), and *Arabidopsis thaliana* cytochrome P450 reductase (AtCPR). It produced nootkatone in titers of more than 200 mg/l after 108 h in fed-batch bioreactor cultivations (Table 16, entry 1). In 2022, Bo Chen and Shuang Li achieved even higher space–time yields with *Saccharomyces cerevisiae* strains, which they also used for valencene synthesis. By optimizing the HPO/AtCPR activity and applying other metabolic engineering techniques, they reached maximum titers of 1020 mg/l of (+)-nootkatone in biphasic fed-batch fermentation after 198 h (Table 16, entry 2). Similar productivity was achieved in *Escherichia coli* by artificial fusion and expression of the CYP450 BM3 from *Bacillus megaterium* and an alcohol dehydrogenase from *Sphingomonas yanoikuyae* to bifunctional constructs to enable and facilitate cofactor regeneration and improve the in vitro two-step oxidation of (+)-valencene to (+)-nootkatone, as recently reported by Arsenij Kokorin and Vlada B. Urlacher from Heinrich-Heine University Düsseldorf, Germany (Table 16, entry 3).

Market, supply, demand, and prize

Ultimately, it was probably the identification of nootkatone as a small peak in the gas chromatogram of grapefruit oil (Fig. 4) that catapulted this sesquiterpene into the focus of flavor research and made it one of the most valuable citrus aromas over the decades [299]. According to Evolva, the price of food-grade (+)-nootkatone is in the range of US$ 4000/kg [298, 351]. In 2023, the estimated global market size was US$ 29 million. Moreover, owing to its approval as an insecticide and repellent as well as its potential applications in the pharmaceutical, personal, and home care sector (as mentioned in Sect. Pharmacological Properties of Nootkatone), an average annual compound growth rate of 6% is expected, which will result in a market size above

US$ 50 million in 2033 [352]. However, the continuing limited availability and high price of nootkatone as well as the time-consuming and costly approval procedures will pose significant obstacles in certain industries in the future [353]. At present, the top key players in the nootkatone market are Evolva (Switzerland), Isobionics (the Netherlands), International Flavors and Fragrances (USA; including Aromor, Isreal), Vishal Essential Oil and Chemcals (India), and Puyi Biology (China) [354]. The main production sites are located in Asia but also in Europe and North America, and the most important market regions are spread across North and Latin America, Europe, and the Asia–Pacific region [355].

Acknowledgements We are indebted to the reviewers and Dr. Jules Beekwilder (Isobionics), for valuable comments and careful proofreading.

Author contributions B.S. wrote this review, R.R. made valuable suggestions.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflict of interest Bernd Schaefer is topical editor for organic chemistry at ChemTexts, and was therefore excluded from the decision-making process on this manuscript.

References

1. Beaglehole JC (2017) The journals of captain james cook on his voyages of discovery, Volume III, Part 2: the voyage of the resolution and discovery 1776–1780. Taylor and Francis
2. https://www.timescolonist.com/entertainment/our-history-cooks-visit-to-nootka-sound-in-pictures-4652325. Retrieved 11 Feb 2023
3. Currie NE (1994) PhD thesis: captain cook at nootka sound and some questions of colonial discourse, University of British Columbia, Vancouver, Canada
4. Clayton DW (1995) PhD thesis: Islands of Truth: Vancouver Island from captain cook to the beginnings of colonialism, University of British Columbia, Vancouver, Canada
5. Campbell L (1997) American Indian languages: the historical linguistics of native America. Oxford University Press, Oxford, p 396
6. Daley, Paul (29 April 2020). "Commemorating Captain James Cook's arrival, Australia should not omit his role in the suffering that followed". The Guardian, https://www.theguardian.com/australia-news/postcolonial-blog/2020/apr/29/commemorating-james-cooks-arrival-australia-should-not-omit-his-role-in-the-suffering-that-followed. Retrieved 5 Feb 2023
7. Roy, Eleanor Ainge (8 October 2019). "New Zealand wrestles with 250th anniversary of James Cook's arrival". The Guardian https://www.theguardian.com/world/2019/oct/08/new-zealand-wrestles-with-250th-anniversary-of-james-cooks-arrival. Retrieved 5 Feb 2023
8. https://www.gutenberg.org/cache/epub/38010/pg38010-images.html. Retrieved 12 Feb 2023
9. Jewitt JR, Rodgers J (1816) A narrative of the adventures and sufferings of John R. Jewitt: only survivor of the ship *Boston* during a captivity of nearly three years among the Indians of Nootka Sound: with an account of the manners, mode of living and religious opinions of the natives, Middletown, printed by Loomis, and Richards and reprinted by Rowland Hurst, Wakefield, and published by Longman, Hurst, Rees, Orme and Brown, London (https://www.canadiana.ca/view/oocihm.29581/3. Retrieved 26 Feb 2023
10. Vancouver G (1798) Voyage Of Discovery to the north Pacific ocean, and round the world. In: The Years 1791–95, G. G. and J. Robinson, Paternoster-Row, and J. Edwards, Pall. Mall, London (https://www.biodiversitylibrary.org/item/222369#page/8/mode/1up. Retrieved 26 Feb 2023
11. Voyages of George Vancouver—travel guide at Wikivoyage. Retrieved 26 Feb 2023
12. Fryer, MB (1986) Battlefields of Canada, Dundurn Press, 131–140
13. https://conifersociety.org/conifers/cupressus-nootkatensis/. Retrieved 12 Feb 2023
14. Lambert AB (1803) Description of the Genus Pinus, J. White, London; https://www.biodiversitylibrary.org/item/98773#page/1/mode/1up. Retrieved 26 Feb 2023
15. Don D (1828) Description of the Genus Pinus, an appendix, 2nd Ed., Messrs. Weddell, Prospect Row, Walworth, London, 18; https://www.biodiversitylibrary.org/item/97078#page/1/mode/1up. Retrieved 26 Feb 2023
16. https://www.fs.usda.gov/detail/r10/communityforests/?cid=fsbdev2_038767. Retrieved 12 Mar 2023
17. Peattie DC (1934) Trees you want to know. Whitman Publishing Company, Racine, Wisconsin, p 30
18. Smith RS (1970) Canada J Botany 48:1731–1739
19. Newcombe CF (1923) Menzies' Journal of Vancouver's voyage, April to October 1792, William H Collins, Victoria; https://open.library.ubc.ca/collections/bcbooks/items/1.0226118. Retrieved 26 Feb 2023
20. Spach É (1842) Histoire Naturelle des Végétaux. Phanérogames, Paris: Librairie Encyclopédique De Roret. XI: 303–305 and 329–333
21. Oersted AS (1864) Bidrag til Naaletraeernes Morphologi, Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening I Kjobenhavn, Ser. 2, 6:1–36
22. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:20006840-1. Retrieved 12 Mar 2023
23. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:20006839-1. Retrieved 12 Mar 2023
24. Farjon A, Nguyen TH, Harder DK, Phan KL, Averyanov L (2002) Novon 12:179–189. https://doi.org/10.2307/3392953
25. Gadek PA, Alpers DL, Heslewood MM, Quinn CJ (2000) Am J Bot 87:1044–1057
26. Little DP, Schwarzbach AE, Adams RP, Hsieh CF (2004) Am J Bot 91:1872–1881
27. Mill RR, Farjon A (2006) Taxon 55:229–231
28. Debreczy Z, Musial K, Price R, Rćz I (2009) Phytologia 91:140–159

29. Stull GW, Qu XJ, Parins-Fukuchi C, Yang YY, Yang JB, Yang ZY, Hu Y, Ma H, Soltis PS, Soltis DE, Li DZ (2021) Nature Plants 7:1015–1025
30. Rice PJ, Coats JR (1994) J Econ Entomol 87:1172–1179
31. Miyazawa M, Nakamura Y, Ishikawa Y (2000) J Agric Food Chem 48:3639–3641
32. Barton GM (1976) Wood and Fiber 8:172–176
33. Hennon PE (1990) Mycologia 82:59–66
34. De Groot RC, Woodward B, Hennon PE (2000) For Prod J 50:53–59
35. Clark RH, Lucas CC (1926) Trans Roy Soc Can Sec III(20):423–428
36. Cheng YS, von Rudolph E (1970) Phytochem 9:2517–2527
37. Cheng YS, von Rudolph E (1970) Tetrahedron Lett 14:1131–1132
38. https://www.kth.se/che/orgkem/erdtman/holger-erdtman-1902-1989-1.33364. Retrieved 18 Mar 2023
39. Aulin-Erdtman G (1950) Acta Chem Scand 4:1031–1041
40. Carlsson B, Erdtman H, Frank A, Harvey WE (1952) Acta Chem Scand 6:690–696
41. Duff SR, Erdtman H, Harvey WE (1954) Acta Chem Scand 8:1073–1082
42. Erdtman H, Harvey WE, Topliss JG (1956) Acta Chem Scand 10:1381–1392
43. Norin T (1990) Phytochem 29:1713–1714
44. Wang A, Yin R, Zhou Z, Gu G, Dai J, Lai D, Zhou L (2020) Front Chem 8:596889. https://doi.org/10.3389/fchem.2020.596889
45. Erdtman H, Hirose Y (1962) Acta Chem Scand 16:1311–1314
46. Norin T (1963) Ark Kemi 22:123–128
47. MacLeod WD (1965) Tetrahedron Lett 6:4779–4783
48. Xiong Y (2000) Master Thesis: essential oil components of alaska cedar heartwood, Oregon State University
49. Khasawneh MA, Xiong Y, Peralta-Cruz J, Karchesy JJ (2011) Molecules 16:4775–4785
50. http://www.perfumeprojects.com/museum/marketers/Fritzsche.shtml. Retrieved 19 Mar 2023
51. Guenther E (1949) Essential oils of the genus citrus, the essential oils, Van Nostrand, New York, 3:347–357. https://archive.org/details/the-essential-oils-vol-3-ernest-guenther/page/n184/mode/1up. Retrieved 20 Mar 2023
52. Ebel S (1986) Chromatographia 21:559–559
53. Touchstone JC (1992) Practice of thin layer chromatography, 3rd edn. Wiley, New York, NY, pp 3–4
54. Kirchner JG, Miller JM, Rice RG, Keller GJ, Fox MM (1953) J Agr Food Chem 1:510–512
55. Kirchner JG, Miller JM (1953) J Agr Food Chem 1:512–518
56. Stanley WL, Ikeda RM, Vannier SH, Rolle LA (1961) J Food Sci 26:43–48
57. Ikeda RM, Stanley WL, Rolle LA, Vannier SH (1962) J Food Sci 27:593–596
58. Ikeda RM, Rolle LA, Vannier SH, Stanley WL (1962) J Agr Food Chem 10:98–102
59. Hunter GLK, Brogden WB Jr (1964) Analytical Chem 36:1122–1123
60. https://en.wikipedia.org/wiki/Sunkist_Growers,_Incorporated. Retrieved 19 Mar 2023
61. MacLeod WD Jr, Buigues NM (1964) J Food Sci 29:565–568
62. Uzun A, Yesiloglu T (2012) Genetic diversity in plants, https://www.intechopen.com/chapters/31479. Retrieved 10 Apr 2023. https://doi.org/10.5772/32885
63. Wu GA, Terol J, Ibanez V, López-García A, Pérez-Román E, Borredá C, Domingo C, Tadeo FR, Carbonell-Caballero J, Alonso R, Curk F, Du D, Ollitrault P, Roose ML, Dopazo J, Gmitter FG, Rokhsar DS, Talon M (2018) Nature 554:311–316
64. Zech-Matterne V, Fiorentino G (2017) AGRUMED: archaeology and history of citrus fruit in the Mediterranean. Publications du Centre Jean Bérard, Naples
65. Langgut D (2017) HortScience 52:814–822
66. Velasco R, Licciardello C (2014) Nat Biotechnol 32:640–642
67. Kumamoto J, Scora RW, Lawton HW, Clerx WA (1987) Econ Bot 41:97–107
68. Watts D (1966) Man's influence on the vegetation of Barbados, 1627–1800. University Hull, Yorkshire, England
69. Sloane H (1696) Catalogus plantarum quae in insula Jamaica sponte proveniunt. Brown, London, 208–212
70. Sloane H (1707) A Voyage to the islands of Madeira, Barbados, Nieves, St. Christophers, and Jamaica. Printed by British Museum for the author, London
71. Hughes G (1750) The natural history of Barbados, book V. Printed for the author, London
72. Washington G, Toner JM (ed.) (1892) Washington's Barbadoes Journal, 1751, Joel Munsell's sons, Albany, NY
73. https://upload.wikimedia.org/wikipedia/commons/8/8c/The_daily_journal_of_Major_George_Washington%2C_in_1751-2%2C_kept_while_on_a_tour_from_Virginia_to_the_island_of_Barbadoes%2C_with_his_invalid_brother%2C_Maj_%28IA_dailyjournal00washrich%29.pdf. Retrieved 17 Apr 2023
74. Lunan J (1814) Hortus Jamaicensis, vol 2, St. Jago de la Vega Gaz, Jamaica, 171–173
75. Macfadyen J (1830) Bot Misc 1:295–304
76. Macfadyen J (1837) The flora of Jamaica; a description of the plants of that island, Longman, Orme, Brown, Green, and Longmans, London, 131–132
77. https://floridacitrushalloffame.com/inductees/odet-philippe/. Retrieved 17 Apr 2023
78. https://www.bradentongulfislands.com/pages/246/an-empire-built-on-grapefruit. Retrieved 17 Apr 2023
79. https://original-ufdc.uflib.ufl.edu/UF00086639/00051/4j. Retrieved 19 Apr 2023
80. Da Graca JV, Louzada ES, Sauls JW (2004) Proc Int Soc Citriculture 1:369–374
81. http://ufdcimages.uflib.ufl.edu/IR/00/00/46/39/00001/CH06300.pdf. Retrieved 18 Apr 2023
82. Rouse RE, Wutscher HK, Youtsey CO (2001) Subtrop Plant Sci 53:1–3
83. https://www.fao.org/faostat/en/#data/QCL. Retrieved 18 Apr 2023
84. Bowman KD, Gmitter FG Jr (1990) Econ Bot 44:165–173
85. Biolatto A, Sancho AM, Cantet RJC, Güemes DR, Pensel NA (2002) J Agric Food Chem 50:4816–4819
86. Matlack MB (1928) Am J Pharm 100:243–246
87. Matlack MB (1928) J Biol Chem 110:249–253
88. Lee HS (2000) J Agric Food Chem 48:1507–1511
89. Njoroge SM, Koaze H, Karanja PN, Sawamura M (2005) J Agric Food Chem 53:9790–9794
90. Ghani A, Mohtashami S, Jamalian S (2021) J Food Measur Charact 15:4917–4928
91. Demole E, Enggist P, Ohloff G (1982) Helv Chim Acta 65:1785–1794
92. Buettner A, Schieberle P (1999) J Agric Food Chem 47:5189–5193
93. Lehmann D, Dietrich A, Hener U, Mosandl A (1994) Phytochem Anal 6:255–257
94. Buettner A, Schieberle P (2001) J Agric Food Chem 49:2387–2394
95. Peterson JJ, Beecher GR, Bhagwat SA, Dwyer JT, Gebhardt SE, Haytowitz DB, Holden JM (2006) J Food Compos Anal 19:S74–S80

96. Castro-Vazquez L, Alañón ME, Rodríguez-Robledo V, Pérez-Coello MS, Hermosín-Gutierrez I, Díaz-Maroto MC, Jordán J, Galindo MF, Del Mar A-J (2016). Oxid Med Cell Longev. https://doi.org/10.1155/2016/8915729
97. Yusof S, Ghazali HM, King GS (1990) Food Chem 37:113–121
98. Bronner WE, Beecher GR (1995) J Chromatogr A 705:247–256
99. Linke HAB, Eveleigh DE (1975) Z Naturforsch 30b:606–608
100. Akira T, Nakata T, Ohtsuka Y (1971) Nature 233:619–620
101. Cisak A, Mielczarek C (1992) J Chem Soc Perkin Trans 2:1603–1607
102. Ribeir MH (2011) Appl Microbiol Biotechnol 90:1883–1895
103. Narnoliya LK, Jadaun JS (2019) Biotechnological avenues for fruit juices debittering. In: Parameswaran B, Varjani S, Raveendran S (eds) Green Bio-processes. Springer Nature Singapore
104. Bailey DG, Spence JD, Edgar B, Arnold JM (1989) Clin Invest Med 12:357–362
105. Bailey DG, Spence JD, Munoz C, Arnold JM (1991) Lancet 337:268–269
106. Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, Kim RB (2002) Clin Pharmacol Ther 71:11–20
107. Bailey DG, Dresser GK, Leake BF, Kim RB (2007) Clin Pharmacol Ther 81:495–502
108. Dolton MJ, Roufogalis BD, McLachlan AJ (2012) Clin Pharmacol Ther 92:622–630
109. Valussi M, Donelli D, Firenzuoli F, Antonelli M (2021) Encyclopedia 1:152–176
110. Bailey DG, Dresser G, Arnold JMO (2013) Can Med Assoc J 185:309–316
111. Fan J, Liu Z, Xu S, Yan X, Cheng W, Yang R, Guo Y (2022). Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2021.114490
112. Uysal A, Zengin G, Aktumsek A, Rigano D, Senatore F, Sanda MA (2017) Int J Food Prop 20:549–559
113. Kelsey RG, González-Hernández MP, Karchesy J, Veluthoor S (2015) Ann For Sci 72:349–355
114. Bazzali O, Thai TH, Hoi TM, Khang NS, Hien NT, Casanova J, Bighelli A, Tomi F (2016). Molecules. https://doi.org/10.3390/molecules21070840
115. Jin JH, Min BS, Ah JH, Jae SC (2017) Nat Prod Commun 23:208–212
116. Andersen NH (1970) Phytochemistry 9:145–151
117. Xie J, Wang S, Sun B (2009) Food Chem 117:375–380
118. Liu L, Ye WP, Li SM, Zhang F, Ao H, Feng SP (2018) West China J Pharm Sci 33:75–77
119. Tsoyi K, Jang HJ, Lee YS, Kim YM, Kim HJ, Seo HG, Lee JH, Kwak JH, Lee DU, Chang KC (2011) J Ethnopharmacol 137:1311–1317
120. Jaiswal Y, Liang Z, Guo P, Ho HM, Chen H, Zhao Z (2014) J Agric Food Chem 62:7302–7316
121. Kavaz D, Idris M, Onyebuchi C (2019) Int J Biol Macromol 123:837–845
122. Basappa G, Kumar V, Sarojini BK, Poornima DV, Gajula H, Sannabommaji TK, Rajashekar J (2015) Ind Crop Prod 77:89–96
123. Haring HG, Rijkens F, Boelens H (1972) J Agric Food Chem 20:1018–1021
124. Boelens MH, Boelens H, van Gemert LJ (1993) Perfumer Flavorist 18:1–16
125. http://www.leffingwell.com/chirality/nootkatone.htm. Retrieved 07 May 2023
126. Berry RE, Wagner CJ, Moshonas MG (1967) J Food Sci 32:75–78
127. Zviely M (2009) Perfumer Flavorist 34:20–22
128. Hall RL, Oser BL (1970) Food Technol 24:25–34
129. https://uspto.report/TM/72149362. Retrieved 11 June 2023
130. Allen BJ (1964) US3140187 (Coca-Cola Co)
131. https://www.coca-colacompany.com/media-center/fresca-lived-up-to-its-tagline-for-1967-new-york-debut. Retrieved 11 June 2023
132. Shaw PE (1978) Perfum Flavor 3:35–40
133. file:///C:/Users/SCHAEFB/Downloads/50YearsofFlavorChemistry.pdf. Retrieved 11 June 2023
134. https://www.scentspiracy.com/synthetics/nootkatone. Retrieved 11 June 2023
135. https://www.fresca.com/. Retrieved 11 June 2023
136. Ghosh S, de March CA, Branciamore S, Kaleem S, Matsunami H, Vaidehi N (2022) Biophys J 121:830–840
137. Goldblum S, Warren CB (2014) WO 2014/031790 (Allylix Inc.)
138. Zhu BCR, Henderson G, Chen F, Maistrello L, Laine RAJ (2001) Chem Ecol 27:523–531
139. Zhu BCR, Henderson G, Sauer AM, Yu Y, Crowe WE, Laine RAJ (2003) Chem Ecol 29:2695–2701
140. https://www.epa.gov/pesticides/nootkatone-now-registered-epa. Retrieved 30 July 2023
141. Pretel AG, del Pérez PH, Olmeda AS, Gonzalez-Coloma A, Barrero AF, del Quílez MJF (2019). Biomolecules. https://doi.org/10.3390/biom9110742
142. Guo Y, Zhang Q, Liu Z, Bao C, Fan J, Yang R (2019). Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2019.111706
143. Guo Y, Liu Z, Hou E, Ma N, Fan J, Jin CY (2020). Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2020.112864
144. http://sitem.herts.ac.uk/aeru/bpdb/Reports/3496.htm. Retrieved 30 July 2023
145. Norris EJ, Gross AD, Kimber MJ, Bartholomay L, Coats J (2018) Advances in the biorational control of medical and veterinary pests. ACS Symp Ser 1289:219–235. https://doi.org/10.1021/bk-2018-1289.ch012
146. Erspamer V (1948) Acta Pharmacol Toxicol 4:213–223
147. Erspamer V, Boretti G (1951) Arch Int Pharmacodyn 88:296–332
148. Bauknecht P, Jékely G (2017) BMC Biol 15:6. https://doi.org/10.1186/s12915-016-0341-7
149. Li Y, Fink C, El-Kholy S, Roeder T (2015) Arch Insect Biochem Physiol 88:168–178
150. Luo J, Lushchak OV, Goergen P, Williams MJ, Nässel DR (2014) PLoS ONE 9:e99732
151. Orchard I, Ramirez JM, Lange AB (1993) Ann Rev Entomol 38:227–249
152. Selcho M, Pauls D (2019) Curr Opinion Insect Sci 36:125–130
153. Greenfield MD (2001) BioEssays 23:992–995
154. Trimmer BA, Aprille JR, Dudzinski DM, Lagace CJ, Lewis SM, Michel T, Qazi S, Zayas R (2001) Science 292:2486–2488
155. Ormerod KG, Hadden JK, Deady LD, Mercier AJ, Krans JL (2013) J Neurophysiol 110:1984–1996
156. Fields PE, Woodring JP (1991) J Insect Physiol 37:193–199
157. David JC, Coulon JF (1985) Prog Neurobiol 24:141–185
158. Hardwick BC, Axelrod B (1969) Plant Pysiol 44:1745–1746
159. Stewart I, Wheaton TA (1964) Science 145:60–61
160. Stewart I, Newhall WF, Edwards GJ (1964) J Biol Chem 239:930–932
161. Tang F, Tao L, Luo X, Ding L, Guo M, Nie L, Yao S (2006) J Chromatogr A 1125:182–188
162. Jagiełło-Wójtowicz E (1979) Pol J Pharmacol Pharm 31:509–516
163. Muth F, Philbin CS, Jeffrey CS, Leonard AS (2022). iScience. https://doi.org/10.1016/j.isci.2022.104765
164. Carpenter DO, Gaubatz GL (1974) Nature 252:483–485
165. Nathanson JA, Greengard P (1973) Science 180:308–310
166. Robertson HA, Juorio AV (1976) Int Rev Neurobiol 19:173–224
167. Axelrod J, Saavedra JM (1977) Nature 265:501–504
168. Evans PD, O'Shea M (1977) Nature 270:257–259
169. Evans PD, O'Shea M (1978) J exp Biol 73:235–260
170. Evans PD, Maqueira B (2005) Invert Neurosci 5:111–118

171. Lee HG, Rohila S, Han KA (2009). PLoS ONE. https://doi.org/10.1371/journal.pone.0004716
172. Wu S, Jv X, Li J, Xu G, Cai X, Gao C (2017) Insect Biochem Mol Biol 87:55–64
173. Hana S, Lange AB (2017). Front Physiol. https://doi.org/10.3389/fphys.2017.00744
174. Röder T (1999) Prog Neurobiol 59:533–561
175. Pflüger HJ, Stevenson PA (2005) Arthropod Struct Develop 34:379–396
176. https://en.wikipedia.org/wiki/Bilateria. Retrieved 16 July 2023
177. Matsumura F, Ghiasuddin SM (1983) J Environ Sci Health B 18:1–14
178. Norris EJ, Chen R, Geldenhuys W, Boomquist JR, Swale DR (2022). Pesticide Biochem Physiol. https://doi.org/10.1016/j.pestbp.2022.105085
179. https://www.cloud-clone.com/items/O685.html. Retrieved 07 Aug 2023
180. Hodgson E (2012) Toxins and venoms in progress in molecular biology and translational science, vol 12. Elsevier Inc
181. https://datasheets.scbt.com/sc-202765.pdf. Retrieved 30 July 2023
182. Palatnick W, Meatherall R, Sitar D, Tenenbein M (2008) J Toxicol Clin Toxicol 35:617–620
183. Rho JM, Donevan SD, Rogawski MA (1996) J Physiol 497:509–522
184. Olsen RW (2006) Proc Natl Acad Sci USA 103:6081–6082
185. Nilsson E, Eyrich B (2009) Acta Med Scand 137:381–389
186. Weikert S, Hoelzl M, Scherer H (2005) Picrotoxin als Therapeutikum bei M Menière? in HNO-Informationen 84. Thieme Verlag
187. https://pubchem.ncbi.nlm.nih.gov/source/hsdb/6385. Retrieved 30 July 2023
188. Yamaguchi T (2019). Nat Prod Commun. https://doi.org/10.1177/1934578X19859999
189. Farha AK, Yang QQ, Kim G, Zhang D, Mavumengwana V, Habimana O, Corke HB, Li H, Gan RY (2020). Food Control. https://doi.org/10.1016/j.foodcont.2020.107154
190. Alkhaibari IS, Raj KCH, Alnufaie R, Gilmore D, Alam MA (2021) ChemMedChem 16:2628–2637
191. Xu HB, Ma YB, Huang XY, Geng CA, Wang H, Zhao Y, Yang TH, Chen XL, Yang CY, Zhang XM, Chen JJ (2015) J Ethnopharmacol 171:131–140
192. Vincent S, Arokiyaraj S, Saravanan M, Dhanraj M (2020). Front Mol Biosci. https://doi.org/10.3389/fmolb.2020.613401
193. Bezerra RD, Silva ALM, da Silva CP, Alcantara IS, de Correia OMR, Oliveira B, Pereira Bezerra MA, Ribeiro-Filho J, Coutinho HDM, Rocha Santos PF, Quintans-Junior LJ, de Alencar MIR, Pezzani R, Vitalini S (2020). Molecules. https://doi.org/10.3390/molecules25092181
194. Xu Y, Zhang MF, Yang WL, Xia BW, Wang WH, Pan X (2021). Int Immunopharmacol. https://doi.org/10.1016/j.intimp.2021.108119
195. Yoo E, Lee J, Lertpatipanpong P, Ryu J, Kim CT, Park EY, Baek SJ (2020). BMC Cancer. https://doi.org/10.1186/s12885-020-07379-y
196. Zhu XX, Li XY, Chen Z (2020) J Buon 25:427–431
197. Meeran MFN, Azimullah S, Adeghate E, Ojha S (2021). Phytomedicine. https://doi.org/10.1016/j.phymed.2020.153405
198. Al-Salam S, Kandhan K, Sudhadevi M, Tariq S (2022) Cell Physiol Biochem 56:401–417
199. Wang YL, Wang MS, Xu MJ, Li TD, Fan KY, Yan TX, Xiao F, Bi KS, Jia Y (2018) Int Immunopharmacol 62:77–85
200. Yao Z, Li J, Bian L, Li Q, Wang X, Yang X, Wei X, Wan G, Wang Y, Shi J, Shi J (2022) Phytother Res 36:4183–4200
201. Kurdi A, Hassan K, Venkataraman B, Rajesh M (2018). J Biochem Mol Toxic. https://doi.org/10.1002/jbt.22017
202. Chen CM, Lin CY, Chung YP, Liu CH, Huang KT, Guan SS, Wu CT, Liu SH (2021). Nutrients. https://doi.org/10.3390/nu13113921
203. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Hamadi N, Ali BH (2018). Nutrients. https://doi.org/10.3390/nu10030263
204. Ali BH, Al-Salam S, Adham SA, Al Balushi K, Al Zaabi M, Beegam S, Yuvaraju P, Manoj P, Nemmar A (2019). Oxid Med Cell Longev. https://doi.org/10.1155/2019/2416935
205. Murase T, Misawa K, Haramizu S, Minegishi Y, Hase T (2010) Am J Physiol-Endoc M 299:E266–E275
206. Guo Y, Hou EH, Ma NN, Liu ZY, Fan JP, Yang RG (2020). Bioorg Chem. https://doi.org/10.1016/j.bioorg.2020.104294
207. Jin JH, Lee DU, Kim YS, Kim HP (2011) Arch Pharm Res 34:223–228
208. Lee JM, Kim J, Park SJ, Nam JH, Kim HJ, Kim WK (2024). Int J Mol Sci. https://doi.org/10.3390/ijms25105240
209. Wang YB, Li JL, Xu FF, Han XD, Wu YS, Liu B (2022) Curr Chin Sci 2:129–142
210. Zhang LL, Chen Y, Li ZJ, Fan G, Li X (2023) J Agric Food Chem 71:121–142
211. http://www.thegoodscentscompany.com/data/rw1006341.html. Retrieved 28 Dec 2023
212. Beekwilder J, van Houwelingen A, Cankar K, van Dijk AD, de Jong RM, Stoopen G, Bouwmeester H, Achkar J, Sonke T, Bosch D (2014) Plant Biotechnol J 12:174–182
213. Hunter GLK, Brogden WB Jr (1965) J Food Sci 30:876–878
214. Dauben WG, Lobrer M, Fullerton DS (1969) J Org Chem 34:3587–3592
215. Anonymous (1971) Nachr Chem Techn 24:446–448
216. Wilson CW III, Shaw PE (1978) J Agric Food Chem 26:1430–1432
217. Salvador JAR, Clark JH (2002) Green Chem 4:3352–3356
218. Silvestre SM, Salvador JAR (2007) Tetrahedron 63:2439–2445
219. García-Cabeza AL, Marín-Barrios R, Moreno-Dorado FJ, Ortega M, Massanet GM, Guerra FM (2014) Org Lett 16:1598–1601
220. Wang Y, Kuang Y, Wang Y (2015) Chem Commun 51:5852–5855
221. Hong B, Lebeuf R, Delbaere S, Alsters P, Nardello-Rataj V (2016). Catalysts. https://doi.org/10.3390/catal6120184
222. Horn EJ, Rosen BR, Chen Y, Tang J, Chen K, Eastgate MD, Baran PS (2016) Nature 533:77–81
223. Kawamata Y, Yan M, Liu Z, Bao DH, Chen J, Starr JT, Baran PS (2022) J Am Chem Soc 139:7448–7451
224. Wang Y, Chen X, Jin H, Wang Y (2019) Chem Eur J 25:14273–14277
225. Aberkouks A, Mekkaoui AA, Ali MA, Firdoussi LE, Houssame SE (2020). J Chem. https://doi.org/10.1155/2020/1241952
226. de Nunes MC, Moreira MA, da Santos SV, Robles A, DeFreitas-Silva G (2021). Inorg Chim Acta. https://doi.org/10.1016/j.ica.2020.120031
227. Liu C, Cui X, Chen W, Ma X, Prather KJ, Zhou K, Wu J (2022). Chem Eur J. https://doi.org/10.1002/chem.202201230
228. Nakamura A, Nakada M (2013) Synthesis 45:1421–1451
229. Treibs W, Schmidt H (1928) Ber Dtsch Chem Ges 61:459–465
230. Riley HL, Morley JF, Friend NAC (1932) J Chem Soc 1875–1883
231. Weidmann V, Maison W (2013) Synthesis 45:2201–2221
232. Pesaro M, Bozzato G, Schudel P (1968) Chem Commun 1152–1154
233. Hikino H, Suzuki N, Takemoto T (1968) Chem Pharm Bull 16:832–838
234. Coates RM, Shaw JE (1970) J Am Chem Soc 92:5657–5664
235. Coates RM, Shaw JE (1970) J Org Chem 35:2597–2601
236. Marshall JA, Ruden RA (1970) Tetrahedron Lett 15:1239–1242
237. Marshall JA, Ruden RA (1971) J Org Chem 36:594–596
238. Van Der Gen A, Van Der Linde LM, Witeveen JG, Boelens H (1971) Rec Trav Chim Pays-Bas 90:1034–1044

239. Van Der Gen A, Van Der Linde LM, Witeveen JG, Boelens H (1971) Rec Trav Chim Pays-Bas 90:1045–1054
240. Dastur KP (1973) J Am Chem Soc 95:6509–6510
241. Birch AJ (1974) J Agr Food Chem 22:162–167
242. Odom HC, Pinder AR (1969) Chem Commun 26–27
243. McGuire HM, Odom HC Jr (1974) Pinder. JCS Perkin I:1879–1883
244. Takagi Y, Nakahara Y, Matsui M (1978) Tetrahedron 34:517–521
245. Hiyama T, Shinoda M, Nozaki H (1979) Tetrahedron Lett 37:3529–3532
246. Yanami T, Miyashita YA (1980) J Org Chem 45:607–612
247. Hiyama T, Shinoda M, Saimoto H, Nozaki H (1981) Bull Chem Soc Jpn 54:2747–2758
248. Torii S, Inokushi T, Hando K (1982) Bull Chem Soc Jpn 55:887–890
249. Inokuchi T, Asanuma G, Torii S (1982) J Org Chem 47:4622–4626
250. Majetich G, Behnke M, Hull K (1985) J Org Chem 50:3615–3618
251. Sauer AM, Crowe WE, Henderson G, Laine RA (2009) Org Lett 11:3530–3533
252. Sauer AM, Crowe WE, Laine RA, Henderson G (2006) US 2006/0235247
253. Handore KL, Seetharamsingh Reddy DS (2013) J Org Chem 78:8149–8154
254. Reddy DS, Handore KL, Balamkundu SS, Sen A, Pawar PV, Joseph M (2014) WO 2014/170915 (Council of scientific and industrial research)
255. Laine R (2019) US 10435344 (Louisiana State University and Agricultural and Mechanical College)
256. Rapson WS, Robinson R (1935) Journal of the Chemical Society, 1285–1288
257. Heravi MM, Alipour B, Zadsirjan V, Malmir M (2023). Asian J Org Chem. https://doi.org/10.1002/ajoc.202200668
258. Acheson RM, Robinson R (1952) J Chem Soc, 1127–1133
259. Hajos ZG, Parrish DR (1971) German Patent DE 2102623
260. Hajos ZG, Parrish DR (1974) J Org Chem 39:1615–1621
261. Eder U (1971) Angew Chem, Int Ed Engl 10:496–497
262. Bui T, Barbas CF (2000) Tetrahedron Lett 41:6951–6954
263. List B (2002) Tetrahedron 58:5573–5590
264. Constantino MG, Losco P, Castellano EE (1989) J Org Chem 54:681–683
265. Constantino MG, Beltrame M Jr, Da Silva GVJ, Zukerman-Schpector J (1996) Synth Commun 26:321–329
266. Li P, Payette JN, Yamamoto H (2007) J Am Chem Soc 129:9534–9535
267. Zhang Q, Zhang FM, Zhang CS, Liu SZ, Tian JM, Wang SH, Zhang XM, Tu YQ (2019). Nat Commun. https://doi.org/10.1038/s41467-019-10398-4
268. Zhang Q, Zhang FM, Zhang CS, Liu SZ, Tian JM, Wang SH, Zhang XM, Tu YQ (2019) J Org Chem 84:12664–12671
269. Robinson R (1909) J Chem Soc 95:2167–2174
270. Gabriel S (1910) Ber Dtsch Chem Ges 43:134–138
271. Allan J, Robinson R (1924) J Chem Soc 125:2192–2195
272. Robinson R (1917) J Chem Soc Trans 111:762–768
273. Cardwell HME, Cornforth JW, Duff SR, Holtermann H, Robinson R (1951) Chemistry Industry 389–390
274. Dodds EC, Goldberg L, Lawson W, Robinson R (1938) Nature 141:247–248
275. Gulland JM, Robinson R (1925) Memoirs and proceedings of the literary and philosophical society of manchester, 69, 79–86
276. Abraham EP (1987) Nat Prod Rep 4:41–46
277. Kermack WO, Robinson R (1922) J Chem Soc Trans 121:427–440
278. Gilman H, Jones RG, Woods LA (1952) J Org Chem 17:1630–1634
279. Hosomi A, Sakurai H (1977) J Am Chem Soc 99:1673–1675
280. https://pubchem.ncbi.nlm.nih.gov/compound/5367510#section=Chemical-Vendors. Retrieved 21 Dec 2023
281. https://pubchem.ncbi.nlm.nih.gov/compound/Tiglic-aldehyde#section=Food-Additives-and-Ingredients. Retrieved 21 Dec 2023
282. Singh V, Vedantham P (2001) Ind J Chem 40B:144–1147
283. Chappell J (2004) Trends Plant Sci 9:266–269
284. Lynen F (1967) Pure Appl Chem 14:137–167
285. Takahashi S, Kuzuyama T, Watanabe H, Seto H (1998) Proc Natl Acad Sci USA 95:9879–9984
286. Rohmer M (1999) Nat Prod Rep 16:565–574
287. Sharon-Asa L, Shalit M, Frydman A, Bar E, Holland D, Or E, Lavi U, Lewinsohn E, Eyal Y (2003) Plant J 36:664–674
288. Lücker J, Bowen P, Bohlmann J (2004) Phytochemistry 65:2649–2659
289. Schalk M, Clark A (2004) Patent WO2004/0213832
290. Ye ZL, Huang YL, Shi B, Xiang ZL, Tian Z, Huang M, Wu LL, Deng ZX, Shen K, Liu TG (2022) Metab Eng 72:107–115
291. Faraldos JA, González V, Senske M, Allemann RK (2011) Org Biomol Chem 9:6920–6923
292. Starks CM, Back K, Chappell J, Noel JP (1997) Science 277:1815–1820
293. de Kraker JW, Schurink M, Franssen MCR, König WA, de Groot A, Bouwmeester HJ (2003) Tetrahedron 59:409–418
294. Cankar K, van Houwelingen A, Bosch D, Sonke T, Bouwmeester H, Beekwilder J (2011) FEBS Lett 585:178–182
295. Cankar K, van Houwelingen A, Goedbloed M, Renirie R, de Jong RM, Bouwmeester H, Bosch D, Sonke T, Beekwilder J (2014) FEBS Lett 588:1001–1007
296. Meng X, Liu H, Xu W, Zhang W, Wang Z, Liu W (2020). Microb Cell Fact. https://doi.org/10.1186/s12934-020-1295-6
297. Li Z, Jiang Y, Guengerich FP, Ma L, Li S, Zhang W (2020) J Biol Chem 295:833–849
298. Liu T, Li W, Chen H, Wu T, Zhu C, Zhuo M, Li S (2022) J Agric Food Chem 70:15548–15559
299. Deng X, Ye Z, Duan J, Chen F, Zhi Y, Huang M, Huang M, Cheng W, Dou Y, Kuang Z, Huang Y, Bian G, Deng Z, Liu T, Lu L (2024) New Phytol 241:779–792
300. Durairaj J, Di Girolamo A, Bouwmeester HJ, de Ridder D, Beekwilder J, van Dijk ADJ (2018). bioRxiv. https://doi.org/10.1101/466839
301. Bhaskar P, Sareen D (2020). World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-020-02918-y
302. Kersten RD, Diedrich JK, Yates JR III, Noel JP (2015) ACS Chem Biol 10:2501–2511
303. Demin L (2018) CN109369363 (Zhejiang Haotang Industry)
304. Demin L (2018) CN109369362 (Zhejiang Haotang Industry)
305. https://www.linkedin.com/pulse/nootkatone-fact-checking-julien-drone/?trk=public_post. Retrieved 27 Jan 2024
306. Ohloff G (1968) GB1208090 (Firmenich)
307. Schulte-Elte KH, Frachebaud MG, Ohloff G (1969) DE2035901 (Firmenich)
308. Muller B, Dean C, Schmidt C, Kuhn JC (1998) WO97/22575 (Firmenich)
309. Huang R, Christenson PA, Labuda IM (2001) US6200786 (Givaudan)
310. Schröder F (2020) WO2020/245145 (Givaudan)
311. Guo X, Wang B, Chen Y, Wang H, Zhang Z, Guo X, Zhao Z (2022) CN115260014 (Mianyang Simaiergu Biotechnology)
312. Foricher J, Fürbringer G, Pfoertner K (1986) EP0198351 (Hoffmann-La Roche)
313. Kitayama K, Tatsumi A, Terada M, Hirai N (2001) EP1164131 (Daicel)
314. Kitayama K, Sugahara M (2002) JP2002265406 (Daicel)
315. Kitayama K, Sekiguchi K (2003) JP2003321413 (Daicel)
316. Rivas Bascón N, Rodriguez Ferrol R, Ruiz Sanchez A (2021) WO2021/213828 (Sensient Fragrances)

317. Takagi K, Mizutani S, Hayashi K (1984) JP590031728 (Hasegawa)
318. Tarbit B, Hutchings GJ, Edwards JK, Miedziak P (2011) WO2011/106166 (Vertellus Specialties)
319. Julien BN, Wallace DM (2008) WO 2008/116056 (Allylix)
320. Serra S (2013) EP2813484 (INT Trading)
321. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1334-20230321. Retrieved 25 Dec 2023
322. Fraatz MA, Berger RG, Zorn H (2009) Appl Microbiol Biotechnol 83:35–41
323. Achkar J, Sonke T, Beekwilder MJ, Bouwmeester HJ, Bosch HJ (2016) US9260709 (Isobionics)
324. Saran D, Park G (2014) WO2014/150599 (Allylix, Evolva)
325. Chen HF, Zhu CY, Zhu MZ, Xiong JH, Ma H, Zhuo M, Li S (2019). Microb Cell Fact. https://doi.org/10.1186/s12934-019-1246-2
326. Cao CY, Cao X, Yu W, Chen YX, Lin XP, Zhu BW, Zhou YJ (2022) J Agr Food Chem 70:7180–7187
327. Cha YP, Li W, Wu T, You X, Chen HF, Zhu CY, Zhuo M, Chen B, Li S (2022) J Agr Food Chem 70:815–825
328. Zhu CY, You X, Wu T, Li W, Chen HF, Cha YP, Zhuo M, Chen B, Li S (2022) Green Chem 24:4614–4627
329. Dhavlikar, Albroscheit G (1973) Dragoco Report 12:251–258
330. Okuda M, Sonohara H, Takigawa H, Tajima K, Ito S (1994) JP 6303967
331. Sowden RJ, Yasmin S, Rees NH, Bell SG, Wong LL (2005) Organic Biomol Chem 3:57–64
332. Drawert F, Berger RG (1982) Chem Mikrobiol Technol Lebensm 7:143–147
333. Drawert F, Berger RG, Godelmann R (1984) Plant Cell Rep 3:37–40
334. Li X, Ren JN, Fan G, Thang LL, Pan SY (2021). J Ind Microbiol Biotechnol. https://doi.org/10.1093/jimb/kuab046
335. Asakawa Y, Hashimoto T, Noma Y, Furusawa M (2013) Nat Prod Commun 8:859–862
336. Sakamaki H, Itoh K, Taniai T, Kitanaka S, Takagi Y, Chai W, Horiuchi CA (2005) J Mol Catal B Enzym 32:103–106
337. Palmerin-Carreno DM, Castillo-Araiza CO, Rutiaga-Quinones OM, Verde Calvo JR, Trejo-Aguilar GM, Dutta A, Huerta-Ochoa S (2016) J Chem Technol Biot 91:1164–1172
338. Castillo-Araiza CO, Palmerin-Carreno D, Prado-Barragan A, Huerta-Ochoa S (2017) Chem Eng Process 122:493–507
339. Kolwek J, Behrens C, Linke D, Krings U, Berger RG (2018) J Ind Microbiol Biot 45:89–101
340. Girhard M, Machida K, Itoh M, Schmid RD, Arisawa A, Urlacher VB (2009). Microb Cell Fact. https://doi.org/10.1186/1475-2859-8-36
341. Gavira C, Hofer R, Lesot A, Lambert F, Zucca J, Werck-Reichhart D (2013) J Biotechnol 18:25–35
342. Kumaran AP, Lim CG, Li L, Ghosh S, Pirie C, Qualley A, Marshall-Hill G, Preininger M (2020) US 2020/0224224 (Givaudan, Manus Biosynthesis)
343. Wriessnegger T, Augustin P, Engleder M, Leitner E, Muller M, Kaluzna I, Schurmann M, Mink D, Zellnig G, Schwab H, Pichler H (2014) Metab Eng 24:18–29
344. Ouyang X, Cha Y, Li W, Zhu C, Zhu M, Li S, Zhuo M, Huang S, Li J (2019) RSC Adv 9:30171–30181
345. Milhim M, Hartz P, Gerber A, Bernhardt R (2019) J Biotechnol 301:52–55
346. Fraatz MA, Riemer SJ, Stöber R, Kaspera R, Nimtz M, Berger RG, Zorn H (2009) J Mol Catal B Enzym 61:202–207
347. Omarini AB, Plagemann I, Schimanski S, Krings U, Berger RG (2014) Biores Technol 171:113–119
348. Krügener S, Krings U, Zorn H, Berger RG (2010) Biores Technol 101:457–462
349. Kaspera R (2004) Oxyfunktionalisierung von Terpenkohlenwasserstoffen zu aromaaktiven Terpenoiden durch selektive Biokatalyse, Dissertation, Leibniz University, Hannover
350. Kokorin A, Urlacher VB (2022). ChemBioChem. https://doi.org/10.1002/cbic.202200065
351. Waltz E (2020) Nat Biotechnol 38:1368–1369
352. https://www.futuremarketinsights.com/reports/nootkatone-market. Retrieved 10 Mar 2024
353. https://www.businessresearchinsights.com/market-reports/nootkatone-cas-4674-50-4-market-104668. Retrieved 10 Mar 2024
354. https://www.globenewswire.com/news-release/2022/11/2356132 5/0/en/Nootkatone-Market-Growth-USD-29-Million-by-2028-Top-Regions-Development-Key-Players-Market-Dynamics-Sales-Price-Revenue-Gross-Margin-Cost-Analysis-Future-Investment-Expansion-Plan.html. Retrieved 10 Mar 2024
355. https://www.linkedin.com/pulse/nootkatone-market-regional-perspectives-forecast-gogyf. Retrieved 30 June 2024

Raghavendra (Raj) Ramachanderan graduated with a Masters in Molecular Biotechnology from the Ruprecht Karl University of Heidelberg and is currently a doctoral candidate at the Department of Biosystems Science and Engineering (D-BSSE) of ETH Zurich. His research interests include developing bacterial biosensors and other tools to investigate the gut microbiome.

Bernd Schaefer Senior Principal Scientist at BASF SE, studied chemistry in Kaiserslautern and received his PhD from the Max-Planck-Institut für Kohlenforschung in Mülheim, Germany. His professional career at BASF covers research and development in the areas of plant protection, pharmaceutical chemistry, and fine chemicals (e.g., carotenoids and retinoids). In recent years his research has focused on industrial LED photochemistry and photoredox catalysis. In 2000, Bernd Schäfer was appointed Lecturer and in 2009 Honorary Professor at the Ruprecht-Karls-Universität in Heidelberg. He is the author of the advanced textbook Natural Products in the Chemical Industry, Springer, Heidelberg, 2014. In 2019, he became Topical Editor for Organic Chemistry of ChemTexts.