4.16 Human–Environment Interactions – Taste

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4.16.1 Introduction

Humans have evolved to consume plants and animals in their environment and have acquired a sense of taste. It has been suggested that our ability to taste bitter and sour evolved to identify potentially dangerous food. On the contrary, our ability to recognize a sweet taste developed to identify an energy source, while salty and umami tastes are a signal of minerals and proteins, respectively. As time progressed, humans began to use bitter, pungent, and

even astringent tastes that were initially considered to be unpleasant. This may have been because we became aware that such substances were effective at improving health or even treating disease. Human food culture progressed further with the development of cooking methods that use spices and more dramatically through the enjoyment of fermentation products such as cheese, beer, wine, soy sauce, and miso (fermented soybean paste).

Henning[1] proposed, along the lines of a concept that was first introduced by the ancient Greeks, that the four tastes, that is, sweet, sour, salty, and bitter, constitute a psychological continuum, and mixed tastes can be placed on the surface of a psychological continuum in which each of the four tastes is placed at one of the apexes of a regular tetrahedron. Prior to Henning's tetrahedron theory, Ikeda[2] had discovered that monosodium glutamate (MSG) was a savory-tasting substance and named it umami in 1909. Since the umami taste sensation could not be explained by the four basic tastes theory, the umami taste was recognized as a fifth taste modality in Japan. However, until recent molecular-biological findings, only sweet, sour, salty, and bitter have been regarded as the basic taste qualities in the rest of the world. Although umami has previously been referred to as flavor and not taste, recently the term 'umami' has become accepted worldwide as one of the five basic tastes.[3–5]

Recently, a series of major discoveries on taste receptors have been reported. Specifically, the receptors that corresponded to sweet,[6] bitter,[7] and umami[8] were identified and characterized. These receptors are distributed in taste buds, which are flower-bud-shaped organs containing taste receptor cells in taste papillae on the tongue. On the apical surface of taste receptor cells, taste receptor proteins provide molecular specificity to taste receptor cells, which are innervated by afferent nerve endings that transmit information to the taste centers of the cortex through synapses in the brain stem and thalamus. More recently, it was revealed independently by two groups that the receptor for sour taste consisted of polycystic-kidney-disease-like ion channel (PKD2L1) molecule.[9,10] The receptor for salty taste is also a subject of intense scrutiny, with particular focus on epithelial Na^+ channels (ENaCs).[11]

Taste intensity changes in proportion to the level of the stimulus. The recognition threshold is defined as the minimum concentration of a substance at which a particular taste can be recognized by a significant number of taste panels. The threshold value cannot be used to determine the relative taste intensity, since the relative taste intensity and spectrum of a substance change with concentration. Generally, the bitter taste is the most sensitive to concentration, followed by salty, sour, and sweet. Bitter and salty sensations have a wide range of responses to the concentration of the substance. However, the perception of sweet and sour occurs within a much narrower concentration range and can become saturated, for example, by a high concentration of sugar. In addition, quinine and caffeine have different thresholds even though they offer the same kind of taste sensation. On the contrary, a taste may actually change with the concentration. For example, saccharin tastes sweet at low concentration, but is bitter at high concentration. It is also well known that a salty-tasting compound can sometimes enhance sweet sensation. Taste-modifying substances are also known, and both gymnemic acid and miraculin are typical compounds that exhibit such activity. It has been reported that gymnemic acid suppresses sweet sensation probably by competitive binding with the receptor, while miraculin can modify the sour taste of citrate to a sweet taste.[12]

On the contrary, somatosensory stimuli such as pungency, astringency, tingling, and cooling sensations are believed to be transmitted directly to the brain through trigeminal nerve endings in the mouth, although this notion is still controversial.

Several review articles have described the chemistry of taste and structure–activity relationships.[13,14] Two comprehensive reviews have been published in Japan (in Japanese), with particular focus on taste compounds.[15,16] However, there has been no recent comprehensive review written from the perspective of natural products chemistry. In this chapter, several taste sensations found in natural products are described along with their structures. Unfortunately, however, it is still very difficult to anticipate the taste quality and intensity from the structure of an organic compound, even for the thoroughly studied sweet and bitter sensations, although some regularity has been observed. It is expected that recent progress in the study of receptors will contribute to a full understanding of the relationship between taste sensation and chemical structure.

4.16.2 Natural Products Associated with Sweetness

Sweet substances are the most desirable taste for humans, who have enjoyed them in fruits and honey since ancient times. It is believed that the derivation of sugar from sugarcane and sugar beet is a fairly recent practice and was started only 500–600 years ago. Initially, purified sugar was very expensive and could be enjoyed only

by the rich. However, the volume of sugar produced is currently enormous due to an increase in the cultivated area of sugarcane and sugar beet, and therefore the price is not so high. In addition, the discovery of new low-calorie artificial sweeteners such as aspartame, acesulfame K, and sucralose has expanded the sweetener market.

Meanwhile, intensive studies have been performed on the structure–sweetness relationship and many hypotheses have been proposed.[17,18] Based on these hypotheses, new, highly intense sweeteners are currently being designed. As a result, several compounds that are hundreds of thousands of times sweeter than sugar have been synthesized.[19]

Very recently, intensive studies on the receptor for sweet molecules using gene technology and knockout mice have been performed to clarify the mechanism of the perception of a sweet taste. For instance, the recognition of sugars is the function of specialized G protein-coupled receptors (GPCRs) in the gustatory system. Recently, three members of a novel subfamily of GPCRs (T1R1, T1R2, T1R3) have been proposed to function as taste receptors based on their expression in taste cells. The subfamily found in human and mouse contains a long extracellular region composed of a highly conserved amino acid sequence with about 570 residues and is called the T1R family. Notably, while each receptor expressed by cultured cells does not react at all with sweet substances by itself, cells that coexpress T1R2 and T1R3 show this reactivity. Based on these results, it is believed that a heterodimer of T1R2 and T1R3 acts as a sweet receptor.[20]

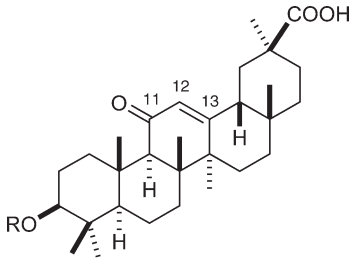
This section describes sweet-tasting natural products that have been found to date. Taste-modifying compounds and antisweet substances are also discussed.

4.16.2.1 Low-Molecular-Weight Sweet Substances

4.16.2.1.1 Glycyrrhizin

Liquorice (licorice) is the root of *Glycyrrhiza glabra* and *Glycyrrhiza uralensis*, from which a sweet substance can be extracted. The licorice plant is a legume (related to beans and peas) and is native to southern Europe and parts of Asia. Licorice extract (derived from the ancient Greek words for 'sweet root') is traded in both solid and syrup forms. Its major active component is an oleanane-type triterpene glycoside, glycyrrhizin, which is used as a sweetener and is more than 50 times sweeter than sugar (sucrose).[21] Glycyrrhizin and its ammonium salt also have a variety of pharmaceutical effects and are used particularly for the treatment of peptic ulcers and as expectorants. Although glycyrrhizin is sweet, its taste is different from that of sugar. The sweetness of glycyrrhizin has a slower onset than sugar and lingers in the mouth for sometime. In the United States, glycyrrhizin is 'generally recognized as safe' (GRAS) as a flavoring agent, but not as a sweetener. Glycyrrhizin is used as a flavoring in some candies, pharmaceuticals, and tobacco products.

Monoglucuronide of glycyrrhetinic acid (MGGR) was found to be 941 times sweeter than sucrose.[22] MGGR is produced from glycyrrhizin by selective removal of the terminal glucuronide unit of glycyrrhizin by an enzyme from *Cryptococcus magnus* MG-27 (yeast).



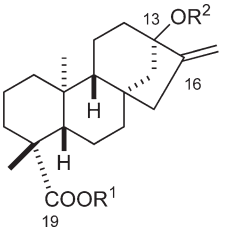
Glycyrrhizin: β-glcA^{2}–β-glcA
MGGR: β-glcA
GlcA = D-glucuronopyranosyl

4.16.2.1.2 Stevioside

Several *ent*-kaurenoid diterpene glycosides with steviol as a common aglycon have been isolated from *Stevia rebaudiana*, which is native to subtropical and tropical South America and Central America.[23–27] Among the

glycosides, stevioside is the most abundant followed by rebaudioside A. Stevioside is 140 times sweeter than sucrose, while rebaudioside is 240 times sweeter. Rebaudioside A has a better quality of sweetness. In Japan, stevia sweeteners have been produced commercially and are widely used in food products such as soy sauce, pickles, and boiled fish paste. Steviol glycosides are stable enough to remain sweet in processed foods.

The leaves of *Rubus suavissimus* S. Lee (Rosaceae), which is found wild in Guang Xi province in China, show potent sweetness and are used as a drink (sweet tea; *tian-cha*). Rubusoside has been isolated as a major sweet component from the leaves.[28] This compound has the same aglycon structure as stevioside but with one glucose less and can be obtained from stevioside by enzymatic transformation. Rubusoside is 130 times sweeter than sucrose. A comprehensive review of stevioside has been published.[29]



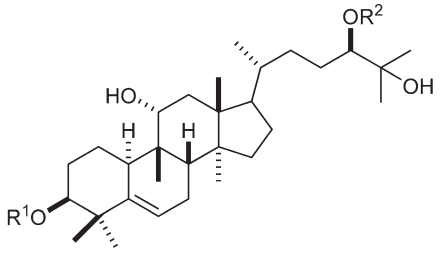
Stevioside: R^1 = β-glc, R^2 = β-glc^2-β-glc
Rebaudioside A: R^1 = β-glc, R^2 = β-glc^2-β-glc
|
β-glc^3
Rebaudioside B: R^1 = H, R^2 = β-glc^2-β-glc
|
β-glc^3
Rebaudioside C: R^1 = β-glc, R^2 = β-glc^2-α-rha
|
β-glc^3

Rebaudioside D: R^1 = β-glc^2-β-glc, R^2 = β-glc^2-β-glc
|
β-glc^3
Rebaudioside E: R^1 = β-glc^2-β-glc, R^2 = β-glc^2-β-glc
Dulcoside A: R^1 = β-glc, R^2 = β-glc^2-α-rha
Steviolbioside: R^1 = H, R^2 = β-glc^2-β-glc
Rubusoside: R^1 = β-glc, R^2 = β-glc

glc = D-glucopyranosyl; rha = L-rhamnopyranosyl

4.16.2.1.3 Mogroside

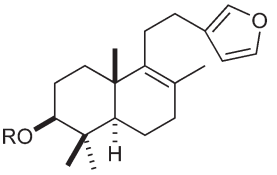
Siraitia grosvenorii is an herbaceous perennial vine that is native to southern China and is best known for its fruit, the lo han kuo (luo han guo). The fruit extract is nearly 300 times sweeter than sucrose and has been used as a natural sweetener in China for nearly a millennium due to its flavor. It has also been used in traditional Chinese medicine for the treatment of cold and sore throat. It is also used as an additive for drinks and candies in Japan and the United States. Two cucurbitane-type triterpene glycosides, mogrosides IV and V, were isolated as major sweet components of this fruit, and have been found to be 233–392 and 250–425 times sweeter than sucrose, respectively.[30,31] Mogrosides are classified by the US Food Drug Administration (FDA) as a GRAS product. There are no restrictions on consuming the fruit or its extracts. Mogroside V has been reported to be nonmutagenic.



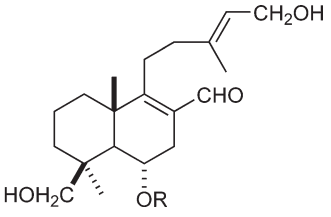
Mogroside IV: R^1 = β-glc^6–β-glc, R^2 = β-glc^2–β-glc
Mogroside V: R^1 = β-glc^6–β-glc, R^2 = β-glc^2–β-glc
|
β-glc^6

4.16.2.1.4 Baiyunoside and gaudichaudioside A

Baiyunoside, a labdane-type diterpene glycoside, was isolated from the root of *Phlomis betonicoides*, which is native to southern China and Tibet and which has been used as a traditional Chinese medicine.[32] Baiyunoside is 500 times sweeter than sucrose and has a long-lasting taste. Nishizawa and Yamada.[33] reported the synthesis of the aglycon, (+)-baiyunol, as well as its isomer, *ent*-baiyunol, and the structure–activity relationship in a series of glycosides. In addition, gaudichaudiosides A–F were isolated as other labdane-type diterpene glycosides from the stem of *Baccharis gaudichaudiana* DC. (Compositae), which is native to Paraguay. Among them, gaudichaudioside A was found to be 55 times sweeter than sucrose.[34,35]



Baiyunoside: R = β-glc^2-β-xyl
glc = D-glucopyranosyl
xyl = D-xylopyranosyl



Gaudichaudioside A: R = α-ara
ara = L-arabinose

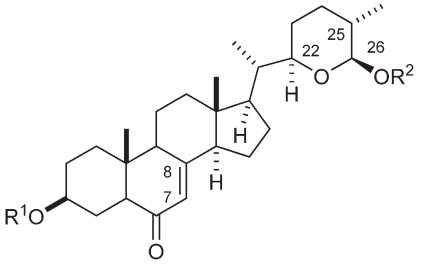
4.16.2.1.5 Steroidal saponins

Osladin, a steroidal saponin, was isolated as a sweet principle of the fern *Polypodium vulgare* L. (Polypodiaceae). Later, Nishizawa and Yamada.[36] reinvestigated the structure of osladin and revised the stereochemistry at C-22, C-25, and C-26. Although osladin has been reported to be 3000 times sweeter than sucrose, this value was also revised to 500 times.

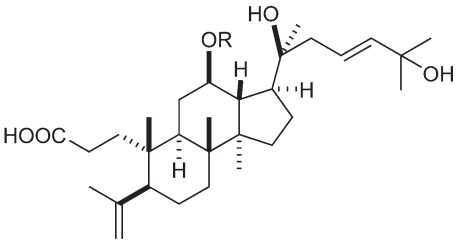
Three steroidal saponins, polypodosides, were isolated from the rhizomes of *Polypodium glycyrrhiza* DC. Eaton (Polypodiaceae).[37,38] According to the corrected structure of osladin, the structure of polypodoside was also revised.[39] Polypodoside A was shown to be highly sweet (600 times sweeter than sucrose).

4.16.2.1.6 Pterocaryosides A and B

Secodammarane saponins, pterocaryosides A and B, were isolated from *Pterocarya paliurus* Batal. (Juglandaceae), which is native to China.[40] Pterocaryosides A and B differ only in the structure of the sugar moiety bound to aglycon. Pterocaryosides A and B have been reported to be nontoxic in terms of mutagenicity and an acute toxicity test. Pterocaryoside A is 50 times sweeter than sucrose (2% solution) while pterocaryoside B is 100 times sweeter. In addition, dammarane glycoside, cyclocaryoside A, which is 200 times sweeter than sucrose, was isolated from the leaves of *Pterocarpa* (*Cyclocarya*) *paliurus*, which is native to China.[41]



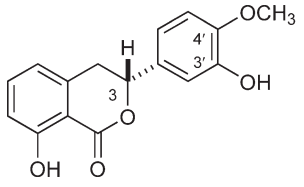
Osladin: R^1 = β-glc^2-α-rha, R^2 = α-rha, 7,8-dihydro
Polypodoside A: R^1 = β-glc^2-α-rha, R^2 = α-rha
glc = D-glucopyranosyl
rha = L-rhamnopyranosyl



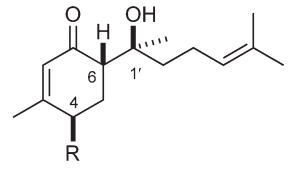
Pterocaryoside A: R = β-qui
Pterocaryoside B: R = α-ara
qui = D-quinovopyranosyl
ara = L-arabinopyranosyl

4.16.2.1.7 Phyllodulcin

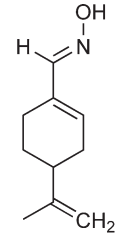
Phyllodulcin-8-*O*-β-D-glucoside, which is found in the leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (Saxifragaceae), does not show any sweet taste. However, its aglycon, D-(+)-phyllodulcin, produced by enzymatic hydrolysis, is intensely sweet.[42] This compound is called 'amacha (sweet tea)' and is used for the sweet flavor of ceremonial tea. Phyllodulcin is structurally dihydroisocoumarin and was found to be a 3*R*-stereoisomer in 1959. In subsequent studies, it was revealed that unprocessed leaves contained a mixture of *R* and *S* isomers in a ratio of 5:1.[43] Phyllodulcin is reported to be 600–800 times sweeter than sucrose. Purified phyllodulcin has no mutagenicity and its acute oral toxicity in mouse is greater than 2 g per kg body weight. One drawback for its use as a sweetener is its very low solubility in water.



Phyllodulcin



Hernandulcin: R = H
4β-Hydroxyhernandulcin: R = OH



Perillartine

4.16.2.1.8 Hernandulcin

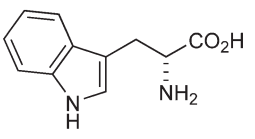
Hernandulcin is a bisabolane sesquiterpene isolated from the herb *Lippia dulcis* Trev. (Verbenaceae), which is native to Mexico, and has been reported to be 1500 times sweeter than sucrose.[44,45] The natural product has a 6*S*, 1′*S* configuration, and of the four possible stereoisomers, only this one has intense sweetness.[46,47] Another sweet substance, 4β-hydroxyhernandulcin, was isolated from a sample native to Panama.[46] The sweetness and bitterness of hernandulcin have been reported to linger in the mouth for sometime. This compound is rather thermolabile.

4.16.2.1.9 Perillartine

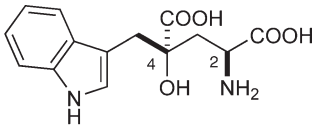
Perillartine, α-*syn*-oxime of perillaldehyde, is reported to be 2000 times sweeter than sucrose.[48,49] Perillaldehyde is a principal volatile oil of *Perilla frutescens* (L.) Britton (Labiatae) and is reported to be only slightly sweet. Perillartine is used as a replacement for maple syrup and licorice for the sweetening of tobacco in Japan. However, due to its low solubility in water as well as a menthol-licorice off-taste, there is some limitation to its use.

4.16.2.1.10 Amino acids

4.16.2.1.10(i) Proteinogenic amino acids The taste of proteinogenic amino acids involving a D-isomer was reviewed by Birch and Kemp,[50] Haefeli and Glaser,[51] and Wieser *et al.*[52] Among L-amino acids, alanine, serine, and glycine are sweet, while most of the D-amino acids are sweet. D-Tryptophan is the most intensely sweet amino acid and is 35 times sweeter than sucrose. Studies on the structure and sweetness–activity relationship have been reported by Shallenberger *et al.*[17] and Kier.[18]



D-Tryptophan



(2*S*,4*S*)-Monatin

4.16.2.1.10(ii) Monatin Monatin is an amino acid-type sweetener isolated from the bark of the roots of a spiny-leafed hardwood shrub, *Schlerochiton ilicifolius*, which is native to the northwestern Transvaal in South Africa.[53] From 160 kg of roots, 1.73 g of a crude mixture of monatin salts was obtained. Recrystallization of the salts from water–acetic acid–ethanol gave free amino acid. After this amino acid was transformed into the lactone derivative (methyl-(2*S*,4*S*)-2-(indol-3-ylmethyl)-4-(2,4-dinitroanilino)-5-oxo-2,3,4,5-tetrahydrofuran-2-carboxylate) and examined by nuclear magnetic resonance (NMR), the relative stereochemistry of the 2- and 4-positions was determined. Its absolute stereochemistry was further determined to be (2*S*,4*S*) by applying the observed optical rotation value to the Clough–Lutz–Jirgenson rule. Monatin is reported to be 800 times sweeter than sucrose at its threshold concentration and 1200–1400 times sweeter in a sucrose solution of 5–10%. It has a slight licorice aftertaste. In the patent literature, it was reported that all four stereoisomers of monatin have a sweet taste, and among them the (2*R*,4*R*) isomer is the most intense, while (2*S*,4*R*) is the weakest.[54] It has also been reported that monatin has no toxicity in the Ames test and no mutagenicity.[55]

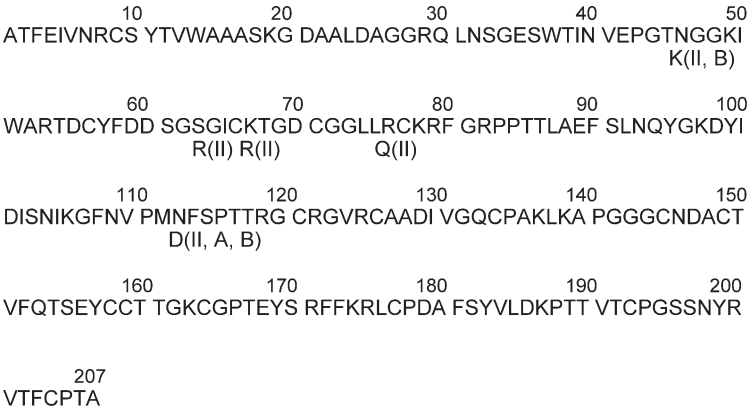
4.16.2.1.11 Sugars

Sugars are the simplest form of carbohydrates, and sugars such as monosaccharides, oligosaccharides, acyclic polyhydroxy alcohols, and cyclic sugar alcohols are well-known sweeteners. Shallenberger[56] has written a good review on the structure–sweetness relationship of sugars.

4.16.2.2 Sweet Proteins

4.16.2.2.1 Thaumatin

Thaumatin is a 22 kDa sweet protein that was isolated from the arils of the katemfe fruit of *Thaumatococcus daniellii* Benth, which is native to West Africa, by van der Wel and Loeve. It is a basic protein with an isoelectric point of approximately 12 and is 1600 times sweeter than sucrose. It also gives a cooling sensation and a slight licorice aftertaste. A water–sweet aftertaste was also reported.[57] Thaumatin is cultivated on a commercial scale and used as a sweetener, flavor enhancer, and flavor modifier. An aqueous solution of commercially available thaumatin is stable under the conditions of pH 2–10. There may be several related proteins in the plant, but there are two main forms: thaumatins I and II. Thaumatins I and II are each composed of 207 amino acids with eight intramolecular disulfide bonds shown in the figure. Thaumatins I and II differ in the amino acid sequence at 46, 63, 67, 76, and 113, which suggests that the two proteins are 98% identical.[58,59] Since the results of amino acid sequencing of the proteins were inconsistent with those of cDNA,[59] Lee *et al.* reinvestigated and isolated two proteins named thaumatins A and B, and found that they differ in only one amino acid at position 46, that is, Asn for A and Lys for B. The residue at 113 in thaumatin I is Asn, whereas it is Asp in thaumatins II, A, and B. Furthermore, thaumatin I did not make a refolding product, while thaumatins A and B expressed in yeast showed intense sweetness after refolding. Therefore, it has been suggested that there might have been an error in the determination of the residue at 113.[60] The tertiary structure of thaumatin I was analyzed by X-ray at resolutions of 3.1[61] and 1.65 Å.[62] It has been reported that thaumatin elicits a sweet taste in humans, and caused a significant electrophysiological response in the chorda tympani and glossopharyngeal nerves in the Old World monkey, but not the guinea pig or rat.[63] However, it was revealed that in Slc:ICR mice, chorda tympani and taste receptor cell response profiles and the behavioral results for monellin and thaumatin are similar to the response profiles for sucrose.[64] Thaumatin has been approved as a sweetener in Israel and Japan. In the United Nations, it is listed in Table III of the Codex General Standard for Food Additives (GSFA), which means that it is permitted for use in food in general.

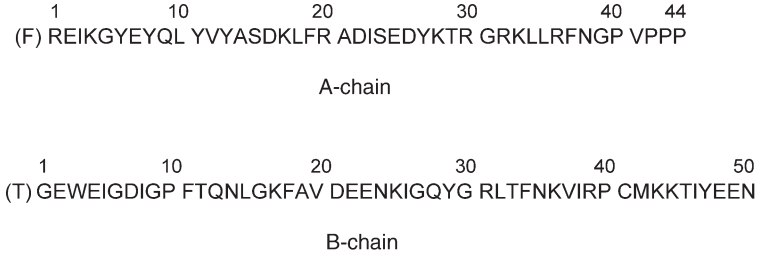


Thaumatin. II, A and B in parentheses correspond with thaumatin II, A and B, respectively. There are eight disulfide bonds between C9–C204, C56–C66, C71–C77, C121–C193, C126–C177, C134–C145, C149–C158, and C159–C164.

4.16.2.2.2 Monellin

Monellin is a sweet protein that was isolated from the fruit of *Dioscoreophyllum cumminsii* (*Stapf*) *Diels*, which is known as the serendipity berry and is native to West Africa. It is a basic protein with an isoelectric point of approximately 9.3 and is 3000 times sweeter than sucrose.[65,66] Perception lasts for more than 1 h and leaves an aftertaste. Heat denatures monellin proteins; they lose their sweetness when heated over 50 °C at low pH. Monellin has a molecular mass of 10.7 kDa. Monellin has two noncovalently associated polypeptide chains: chain A contains 44 amino acid residues and chain B has 50 residues. In 1976, the primary structure of monellin was proposed independently by three groups but their results all differed somewhat.[67–69]

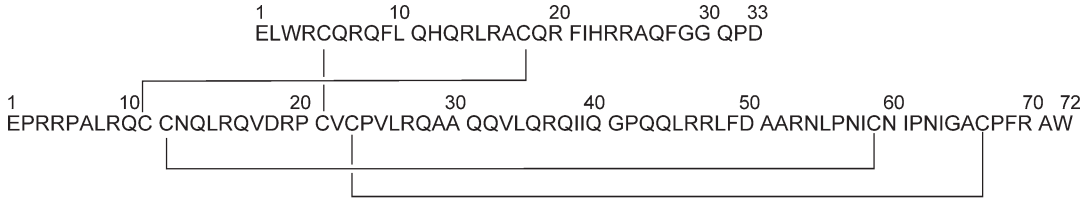
Recently, the amino acid sequence was reinvestigated[70] and it was revealed that chain A was consistent with that of Frank and Zuber,[68] while chain B coincided with the results of Bohak and Li (see figure).[67] The enzymatic hydrolysis product of monellin does not exhibit sweetness.[71] Since chains A and B are not sweet individually,[67,72] it is considered that expression of the sweet taste requires a natural three-dimensional structure. X-ray structural analysis of monellin was carried out at resolutions of 3[73] and 2.75 Å.[74] A structure–sweetness relationship study of monellin analogues strongly suggested that the Asp residue at the 7-position of chain B (AspB7) plays an important role in eliciting a sweet taste.[75–77] Meanwhile, a single-chain monellin was expressed by combining the C-terminus of chain B and the N-terminus of chain A. This compound was reported to be stable at higher temperature and over a wide range of pH,[78] and its tertiary structure was analyzed by X-ray at a resolution of 1.7 Å.[74] The main issue regarding its use as a sweetener is that monellin has no legal status in the European Union or the United States.



Monellin. 10% of A-chain has F in the N-terminus and 19% of B-chain has T in the N-terminus G of N-terminus is deleted in 24% of B-chain.

4.16.2.2.3 Mabinlin

Mabinlins are sweet-tasting proteins extracted from the seed of Mabinlang (*Capparis masaikai* Levl.), a Chinese plant that grows in Yunnan province. They have long-lasting but weak sweetness of 0.1% threshold.[79] There are at least five homologues. Mabinlin-I, Mabinlin-III, and Mabinlin-IV have molecular masses of 12.3, 12.3, and 11.9 kDa, respectively. Mabinlin-II is a 10.4 kDa protein and the most abundant homologue in nature.[80] It is also a basic protein with an isoelectric point of approximately 11.3 and is a heterodimer consisting of two different chains, A and B, like monellin. Chain A is composed of 33 amino acid residues and chain B is composed of 72 amino acid residues. Chain B contains two intramolecular disulfide bonds and is connected to chain A through two intermolecular disulfide bridges shown in the figure.[81] Mabinlin-II was estimated to be about 400 times sweeter than sucrose on a weight basis, which makes it less sweet than thaumatin (3000 times), although it elicits a similar sweetness profile. (Mabinlin-II is 375 times sweeter than sucrose on a molar basis and 10 times sweeter on a weight basis, and therefore mabinlin is not as sweet as other sweet proteins.) It has also been suggested that the difference in the heat stability of the different mabinlin homologues is due to the presence of an arginine residue (heat-stable homologue) or glutamine (heat-unstable homologue) at position 47 in chain B.[82] It has been reported that the precursor of mabinlin-II is a single-chain protein composed of 155 amino acid residues.[83]

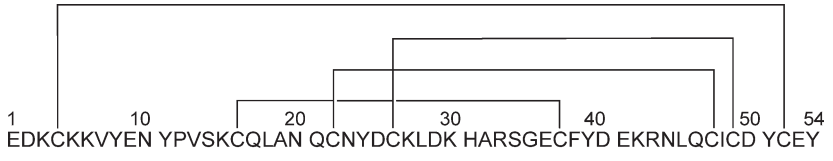


Mabiblin II

4.16.2.2.4 Brazzein

Brazzein is a sweet protein that was isolated from the fruit of the West African climbing plant Oubli (*Pentadiplandra brazzeana* Baillon). Along with pentadin, which was discovered in 1989, brazzein is the second sweet protein that was discovered in this fruit. Like other natural sweet proteins such as monellin and thaumatin, it is highly sweet. On a weight basis, brazzein is 500 times sweeter than sucrose when compared to 10% sucrose solution and 2000 times sweeter when compared to 2% sucrose solution. Its sweet perception is more similar to that of sucrose than that of thaumatin, and it presents a clean sweet taste with a lingering aftertaste. Brazzein is stable over a broad pH range from 2.5 to 8 and is heat stable at 80 °C for 4 h.[84]

The monomer protein, consisting of 54 amino acid residues with eight disulfide bonds shown in the figure,[84,85] is the smallest among the sweet proteins, with a molecular mass of 6.4 kDa. Chemical synthesis[86] of brazzein was performed and recombinant proteins were successfully produced by *Escherichia coli*.[87] Based on X-ray analysis[88] and NMR studies,[89] residues 29–33 and 39–43, plus residue 36, as well as the C-terminus were found to be involved in the sweet taste of the protein. The charge of Arg-43 in the protein also plays an important role in its interaction with the sweet taste receptor.[90]



Brazzein

4.16.2.2.5 Pentadin

Pentadin is a sweet-tasting protein that was isolated from the fruit of Oubli (*P. brazzeana* Baillon), a climbing shrub that is native to West African countries. Pentadin's molecular mass is estimated to be 12 kDa. It is reported to be 500 times sweeter than sucrose on a weight basis. The primary structure has not yet been determined, although amino acid analyses were carried out.[91]

4.16.2.3 Sweetness-Inducing Proteins and Substances

4.16.2.3.1 Miraculin

Miraculin is a basic glycoprotein that was extracted from the miracle fruit plant, a shrub that is native to West Africa (*Synsepalum dulcificum* or *Richadella dulcifica*). Miraculin itself is not sweet, but the human tongue, once exposed to miraculin, perceives ordinarily sour foods, such as citrus, as sweet for up to 2 h afterward. This small red berry has been used in West Africa to improve the taste of acidic foods. Since the miracle fruit itself has no distinct taste, this taste-modifying function of the fruit had been regarded as a miracle. The active substance, isolated by Kurihara, was named miraculin after the miracle fruit.[92] Miraculin was first sequenced in 1989 and was found to be a glycoprotein consisting of 191 amino acids and some carbohydrate chains.[93] The molecular mass of the glycoprotein is 24.6 kDa, including 3.4 kDa (13.9% of the weight) of sugar consisting (on a molar basis) of glucosamine (31%), mannose (30%), fucose (22%), xylose (10%), and galactose (7%). The sugar is linked with Asp-42 and Asp-186.

Miraculin occurs as a tetramer (98.4 kDa), a combination of four monomers grouped into dimers. Within each dimer, two miraculin glycoproteins are linked by an intramolecular disulfide bridge.[92–94] The formation of three intrachain disulfide bridges at Cys-47–Cys-92, Cys-148–Cys-159, and Cys-152–Cys-155, and one interchain disulfide bridge at Cys-138 was determined by amino acid sequencing and a composition analysis of cystine-containing peptides isolated by high-performance liquid chromatography (HPLC). It was concluded that native miraculin in pure form is a tetramer of a 25 kDa peptide and native miraculin in a crude state or denatured, nonreduced miraculin in pure form is a dimer of the peptide. Both tetramer miraculin and native dimer miraculin in a crude state have taste-modifying activity.

A cDNA clone encoding miraculin was isolated and sequenced. The encoded precursor of miraculin was composed of 220 amino acid residues, including a possible signal sequence of 29 amino acids.[95,96] Attempts have been made to express these proteins in *E. coli*, yeast, and tobacco. While it was confirmed that these proteins were expressed in these organisms, miraculin expressed in *E. coli* and yeast showed no activity.[97] The perception of 0.1 mol l^{-1} of citrate after taking 1 μmol l^{-1} of miraculin corresponds to the sweetness of 0.4 mol l^{-1} of sucrose, which means that it is 400 000 times sweeter than sucrose on a molar basis. Interestingly, a mixture of miraculin with citrate did not elicit sweetness.[97] Miraculin was denied approval for this purpose by the FDA. Miraculin also has no legal status in the European Union.

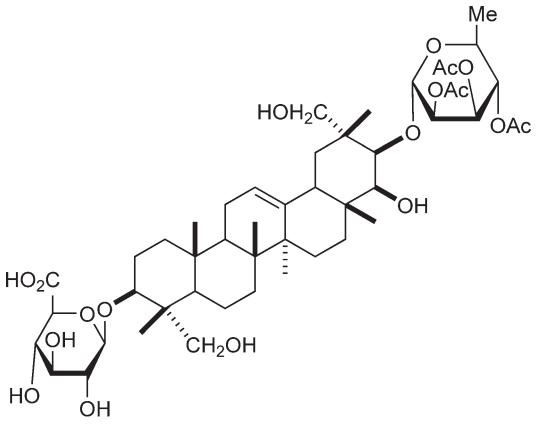
4.16.2.3.2 Curculin and neoculin

Curculin is a sweet protein that was isolated in 1990 from the fruit of *Curculigo latifolia* (Hypoxidaceae), which grows in Malaysia. Like miraculin, curculin exhibits taste-modifying activity. However, unlike miraculin, it also elicits a sweet taste by itself. After the consumption of curculin, water and sour solutions taste sweet. Curculin was reported to be a homodimer of two proteins connected through two disulfide bridges.[98] The molecular mass of curculin monomer is reported to be 12 kDa. It contains a sequence of 114 amino acids with an isoelectric point of 7.1. Curculin was crystallized and an X-ray structural analysis was performed.[99] Although both curculin and miraculin elicit a sweet taste, the two proteins are not homologous. However, based on an enzyme immunoassay and immunoblot analysis of curculin, curculin and miraculin showed cross-reactivity to both antibodies, and therefore it is considered that a common structure elicits sweet activity for both proteins.[100] Curculin is rather stable in acidic solution, and heating at 55 °C for 1 h does not reduce its potency. A 10 μmol l^{-1} curculin solution is as sweet as a 0.35 mol l^{-1} solution of sucrose. Thus, curculin is 35 000 times sweeter than sucrose. After curculin is held in the mouth for a short time, the sweet taste diminishes. However, the sweet taste is elicited again upon exposure to clear water. This can be explained as follows.[101] The sweet taste is suppressed by the reaction of curculin with divalent cations (Ca^{2+} and Mg^{2+}) in saliva. Upon exposure to water, the sweet taste is induced again since water washes the cations away. It is believed that the taste-modifying

protein strongly binds to the membrane surfaces of the taste cells in the presence of an acid such as citric acid.[102] In 1997, curculin was expressed in *E. coli* and yeast, but recombinant curculin did not exhibit 'sweet-tasting' or 'taste-modifying' activity.[103] Recently, neoculin, which is composed of an acidic glycoprotein subunit with 113 amino acid residues and a basic curculin subunit, was isolated from the same fruit as a heterodimer.[104] Another group successfully cloned curculin-2, which is highly homologous to curculin, and demonstrated that the heterodimer of curculin and curculin-2 elicits intense sweetness.[105] These results suggest that curculin itself may also be a heterodimer.

4.16.2.3.3 Strogin

Five new oleanane-type triterpene glycosides, strogins I–V, were isolated by Kurihara and coworkers[106] from the leaves of *Staurogyne merguensis* Kuntze, which is native to Malaysia. Strogin itself has a sweet taste. In addition, after strogins I, II, and IV are held in the mouth, the sweet taste can be recovered with exposure to water, as with curculin. In contrast, strogins III and V had no such activity. A 1.0 mmol l^{-1} strogin solution is as sweet as a 0.15 mol l^{-1} solution of sucrose. Furthermore, sweetness corresponding to 300 mmol l^{-1} sucrose is induced by holding 1.0 mmol l^{-1} strogin followed by water. Strogin's sweetness induction was temperature-dependent[107] while curculin's sweetness induction was independent of temperature. While Ca^{2+} and Mg^{2+} suppressed curculin's induction, strogin's induction was not suppressed by divalent cation. Thus, the mechanism of sweetness induction by strogin is different from that of curculin.

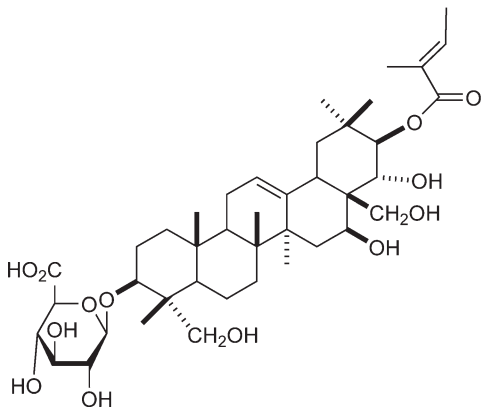


Strogin II

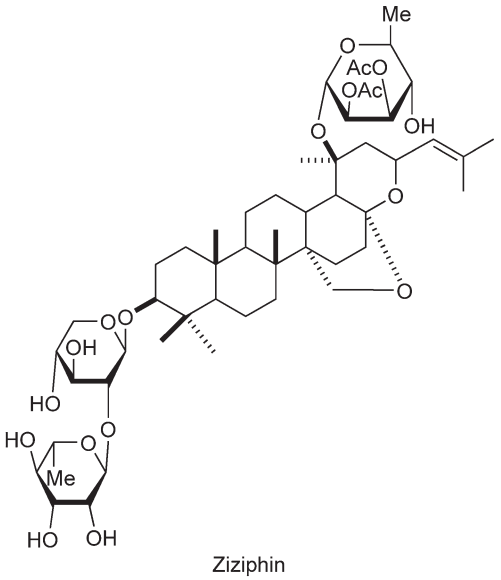
4.16.2.4 Antisweet Substances

4.16.2.4.1 Gymnemic acid

Gymnemic acids were isolated from the leaves of *Gymnema sylvestre* (Asclepiadaceae), which is native to India and southern China.[108,109] Gymnemic acids are glycosides of triterpene that suppress sweetness in humans. After the leaves are chewed, solutions that have been sweetened with sucrose taste like water. It is thought that gymnemic acid inhibits the binding of a sweet substance to the sweet receptor. Several gymnemic acid homologues with different acyl groups were purified from the leaves of *G. sylvestre* and their structures were determined. Interestingly, deletion of the acyl group diminishes the antisweet activity.[110] It suppresses the sweetness of most of sweeteners, including intense artificial sweeteners such as aspartame and natural sweeteners such as thaumatin, a sweet protein. The herb is traditionally used for the treatment of diabetes in India and Gymnema extracts are sold in Japan for the control of obesity.



Gymnemic acid I



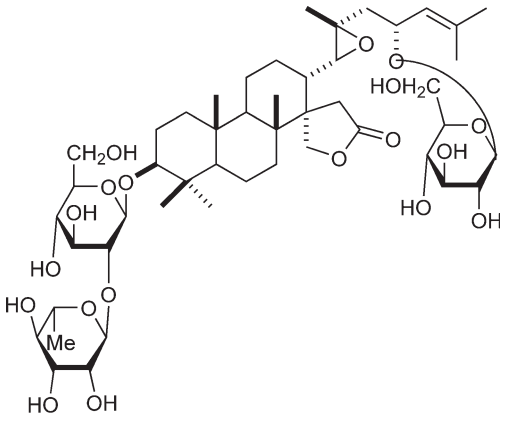
Ziziphin

4.16.2.4.2 Ziziphin

The sweetness-inhibiting substance ziziphin was isolated from the leaves of the plant *Zizyphus jujuba* (Rhamnaceae), which is native to China, by Kurihara *et al.*[111,112] Like gymnemic acids, ziziphin is a glycoside of triterpene that suppresses sweetness in humans. Removal of the acyl group under mild hydrolytic conditions led to complete abolishment of its antisweet activity.

4.16.2.4.3 Hodulcin

Hodulcin was extracted from the leaves of *Hovenia dulcis* (Rhamnaceae), which is native to China and Japan, and has been shown to selectively reduce sweetness perception in humans.[113] Hodulcin appears to be a triterpene glycoside, as are the gymnemic acids and ziziphins. NMR spectra indicated that the aglycon structure of hodulcin is different from that of gymnemic acid and similar to but not the same as that of ziziphin. Later, Arihara and coworkers[114] isolated five new dammarane glycosides named hodulosides I–V (e.g., hoduloside I) from the fresh leaves of *H. dulcis*. Their structures were determined on the basis of chemical and spectral evidence. All the compounds showed antisweet activity.



Hoduloside I

4.16.3 Bitter-Tasting Natural Products

There are many bitter-tasting compounds in nature.[115,116] Many of them are alkaloids, terpenoids and their glycosides (saponins) as well as amino acids and peptides. The threshold values of bitter compounds, as represented by alkaloids such as quinine, are extremely low (as low as ppm concentration) compared with compounds that elicit other basic tastes. It is considered that many bitter compounds possess some toxicity and therefore animals have become highly sensitive at tasting such bitter compounds. In many cases, an appropriate amount of a bitter-tasting compound can have a useful pharmacological effect. For example, it has long been known that the bitter-tasting component extracted from *Gentiana lutea* (Gentianaceae) can directly stimulate acid production by the gastric mucosa.[117]

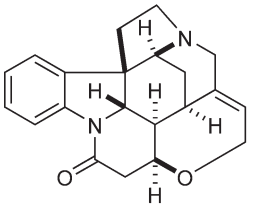
The bitter taste in foods is not always disliked by people. There are unexpectedly many cases in which a bitter taste has a positive effect of adding richness to food such as beer, coffee, and green tea. In these cases, if the bitter taste is eliminated or replaced by other compounds, the intrinsic value of the food might be completely lost. Therefore, the bitter taste is essential for these foods. On the contrary, however, methods to eliminate the unpleasant bitterness of cheese and grapefruit have also been investigated. These efforts also contribute to the progress in research on the bitter taste.[118]

Intensive studies on the bitter taste receptor are also in progress, as with the sweet taste receptor.[119] In fact, it has been reported that T2R receptors are necessary and sufficient for the detection and perception of bitter compounds.

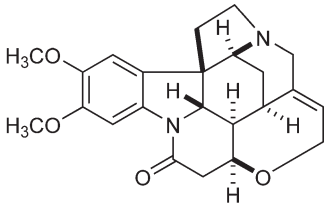
4.16.3.1 Bitter-Tasting Alkaloids

4.16.3.1.1 Strychnine

Strychnine is a highly toxic (LD_{50} i.v. in rats: 0.96 mg kg^{-1}) alkaloid that was isolated from the seeds of *Strychnos nux-vomica*, which is native to India and East Asia, and named after the tree. The structure of strychnine was determined by Woodward in 1948.[120] The total synthesis was also achieved by many chemists including Woodward. While it is barely soluble in water, its hydrochloride and nitric acid salts are water soluble. Strychnine elicits an intense bitter taste with a threshold of 0.000 001 6.[115] In India, China, and Japan, the seeds have long been used as a bitter-tasting gastric medicine, Vomica. Strychnine has also been used as a pesticide, particularly for killing small vertebrates such as rodents. Strychnine causes muscular convulsions and eventually death through asphyxia or sheer exhaustion.[121] At present, it is not used for clinical treatment, but is used in a pharmacological test as an analeptic. In nature, strychnine is produced by biosynthesis from tryptophan.



Strychnine



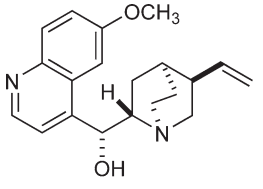
Brucine

4.16.3.1.2 Brucine

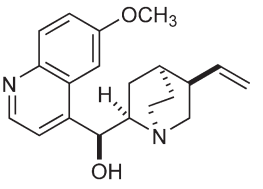
Brucine was also extracted as a principle, along with strychnine,[122] from the seeds of *S. nux-vomica*. Brucine is thought to be the most bitter-tasting alkaloid with a threshold of 0.000 000 7.[115] It is used for the chiral resolution of optically active carboxylic acids by diastereomeric salt formation. Brucine is isostructural to strychnine with methoxy groups, rather than hydrogen, at the 9- and 10-positions of the aromatic ring. Brucine is reported to be less toxic than strychnine. Nevertheless, a human consuming more than 2 mg of pure brucine will almost certainly suffer symptoms similar to strychnine poisoning.[123]

4.16.3.1.3 Quinine

Quinine was first extracted from the bark of the South American cinchona tree and isolated. In 1944, the total synthesis of quinine was achieved by Woodward and Doering.[124] Quinine exhibits specific toxicity against Plasmodium and has antipyretic (fever-reducing) activity. Therefore, it has long been used as an antimalarial drug. Although many other antimalarial drugs such as chloroquine have been developed based on the structure of quinine, it is still widely used since it is the sole compound to which Plasmodium has no resistance. Before 1820, the bark was first dried, ground to a fine powder, and then mixed into a liquid (commonly wine), which was then drunk. Quinine is a flavor component of tonic water, bitter lemon, vermouth, and cocktails. In the United States, the FDA limits quinine in tonic water to 83 ppm. Quinine is used as a standard substance for a bitter taste (threshold of sulfate salt: 0.000 008) in gustatory physiology.[125] It is also useful as an optical resolution agent and as an asymmetric catalyst.[126,127] Quinidine, a diastereomer of quinine, is also extracted from the bark and elicits a bitter taste. It is reported to have an antiarrhythmic effect.[128]



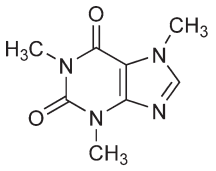
Quinine



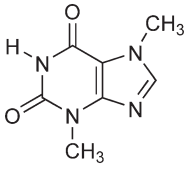
Quinidine

4.16.3.1.4 Caffeine

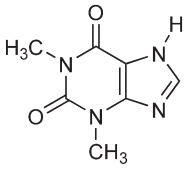
Caffeine is a bitter-tasting purine alkaloid.[129] Its threshold (0.000 7) indicates that the bitter intensity of caffeine is weaker than quinine and brucine. Caffeine was extracted from coffee and named after it. Caffeine is also contained in cola, black tea, green tea, cocoa, chocolate, and so on. It is well known that caffeine has antihypnotic, antipyretic, and diuretic effects.[130] Caffeine is a central nervous system and metabolic stimulant and is also used medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Sometimes, caffeine is incompatible with other drugs. For example, it has been reported that cimetidine decreased the systemic clearance of caffeine. Overdosing of caffeine may lead to a condition known as caffeinism.[131] Caffeinism usually combines caffeine dependency with a wide range of unpleasant physical and mental conditions including nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations. Today, the global annual consumption of caffeine has been estimated at 120 000 tons. The US FDA lists caffeine as a Multiple Purpose GRAS Food Substance.



Caffeine (coffee)



Theobromine (cocoa)



Theophylline (green tea)

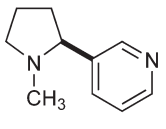
4.16.3.1.5 Theobromine and theophyllin

Theobromine was isolated from the seeds of the cacao tree and then shortly afterward was synthesized from xanthine by Fischer.[132] Theobromine is the primary bitter-tasting alkaloid found in cocoa and chocolate; chocolate contains 0.5–2.7% theobromine. Theobromine is water insoluble and is an isomer of theophylline as well as paraxanthine. Theobromine is categorized as 3,7-dimethylxanthine while theophylline is 1,3-dimethyl-7*H*-purine-2,6-dione and paraxanthine is 1,7-dimethylxanthine. Theophylline is known to be a bitter-tasting principle of green tea. Theobromine is used as a vasodilator (a blood vessel widener), as an aid in urination, and

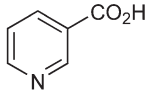
as a heart stimulant. Although the theobromine content in chocolate is small enough to be safely consumed by humans, it is reported that animals such as dogs metabolize theobromine more slowly and may succumb to theobromine poisoning.[133]

4.16.3.1.6 Nicotine

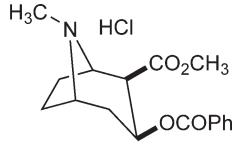
Nicotine is a bitter-tasting alkaloid found in the nightshade family of plants (Solanaceae), predominantly in tobacco. At low doses (an average cigarette yields about 1 mg of absorbed nicotine), nicotine acts as a stimulant in mammals and is one of the main factors responsible for the dependence-forming properties of tobacco smoking.[134] The biosynthesis is carried out from tryptophan via nicotinic acid. Nicotinic acid reacts with a piperidine compound derived from lysine to give anabasine as a homologue of nicotine. Nicotine exists in tobacco leaves as salts with malic acid or citric acid. There are more than 30 analogues of nicotine. Nicotine is essential for the synthesis of a water-soluble vitamin, nicotinic acid (niacin). Nicotine has an addictive nature and has been found to activate reward pathways – the circuitry within the brain that regulates feelings of pleasure and euphoria. The LD_{50} of nicotine is 3 mg kg^{-1} for mice, and 40–60 mg (0.5–1.0 mg kg^{-1}) for adult humans can be a lethal dosage.[135] This means it is deadly poisonous. It is more toxic than many other alkaloids such as cocaine, which has an LD_{50} of 95.1 mg kg^{-1} when administered to mice.



Nicotine



Nicotinic acid



Cocaine

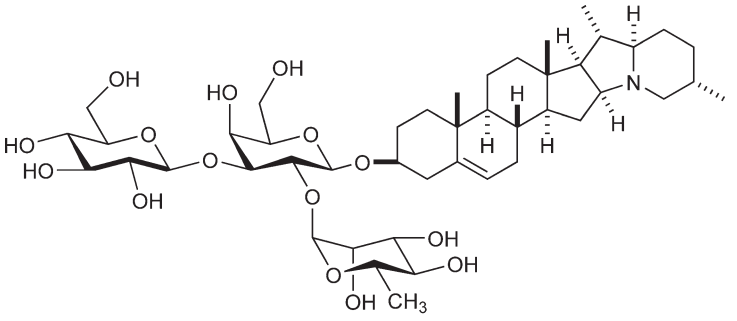
4.16.3.1.7 Cocaine

Cocaine is a bitter-tasting alkaloid extracted from the leaves of the coca plant (*Erythroxylon coca* Lam.).[136] It has a tropane skeleton and is synthesized from ornithine in nature. In medicine, cocaine is used in a limited manner as a topical anesthetic for nasal and lacrimal duct surgery. Cocaine is a potent central nervous system stimulant. Its effects can last from 20 min to several hours, depending upon the dosage. The initial signs of stimulation are hyperactivity, restlessness, increased blood pressure, increased heart rate, and euphoria. The euphoria is sometimes followed by feelings of discomfort and depression and a craving to experience the drug again. Side effects include twitching, paranoia, and impotence. Cocaine addiction is reported to be physical and psychological dependence may develop with regular use. It may result in physiological damage, lethargy, depression, or a potentially fatal overdose.[137] Therefore, its possession, cultivation, and distribution are illegal for nonmedicinal and non-government-sanctioned purposes in virtually all parts of the world. On the contrary, coca herbal infusion (referred to as Coca tea) is used in coca-leaf-producing countries much as any herbal medicinal infusion would be elsewhere in the world. The free and legal commercialization of dried coca leaves in the form of tea bags to be used as 'coca tea', as a drink with medicinal powers,[138] has been actively promoted in Peru and Bolivia. It is also used to help visitors overcome the malaise of high-altitude sickness.

4.16.3.1.8 Solanine

Solanine is a bitter-tasting steroidal alkaloid saponin that has been isolated from all nightshades, including tomatoes, capsicum, tobacco, and eggplant.[139] However, the most widely ingested solanine is from the consumption of potatoes. Potato leaves, stems, and shoots are naturally high in this saponin. When potato tubers are exposed to light, they turn green and increase saponin production. This is a natural defense mechanism to prevent the uncovered tuber from being eaten. It is very toxic even in small quantities. The poisoning is primarily manifested by gastrointestinal and neurological disorders.[140] Symptoms include nausea, diarrhea, vomiting, stomach cramps, burning of the throat, heart arrhythmia, headache, and dizziness. Hallucinations, loss of sensation, paralysis, fever, jaundice, dilated pupils, and hypothermia have been reported in more severe cases. It is suggested that doses of 200–400 mg for adult humans can cause toxic

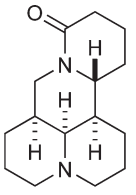
symptoms (20–40 mg for children). Most commercial potatoes have a solanine content of less than 0.2 mg g^{-1}.[141] However, potatoes that have been exposed to light and have started to turn green can show higher concentrations.



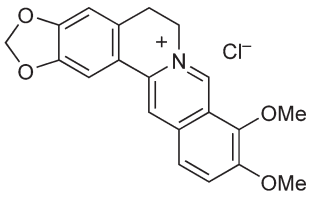
Solanine

4.16.3.1.9 Matrine and berberine

An herbal medicine, kushen, is obtained from the roots of *Sophora japonica* (*Sophora flavescens*) and is used as an anti-inflammatory and bitter-tasting stomachic. The principal component of kushen is the piperidine alkaloid matrine.[142] Berberine, which is isolated from the bark of *Phellodendron amurense* (Rutaceae) as well as *Coptis japonica* Makino (Ranunculaceae), is also an intensely bitter-tasting alkaloid that is named after Berberis.[143] There are a variety of known derivatives such as berberine hydrochloride, hydrosulfate, and tannic acid salt according to the kind of counteranion, and all are used as stegnotic agents. There is some controversy regarding the antibiotic activity of berberine. In Japan, berberine hydrochloride is available commercially. It is contraindicated for use in hemorrhagic colitis and bacteriogeneous diarrhea, since it might make the symptoms worse and prolong the duration of treatment.



Matrine



Berberine chloride

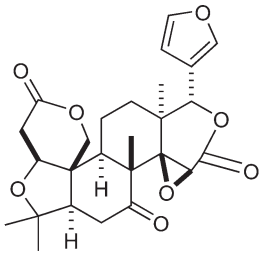
4.16.3.2 Bitter-Tasting Terpenoids

There are many bitter-tasting terpenoids in the plant kingdom. Among them, limonoids in citrus fruits, the cucurbitacin in members of the family Cucurbitaceae, and humulon analogues in hop have been intensively investigated. The structure–activity relationships of bitter-tasting terpenes have been studied for many years, and Kubota[144] and Beets[145] have proposed hypotheses.

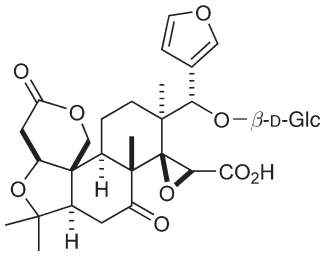
4.16.3.2.1 Limonoids

Several limonoids are known to be bitter principles of citrus (Rutaceae). A typical example is limonin. Although fresh juice does not elicit a bitter taste, sometimes it becomes bitter after heating or storage. This is explained by the formation of bitter-tasting limonin by deglycosylation and further cyclization from limonin glucoside, which is present in citrus fruit tissue and seeds and does not exhibit bitterness.[146] Recently, it was reported that limonin had antitumor activity.[147] Besides limonin, nomilin and obakunone, which are considered to be

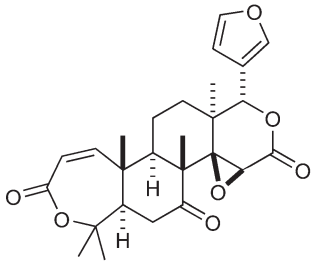
biosynthetic intermediates of limonin, have also been isolated as bitter-tasting components.[148] These three limonoids are formed from squalene via cyclization and oxidation.



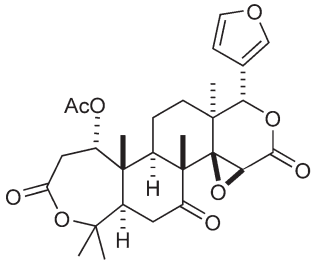
Limonin



Limonin glucoside



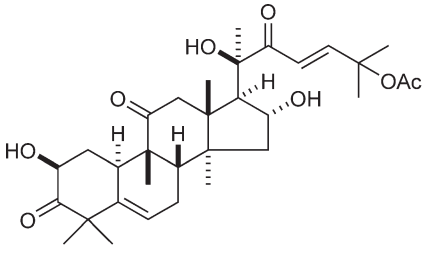
Obacunone



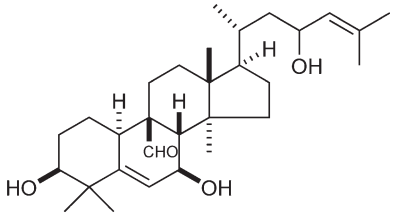
Nomilin

4.16.3.2.2 Cucurbitacin and momordicine

Cucurbitacin is a bitter-tasting principle that can be isolated from members of the family Cucurbitaceae, such as cucumber (*Cucumis sativus*) and melon (*Cucumis melo* L.). In particular, cucurbitacin[149] and momordicine,[150] which have an intensely bitter taste, are contained abundantly in *Momordica charantia* (bitter melon in English, go-yaa in Okinawa, Japan), which people enjoy due to its bitterness. There are more than 18 kinds of cucurbitacin, and among them cucurbitacin B is a typical component. It has been reported that cucurbitacin exhibits anticancer activity.[151] In addition, it is used for the treatment of hepatic disease in traditional Chinese medicine. It is also found in some herbal teas.



Cucurbitacin B

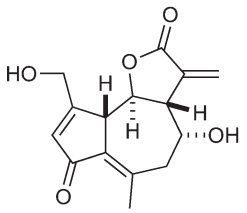


Momordicine I

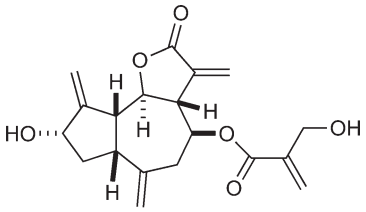
4.16.3.2.3 Lactucin and cynaropicrin

Lactucin is a bitter principle of the leaf vegetable chicory (*Cichorium endivia*), which is cooked or used for salads in western Europe.[152] It is also contained in the form of *p*-hydroxyphenyl acetate as lactucopicrin, which is known to have a sedative effect on the central nervous system.[153] On the contrary, a bitter-tasting sesquiterpene

lactone, cynaropicrin, can be isolated from artichoke (*Cynara scolymus*). Cynaropicrin has been reported to exhibit immunomodulatory effects.[154]



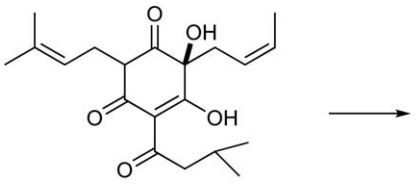
Lactucin (chicory)



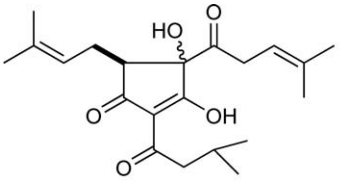
Cynaropicrin (artichoke)

4.16.3.2.4 Humulone

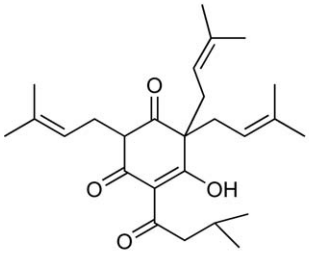
Humulone is a well-known bitter principle of beer. At least 32 derivatives of humulone such as luplone, cohumulone, and adhumulone have been isolated, many of which are not contained in hop (*Humulus lupulus*) but rather are formed during fermentation and preservation.[155] For example, humulone is transformed into isohumulone to elicit a bitter taste in beer. Hop (*Humulus*) is a small genus of flowering plants, native to the temperate Northern Hemisphere. The female flowers, commonly called hops, are used as flavoring and stabilizers during beer brewing. Hop is part of the family Cannabaceae, which also includes the genus *Cannabis* (also known as hemp). Beer has been used as folk medicine in Europe for a long time due to its diuretic and stomachic effect. Recently, new medicinal uses and properties of humulone derivatives are being explored.



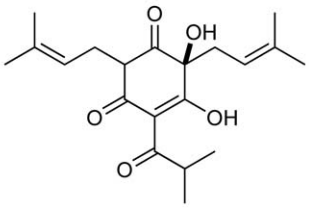
Humulone



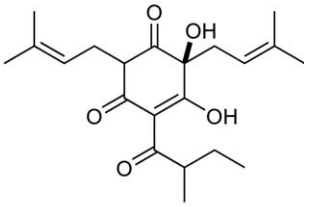
Isohumulone



Lupulone



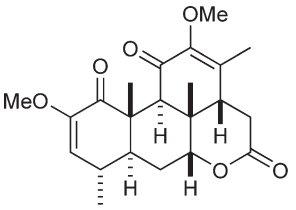
Cohumulone



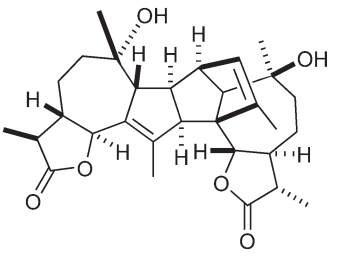
Adhumulone

4.16.3.2.5 Quassin

Quassin is a bitter-tasting substance that can be extracted from the quassia tree (bitter tree, *Picrasma quassioides* Benn).[156] It is said to be the most bitter substance found in nature. Quassin is used in traditional Chinese medicine. Besides quassin, modified triterpenes, the so-called quassinoid, are the principal component of the bitter taste.



Quassin



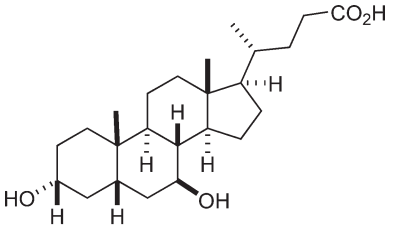
Absinthin

4.16.3.2.6 Absinthin

Absinthin is one of the most bitter substances known and is extracted from various plants of the genus *Artemisia*, but most commonly absinth wormwood.[157] The simple maceration of wormwood in alcohol without distillation produces an extremely bitter drink because of the presence of water-soluble absinthin. Wormwood extract can cause renal failure and death due to excessive amounts of thujone, which in large quantities acts as a convulsive neurotoxin. Absinthe was once portrayed as a dangerously addictive, psychoactive drug; thujone was blamed for most of its deleterious effects. Therefore, it was prohibited in several European countries and the United States. However, it has since been verified that no evidence shows it to be anymore dangerous or psychoactive than ordinary alcohol. An absinthe revival began in the 1990s, as countries in the European Union began to reauthorize its manufacture and sale.

4.16.3.2.7 Ursodiol

Ursodeoxycholic acid (ursodiol), which is found in large quantities in bear bile, and which elicits an intense bitter taste, has long been used in traditional Chinese medicine not only as a stomachic but also as a universal drug for the alimentary system.[158] Currently, the drug is generally derived by chemical synthesis rather than from animals.



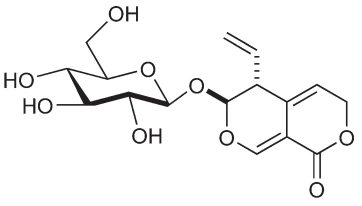
Ursodeoxycholic acid

4.16.3.3 Bitter-Tasting Saponins

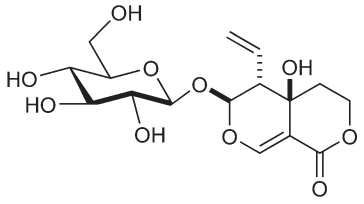
4.16.3.3.1 Secoiridoid saponins

Senburi is a biennial herb, *Swertia japonica* (*Ophelia japonica*), Gentianaceae, that is native to Japan.[159] Senburi is considered as one of the most popular medicinal herbs in Japan and is the most bitter Japanese herb. Senburi, also called 'touyaku' in traditional medicine, literally means 'still bitter after one thousand times infusion'. It is used for the treatment of gastrointestinal disease, diarrhea, and bellyache, and also as a digestive stimulant. The principal components of Senburi are bitter-tasting saponins such as swertiamarin, sweroside, amarogentin, amaroswerin, and gentiopicroside. Among them, amaroswerin is one of the most bitter-tasting natural products.[160]

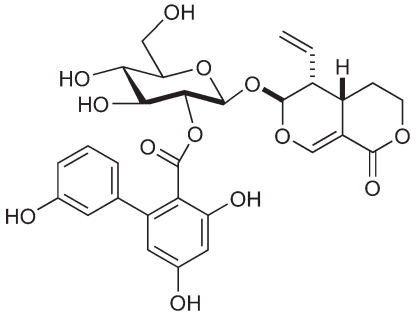
Gentiopicroside is also contained in the herbal medicine Gentiana (Ryutan), which is the extract of the root of *Gentiana lutea* and has gastroprotective effects.[117] It has a secoiridoid structure, which is a common constituent in members of the family Gentianaceae.



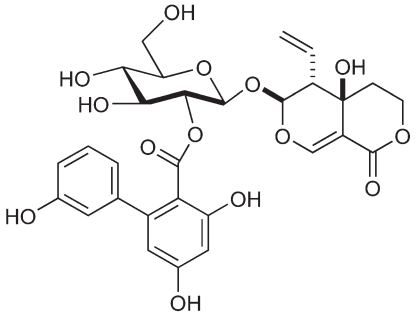
Gentiopicroside



Swertiamarin



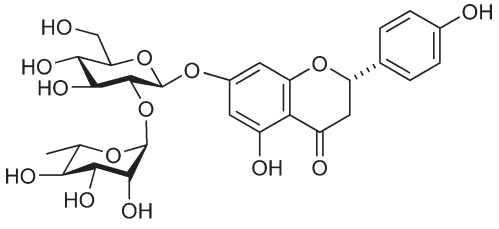
Amarogentin



Amaroswerin

4.16.3.3.2 *Flavonoid glycoside*

Naringin is a flavonoid glycoside that is abundantly contained in the skin of grapefruit and orange and is the origin of their bitterness.[161,162] Its aglycon is naringenin, which is synthesized by a shikimic acid pathway and occurs naturally in citrus fruits. To remove the bitterness of naringin in the production of canned citrus juice, an enzymatic hydrolysis process using naringinase is sometimes employed.[163] It has been reported that naringin exerts a variety of pharmacological effects such as antioxidant activity, anticarcinogenic activity, and inhibition of selected cytochrome P450 enzymes, which may result in several drug interactions *in vitro*. However, this notion is still controversial.[164]



Naringin

4.16.3.4 Bitter-Tasting Amino Acids and Peptides

4.16.3.4.1 *Amino acids*

Ever since Fischer, many chemists have focused their attention on the taste of amino acids. Generally, natural L-amino acids exert either no taste or a bitter taste while unnatural D-amino acids elicit a sweet taste almost without exception. Proteinogenic L-amino acids that exhibit a bitter taste include Trp (0.133%), Phe (0.069%), Tyr (0.017%), Leu (0.011%), Arg, Val, Ile, and Pro, and the remaining amino acids exert either no taste or a sour taste. The values in parentheses show the caffeine concentration that provides the same bitterness as a 0.3% amino acid solution.[165] However, different authors have reported different values for the strength of their

bitterness. A basic amino acid, arginine, exhibits bitterness along with a sweet taste. Glycine and alanine elicit a sweet taste. The bitter taste of an amino acid will be enhanced with an increase in the bulkiness of any substituent.

4.16.3.4.2 Peptides

Studies of bitter-tasting peptides arose from interest in the bitter constituent of cheese. Murray reported that the bitter taste accumulated after casein was hydrolyzed by various proteases and the principal component of its bitterness was peptides.[166] Afterward, several groups successfully isolated the bitter peptides from the hydrolyzate of casein. For example, formation of the bitter peptide QNKIHPFAQTQSLVYPFPGPIP was identified during the maturation of cheddar cheese.[167] Furthermore, hydrolysis of the constituent with peptidase makes the bitter taste worse. On the contrary, Fujimaki and coworkers[168] successfully eliminated the bitter taste of soybean peptides using a plastein reaction. These peptides characteristically contain mainly hydrophobic amino acids as constituents. Interestingly, a diketopiperazine, cyclo (Trp-Leu),[169] exhibits a bitter taste regardless of the optical isomer and cyclo (Val-Phe) and cyclo (Pro-Phe) have been reported to be bitter substances formed from cacao beans during roasting.[170]

4.16.3.5 Masking the Bitter Taste

As described above, many natural products elicit a bitter taste. Thus, it is an important issue in the formulation of pharmaceuticals to determine how to mask this bitterness. Although this issue has been somewhat overcome by coating technology in the manufacture of tablets, pills, and granules, the method used in liquid medicines is still unsatisfactory, though some approaches that involve the addition of high concentrations of sugars and acids have been developed. Kurihara and coworkers[171] reported that the bitter taste in response to quinine could be selectively suppressed with phosphatidyl acid in phospholipids of soybean.

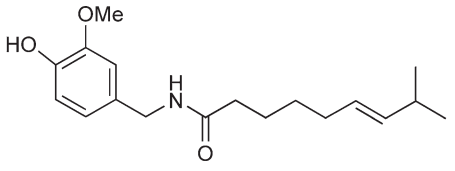
4.16.4 Pungent Natural Products

Pungency is generally associated with fiery stimuli and is sometimes an intolerable sensation for humans. However, it is effective at increasing appetite in many cases and is used in the cuisine of several cultures. Pungency is not one of the five basic tastes, but rather is characterized by trigeminal sensation in the mouth through the sensory modalities of touch, thermal sensation, and pain. There are several subtypes of pungency that basically depend on the chemical properties of the pungent constituents. In addition to pungency (hot sensation), a tingling effect and a cooling sensation on the tongue are important for cuisine. As a measure of the 'hotness', or more correctly, piquancy, of a chili pepper, the Scoville scale has been proposed.[172,173] Fruits of the *Capsicum* genus contain capsaicin, which stimulates chemoreceptor nerve endings in the skin, especially the mucus membranes. The number of Scoville heat units (SHU) indicates the amount of capsaicin present; however, it cannot be used as a measure of piquancy in foods without capsaicin.

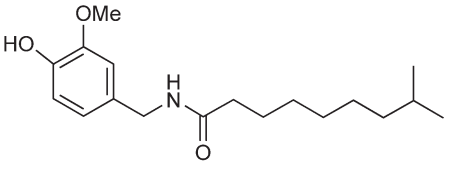
4.16.4.1 Capsaicin

Normally, spices used for cooking provide a pungent sensation. A typical pungent spice is red pepper (hot chili pepper), which contains amide derivatives, including capsaicin, as pungent constituents.[174] It elicits intense pungency and is an interesting compound from the viewpoint of its pharmacological action. Thus, it has been reported that capsaicin exhibits hypermetabolism as well as sweating by promoting the secretion of adrenaline.[175] In addition, it also has strong antibacterial activity along with an antisepsis function.[176] Capsinoids are amides of vanillylamine with a variety of aliphatic acids. Capsaicin is the main capsinoid in chili peppers, followed by dihydrocapsaicin. These two compounds have almost the same potency and other compounds such as nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin have been isolated as minor capsinoids. Dilute solutions of pure capsinoids produce different types of pungency; however, these differences were not noted using more concentrated solutions. Nakatani and Masuda.[177] isolated capsaicinol with only one hydroxy group in capsaicin. Intriguingly, they reported that capsaicinol did not exhibit pungency. Very recently, Yazawa

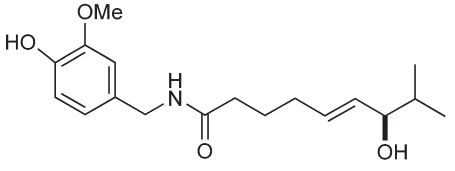
et al.[178] found that a nonpungent red pepper, CH-19 Sweet, contained only a trace amount of capsaicin. Instead, capsiate and dihydrocapsiate, which have an ester bond with vanillyl alcohol, were the major constituents of CH-19 Sweet.[179] Interestingly, the pungency disappeared when the amide was changed to an ester and this finding has attracted considerable attention to solve the mechanism of pungency perception, and intensive studies on the vanilloid receptor subtype 1 (VR1) are in progress.[180] Recently, it has also been shown that the glucosylation of capsaicin diminished its pungency.[181] Therefore, studies on glucosylation are ongoing. Studies on the prevention of obesity by capsinoid and capsaicin glucoside are also in progress.[182]



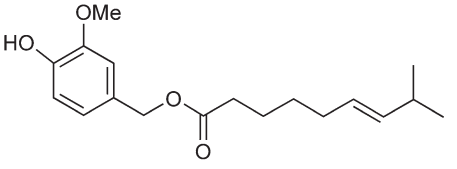
Capsaicin



Dihydrocapsaicin



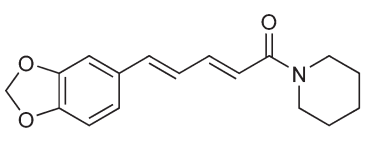
Capsaicinol



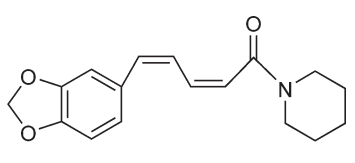
Capsiate

4.16.4.2 Piperine

Piperine is the alkaloid responsible for the pungency of black pepper, *Piper nigrum* (Piperaceae), and *Piper longum* L., commonly known as long pepper.[183] Three geometrical isomers of piperine (chavicine, isochavicine, and isopiperine) and piperanine (dihydro- form of piperine) are other constituents of these plants. Previously, chavicine was believed to cause the particular taste of pepper. However, it was reported later that only piperine has a strong pungent taste.[184] Although an increment in the three isomers in ground pepper after exposure to sunlight was observed by monitoring the MH^+ ion on liquid chromatography/atmospheric pressure chemical ionization mass spectrometer (LC/APCIMS), the degree of increase varied very little. Therefore, it is questionable whether the disappearance of the pungency in older ground pepper is derived from the formation of tasteless isomers by photochemical changes in piperine.[185] Piperine is a solid substance that is essentially insoluble in water. It is initially tasteless, but leaves a burning aftertaste. Piperine belongs to the vanilloid family of compounds, which also includes capsaicin, the pungent substance in hot chili peppers. Piperine may have bioavailability-enhancing activity for some nutritional substances and for some drugs.[186] It has putative anti-inflammatory activity and may be active at promoting digestive processes. Recently, with advances in analytical methods, many piperine derivatives have been isolated and their structures have been determined by Tsuda and coworkers.[187]



Piperine

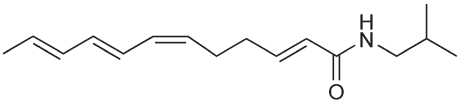


Chavicine

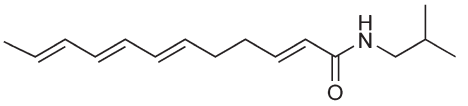
4.16.4.3 Sanshool and Gingerol

Sanshool is the pungent constituent in Sichuan pepper (Sansho in Japan).[188] It is contained in the outer pod of the tiny fruit of several species of the genus *Zanthoxylum* (most commonly *Zanthoxylum piperitum*, *Zanthoxylum simulans*, *Z. piperitum sansho*, and *Zanthoxylum schinifolium*), which are widely grown and consumed in Asia as a spice. Despite the name, it is not related to black pepper or chili peppers. It is widely used in the cuisine of Japan. Several sanshools have been isolated, such as α- and β-sanshool, both of which have an amide structure.[189] Sanshools exhibit several biological activities such as an insecticidal effect. A similar amide, spilanthol, is also found in the aerial part of *Spilanthes acmella* and *S. acmella* var. *oleracea*.[190]

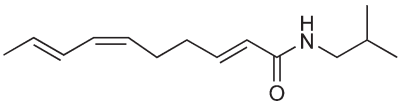
Gingerols are the pungent constituents of the rhizome of ginger, which is often used as a folk medicine and food.[191] Each gingerol has a phenol group as a substituent. The major constituent is 6-gingerol, which has vanillyl ketone (gingerone or zingerone) as a framework, similar to vanillylamine of capsaicin, but without an amide bond. Gingerone itself is also found in ginger root.[192] (6)-Shogaol is also found as a pungent component of ginger and is contained in semidried ginger but is rarely found in fresh ginger.[193]



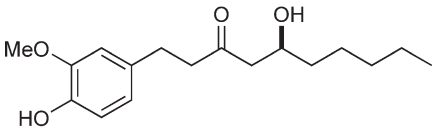
α-Sanshool



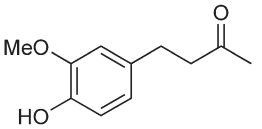
β-Sanshool



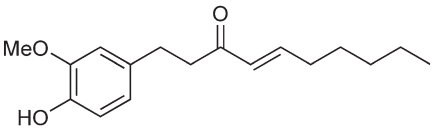
Spilanthol



(S)-(+)-[6]Gingerol



Gingerone (Zingerone)

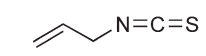


(6)-Shogaol

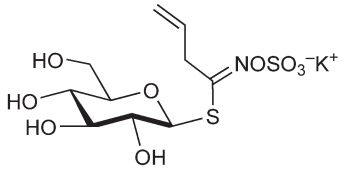
4.16.4.4 Isothiocyanates

Wasabi (*Wasabia japonica*, *Cochlearia wasabi*, or *Eutrema japonica*) is a member of the Brassicaceae family and is also known as Japanese horseradish. The rhizomes are used as a spice. Wasabi is enjoyed with sushi and sashimi, usually accompanied by soy sauce. It has an extremely strong and stimulating flavor with burning sensations. Its hotness is more akin to that of hot mustard than capsaicin in chili pepper, in that it produces vapors that irritate the nasal passages more than the tongue. Allyl isothiocyanate (AITC) and several other isothiocyanates are known to be the pungent constituents, and among them AITC is the main constituent (reported to be $\sim$2.0 g kg^{-1}).[194] It is thought to be beneficial to eat raw fish with wasabi because it has a sterilizing effect. AITC itself does not exist in wasabi, but it is formed immediately upon grating the root very finely. Thus, a glucosinolate (known as sinigrin) present in wasabi reacts with the enzyme myrosinase upon grating, and this leads to the production of AITC.[195] This suggests that sinigrin and myrosinase are present in different parts of the plant tissue. There are almost 30 known glucosinolates other than sinigrin, the biosynthetic pathway of which has also been clarified.[196] Based on its sterilizing effect, AITC is used in Japan to maintain freshness in

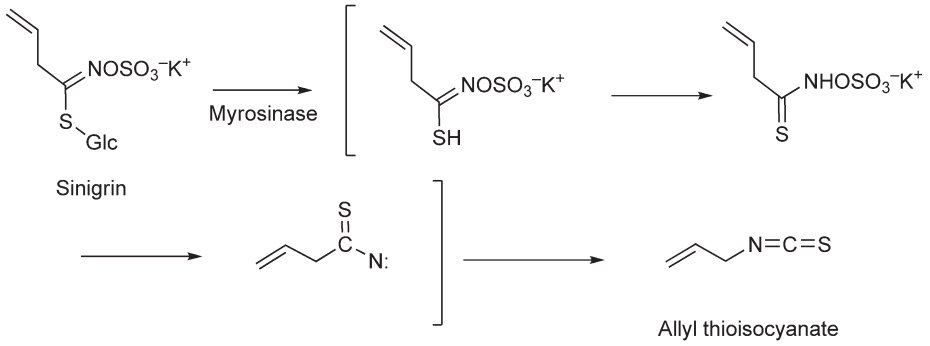
refrigerators. The pungent constituent of the horseradish root is also AITC, and methyl-3-butenyl isothiocyanate is the constituent in the Japanese radish.[197]



Allyl thioisocyanate
pungent taste of wasabi



Sinigrin

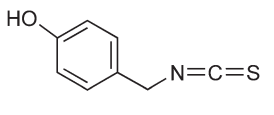


Sinigrin

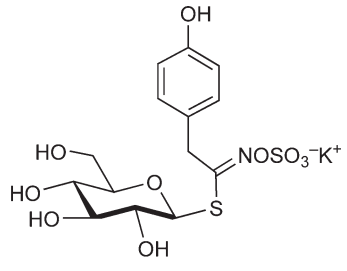
Allyl thioisocyanate

Formation mechanism of allyl thioisocyanate

Mustard is prepared from the ground seeds of mustard plants (white or yellow mustard, *Sinapis hirta*; brown or Indian mustard, *Brassica juncea*; or black mustard, *Brassica nigra*) by mixing them with water and adding ingredients such as flour. Mustard sensation can cause the eyes to water, burn the palate, and inflame the nasal passages. The pungent constituent of black mustard is sinigrin, as in wasabi, whereas that of white mustard is sinalbin.[198] In this case, *p*-hydroxybenzyl isothiocyanate is formed in the reaction with the enzyme myrosinase. The pungency of these spices is represented as hot in red (chili) and black pepper, and as sharp for the isothiocyanate family.



p-Hydroxybenzyl thioisocyanate
(pungent taste of white mustard)



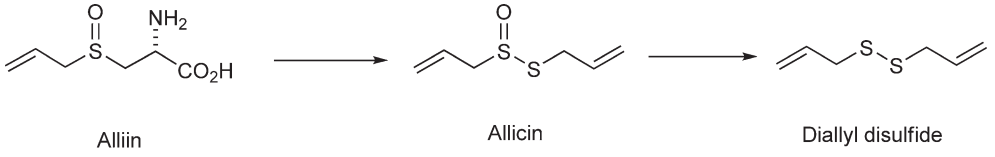
Sinalbin

4.16.4.5 Disulfides

Garlic, *Allium sativum* L., is a species in the onion family, Alliaceae. Onion, shallot, and leek are close relatives. Garlic has been used throughout history for both culinary and medicinal purposes. It has a pungent 'hot' sensation that mellows and sweetens considerably with cooking. A large number of sulfur compounds contribute to the smell and taste of members of the onion family. Diallyl disulfide and diallyl sulfide are

believed to be important odor and flavor components. Allicin has been found to be the constituent that is most responsible for the spiciness of raw garlic.[199] However, alliin, rather than allicin, is present in the plant before the cells are damaged. When a cell is broken, allicinase triggers the breakdown of alliin to give allicin, which has a strong smell and is converted to diallyl disulfide by reduction.[200]

The pungent constituents of onion are somewhat different from those of garlic and this accounts for their different odors and pungency. It has been reported that the major pungent constituents of onion are di-*n*-propyl disulfide and methyl-*n*-propyl disulfide.[201]



Formation mechanism of diallyl disulfide

4.16.5 Astringent Natural Products

4.16.5.1 Tannin

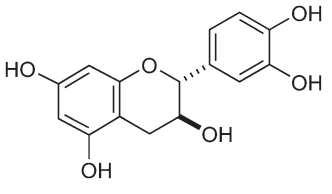
Tannins are astringent-tasting polyphenols found in plants that can bind and precipitate proteins. While the term tannin was originally derived from the use of tannins in tanning animal skins to make leather, the term is widely applied to any polyphenolic compound that forms strong complexes with proteins. The molecular weight of tannins ranges from 500 to more than 3000. There are two types of tannins, hydrolyzable tannins and condensed tannins.[202] At the center of a hydrolyzable tannin, there is a carbohydrate such as D-glucose. The hydroxyl groups of the carbohydrate are partially or totally esterified with phenolic acids such as gallic acid (in gallotannins). Hydrolyzable tannins can be hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids. Condensed tannins, known as proanthocyanidins, are widely distributed in plants and strongly affect the quality of foods. They are polymers of 2–50 (or more) flavonoid units, which are not susceptible to hydrolysis. Some very large condensed tannins are insoluble, while hydrolyzable tannins and most condensed tannins are soluble in water. All phenolic compounds are highly unstable and are rapidly transformed into various reaction products when the plant cells are damaged (for instance, during food processing), thus adding to the complexity of dietary polyphenol compounds. This accounts for the complexity of polyphenols in food.

Holding tannins in the mouth produces a strong sensation of astringency. It is believed that tannins bind to proteins in the tongue and mucous membrane in the oral cavity and then induce their denaturation.[203] This type of denaturation of proteins in the oral mucosa by tannins is called a shrinking effect. Strictly, it is considered that astringency is closely related to the pain and tactile sensation generated by protein denaturation. To feel the astringent effect of tannins, they must be dissolved in saliva. Accordingly, an increase in the molecular weight by polymerization causes a lack of astringency due to the insolubility of tannins. Some persimmons are highly astringent and therefore inedible due to the presence of soluble tannins. However, when they are dried or ripened, the astringency disappears due to the insolubility of tannins.[204] These condensed tannins consist of a high-molecular-weight- (∼15 000) polymer formed from catechins. They exhibit strong protein coagulation activity and therefore are used to clarify Japanese sake as well as a preservative.

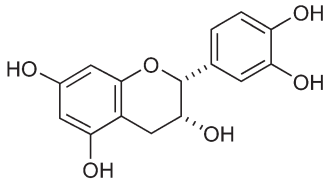
4.16.5.2 Catechins in Green Tea

Astringency in green tea (Japanese tea) is due to catechins, which are a kind of condensed tannins. The largest source is various teas derived from the tea plant *Camellia sinensis*. Catechins have various biological activities,

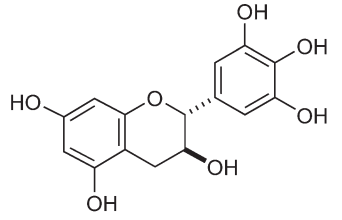
such as antibacterial and antiviral activities. The health benefits of catechins have been studied extensively in humans and in animal models. A reduction of carcinogenesis has been observed *in vitro*. The catechin content in the tea plant, and therefore its astringency, increases with an increase in exposure to sunlight. Although green tea contains several constituents, such as proteins, amino acids, vitamins, and caffeine, catechins are the major constituents and have attracted attention due to their health benefits. The catechins in green tea include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and catechin.[205] The most abundant (50%) of these is EGCG, which exhibits strong antioxidant activity.[206] Recently, an air filtration system against virus was developed in Japan using tea catechins.



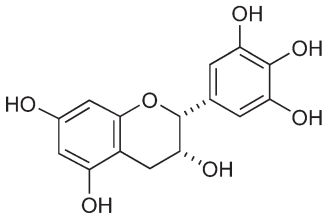
(+)-Catechin



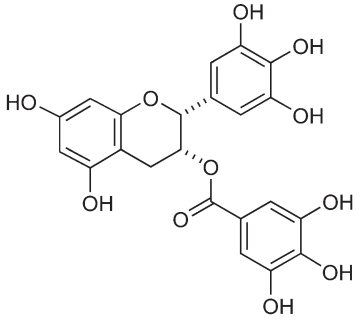
(–)-Epicatechin



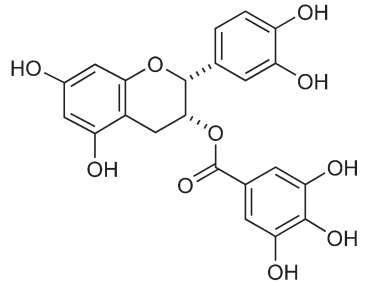
(+)-Gallocatechin



(–)-Epigallocatechin



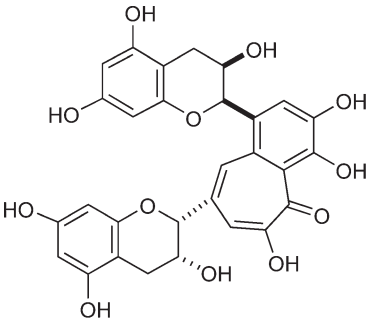
(–)-Epigallocatechin gallate



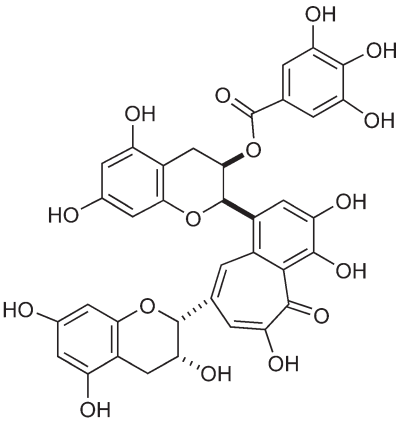
(–)-Epicatechin 3-gallate

4.16.5.3 Theaflavins and Thearubigins in Black Tea

Tannin constituents such as thearubigins and theaflavins present in black tea are formed by the enzymatic oxidation of EC and EGC followed by condensation, and this causes the characteristic astringency.[207] Theaflavins have benzotropolone structures and therefore give a red color in a black tea fusion. It has been reported that the relative proportions of theaflavins in black tea are theaflavin (18%), theaflavin-3-gallate (18%), theaflavin-3′-gallate (20%), theaflavin-3,3′-digallate (40%), and minor derivatives such as theaflavic acids.[208] These compounds are contained in oolong tea (a traditional Chinese tea), but are not as abundant as in green tea. It has been reported that theaflavins have various biological activities, such as antioxidant and anticancer activities. Thearubigins comprise 10–20 % of the dry weight of black tea. However, due to their high solubility in water, they account for 30–60% of the solids in black tea infusion. They are polymeric catechins that are formed during the enzymatic oxidation (called fermentation in the tea trade) of tea leaves. In contrast to theaflavins, thearubigins contain polysaccharides and proteins in the polymer. Gallic acid, cyanidins, and delphinidin are formed by acid hydrolysis and catechins are produced by reductive hydrolysis. These results indicate that thearubigins are a mixture of proanthocynidins containing flavonoid residues.[209]



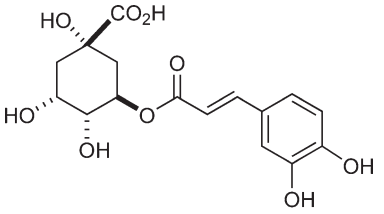
(–)-Theaflavin



Theaflavin 3′-gallate

4.16.5.4 Chlorogenic Acid in Coffee

Chlorogenic acid was isolated from green coffee beans.[210] It has also been found in the seeds and leaves of many dicotyledonous plants. It is thermally unstable and is readily decomposed to quinic acid and caffeic acid. Chlorogenic acid accounts for 5–10% of coffee beans, which is a much larger amount than caffeine (1–2%). Chlorogenic acid strongly influences the taste of coffee, such as astringent, sweet, and sour tastes, which change with the concentration. It is also considered to be the origin of the unpleasant complex taste found after prolonged brewing. It forms greenish-black compounds in the presence of Fe(III) ion. Due to its radical-capturing ability, an antioxidant activity is expected.[211]

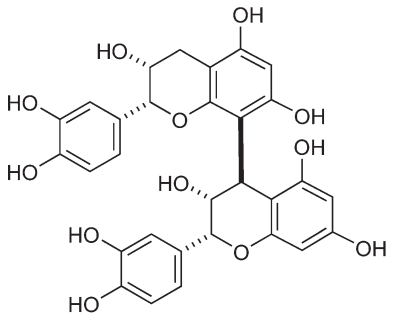


Chlorogenic acid

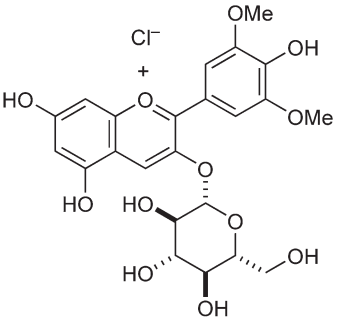
4.16.5.5 Anthocyanins in Red Wine

Anthocyanins (red pigments) and tannins are particularly important components of red wine. The changes in color and taste observed during the aging of red wine have been ascribed to anthocyanin–tannin reactions. The structures and properties of the tannins and pigmented tannins from these reactions are often misunderstood. Current research on phenolic compounds in wine has revealed that (1) reactions of tannins yield both larger polymers and smaller species, (2) anthocyanin reactions can generate colorless species as well as polymeric and various small pigments, (3) some polymeric pigments undergo sulfite bleaching while some low-molecular-weight pigments do not, (4) polymers are both soluble and astringent, so the loss of astringency during aging may involve cleavage rather than polymerization, and (5) sensory properties of anthocyanins and tannins are modulated by interactions with other wine components.[212] However, while great advances have been made in the field of red wine chemistry in recent years, a better understanding of the effect of wine polyphenol–salivary protein interaction is needed to gain a comprehensive understanding of red wine astringency.

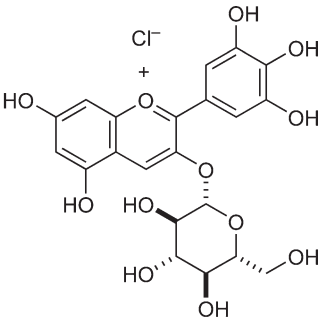
Anthocyanins are known to have antioxidant activity. Recently, they were also reported to have potential effects against cancer, aging and neurological diseases, inflammation, diabetes, and bacterial infections.[213]



Procyanidine B-2



Malvidin 3-glucoside



Delphinidin 3-glucoside

Structures of tannin derivatives in red wine

4.16.6 Umami and Kokumi

4.16.6.1 Umami-Tasting Natural Products

The postulate that umami is a basic taste quality was not generally accepted for a very long time. As a result of mounting research evidence obtained not only by Japanese but also by Western researchers from North America and Europe, the umami taste was finally recognized as the fifth basic gustatory quality at the first International Symposium on Umami held in Hawaii in 1985. Today, the Japanese word 'umami' has been established as the worldwide technical term for this savory taste quality.[4] The analysis of sensory evaluation data carried out by the multidimensional scaling method has provided numerical validation to substantiate the notion that the umami taste of substances such as sodium glutamate lies outside the gustatory space formed by the traditional four basic gustatory qualities. It has been established that (1) taste cells have receptors that bind with umami substances and produce an electrophysiological response to umami substances, (2) there are taste nerves that transmit umami stimuli, and (3) there are sites in the brain that respond to umami stimuli in the same manner as they respond to the other four basic tastes. Recent research has progressed to the point of cloning potential umami taste receptors. Currently, two types of receptors for the umami taste have been proposed: mGluR4[214] and T1R1/T1R3 heterodimer.[215,216]

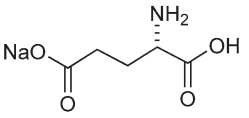
4.16.6.1.1 Monosodium glutamate

In 1908, Ikeda isolated MSG as the principal component of the savory taste contained in soup stock of kelp and named the sensation umami (delicious taste in Japanese).[2] MSG is now widely used as a flavor enhancer and seasoning, and current annual consumption worldwide is estimated to be 1.8 million tons. The present production method relies on fermentation, mainly of glucose. Arai *et al.*[217] reported that the intensity of the umami taste of free glutamic acid and its disodium salt is weak, and the taste was further diminished by esterification or amide formation. Homocysteinic acid with γ-sulfonate in place of γ-carboxylate of glutamic acid also exhibits a strong umami taste. While it has been suggested that the sensitivity to MSG shows racial differences, the threshold for Japanese is 0.015%, which is lower than that for sucrose (0.16%) and closer to that for table salt (0.0086%).[218] In general, the threshold value is lower for bitter- and sour-tasting compounds because of their warning nature, and higher for sweet compounds like sugar, which are taken in large quantities. Glutamic acid is the most abundant among the amino acids that constitute proteins in natural food. It is also contained in a free form in natural food and mother's milk and therefore can be considered a sign of proteins. The sensitivity of glutamate is reinforced tremendously by its 1:1 combination with 5′-mononucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP).[219,220] The synergistic action between MSG and nucleotides has been explained in terms of an allosteric effect by Kurihara *et al.*[221]

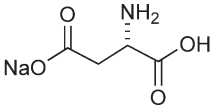
Monosodium aspartate also elicits an umami taste, although its intensity is not as high. It has also been reported to have a synergistic effect with IMP.[222]

4.16.6.1.2 Other proteinogenic amino acids

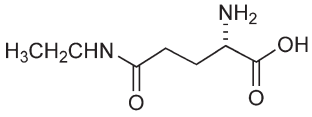
As described in Section 4.16.6.1.1, L-α-amino acids with an acidic side chain, glutamate and aspartate among proteinogenic amino acids, elicit umami taste. Several other proteinogenic amino acids without an acidic side chain, such as glycine, alanine, serine, threonine, asparagine, and glutamine, have umami taste as a side taste though their dominant taste is sweet (see Section 4.16.2.1.10(i)).[223] Their umami intensities are enhanced synergistically by adding 5′-mononucleotide similar to acidic amino acids.[8,224] It has been reported that amino acids such as glutamic acid, glycine, alanine, and arginine as well as guanylic acid, sodium cation, calcium cation, and chlorine anion are essential constituents that form the taste of scallops.[225]



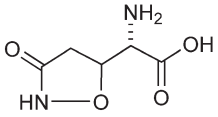
Monosodium glutamate



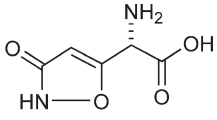
Monosodium aspartate



Theanine



Tricholomic acid



Ibotenic acid

4.16.6.1.3 L-Theanine

L-Theanine is abundantly contained in green tea (*C. sinensis* and *Thea sinensis*) and is an umami-tasting constituent along with glutamic acid.[226] Theanine was named after *T. sinensis*. The theanine content in dry leaves is 1–2% and is much higher in high-grade tea. It has been approved as a food additive in Japan, and theanine produced by fermentation is now commercially available. Besides theanine, green tea also contains many amino acids such as glutamic acid, aspartic acid, arginine, and serine.

4.16.6.1.4 Tricholomic acid and ibotenic acid

L-Tricholomic acid and L-ibotenic acid were isolated as umami taste principles from Japanese mushrooms, *Tricholoma muscarium* and *Amanita strobiliformis*, respectively.[227,228] Their umami intensities are much stronger than that of L-glutamic acid. These nonproteinogenic amino acids were found during the screening of insecticides. Although they have a very strong umami intensity, they are not used as seasonings.

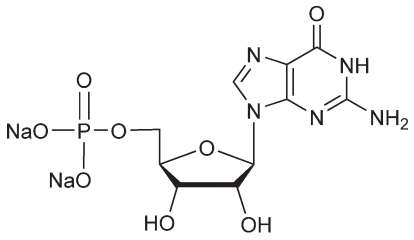
4.16.6.1.5 Organic acids

In the sixteenth century, succinic acid was isolated as colorless crystals by the dry distillation of amber. Takahashi found that a large amount of succinic acid was accumulated during the cultivation of a microorganism, and it had a delicious flavor.[229] Later, Aoki[230] reported that the umami-tasting constituent of shellfish such as *Corbicula japonica* (Asian clams) was succinic acid. Succinic acid is widely distributed in plants and animals and is used as a seasoning as well as a pharmaceutical ingredient. The threshold value is 0.02% and there is no synergistic effect with MSG or IMP.

4.16.6.1.6 Nucleotides

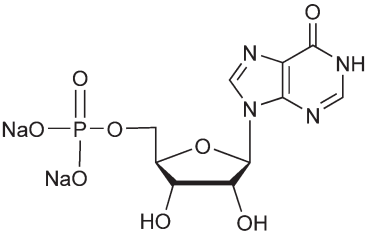
Five years after the discovery of MSG as an umami taste, Kodama, who was a senior pupil of Ikeda, found that inosine 5′-monophosphate (IMP) was an umami-tasting constituent of dried bonito, which has also been used for soup stock in Japan and East Asia.[231] Kuninaka.[232] further studied umami-tasting substances and found that guanosine 5′-monophosphate (GMP) obtained by the enzymatic hydrolysis of yeast RNA had an intense

umami taste. GMP was also found as a constituent of dried mushrooms (*Cortinellus shiitake*) cultivated in Japan.[233] Among natural nucleotides, umami-tasting compounds are 5′-nucleotides with purine as a nucleic acid base, such as IMP, GMP, and AMP. Mononucleotides with phosphate at the 2′- or 3′-position do not elicit an umami taste. Nucleosides and purine bases also do not give a savory taste. The relationship between the nucleotide structure and the intensity of umami was extensively studied.[234,235] As described in Section 4.16.6.1.1, it was confirmed that IMP and GMP have synergistic action with MSG and therefore these nucleotides are used with MSG as seasonings. Both IMP and GMP are industrially produced by enzymatic transformation and fermentation technology. Interestingly, the umami taste increases in stored meat and fish compared to very fresh food. It is thought that ATP present in the meat is transformed to IMP through AMP.



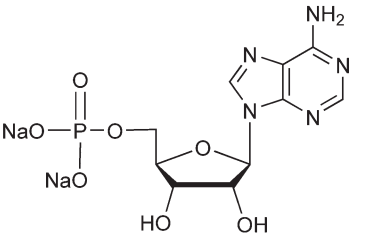
Disodium guanosine monophosphate

(GMP)



Disodium inosine monophosphate

(IMP)



Disodium adenosine monophosphate

(AMP)

4.16.6.1.7 Peptides

It was reported that α-glutamyl peptides, particularly peptides with hydrophilic amino acids, such as Glu–Asp, Glu–Thr, Glu–Ser, and Glu–Glu, elicited an umami flavor.[236] These peptides were isolated from the umami constituents in the enzymatically hydrolyzed products of soybean proteins. The same author also reported that tripeptides such as Glu–Gly–Ser also elicited the umami taste. The threshold value (0.15%) of these peptides is greater than that of MSG. The flavor of meat extract can be reproduced using these peptides with MSG and IMP. Recently, *N*-lactoyl glutamic acid, which is a condensation product of lactic acid with glutamic acid, was shown to elicit a weak umami taste, similar to MSG.[237]

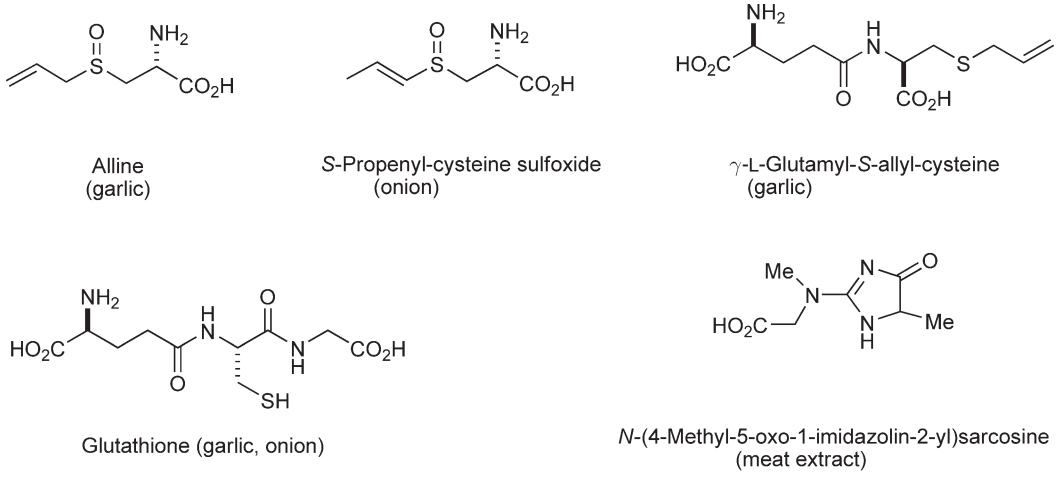
4.16.6.2 Kokumi

In Japan, 'koku' or 'kokumi' refers to the delicious taste of food. In particular, it is used when the flavor cannot be represented by any of the five basic taste qualities. It has been reported that 'kokumi' can be classified more concretely into thickness, continuity, mouthfullness of flavor, and harmony of taste.[238] Previously, there have been many reports on the constituents or fractions that enhance the flavor relevant to kokumi. In this section, examples of the constituents that provide kokumi are described by illustrating their structures.

4.16.6.2.1 Kokumi-inducing natural products

Ueda *et al.*[239] studied the kokumi-inducing effect of garlic and attempted to isolate the kokumi-inducing compound. Through repeated ion exchange chromatographic purification of garlic extracts, they obtained kokumi-inducing fractions as determined by sensory tests. As a result, sulfur-containing amino acids and peptides were characterized as kokumi-inducing constituents of garlic. These compounds are alliin (*S*-allyl-L-cysteine sulfoxide), *S*-methyl-L-cysteine sulfoxide, glutathione (γ-glutamyl-cysteinylglycine), and γ-glutamyl-*S*-allyl-L-cysteine. Ueda *et al.*[240] further investigated the kokumi-inducing constituents of onion using the same methodology and identified them as sulfur-containing amino acids, *S*-(1-propenyl)-L-cysteine sulfoxide and *S*-methyl-L-cysteine sulfoxide, and peptides such as glutathione (γ-glutamyl-cysteinylglycine) and γ-glutamyl-*S*-(1-propenyl)-L-cysteine sulfoxide. By themselves, these compounds exhibit only slight flavor in water solution. However, when they are added to an umami solution or various kind of foods, they substantially enhance the thickness, continuity, and mouthfullness of the taste.

Shima *et al.* noted that the taste of beef bouillon could not be reproduced with combinations of the compounds known to be contained and sought to identify the unknown compound that gives bouillon its brothy taste.[241,242] Broth prepared from beef was analyzed successively by dialysis, electrodialysis, gel filtration chromatography, chelate affinity chromatography, and carbon partition chromatography, and finally three fractions that gave the 'brothy taste' were obtained. One fraction contained the component responsible for this taste in the highest purity. A structural analysis was carried out using positive fast atom bombardment tandem mass spectrometry (FAB-MS) and various NMR methods and the main compound of the fraction was elucidated to be the novel compound *N*-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine. The structure was also confirmed by X-ray structural analysis.[243] This compound is estimated to be synthesized by the reaction of creatine in meat extract with methylglyoxal generated from sugar. This compound does not exhibit a brothy taste by itself in water solution. However, when added to soup stock, it substantially enhances kokumi, such as the thickness, continuity, and mouthfullness of the taste as well as a thick sour taste. It was also reported that glutathione, which is a kokumi-inducing constituent, enhanced the umami flavor response, particularly for IMP, by a neurophysiological approach in which the response of tympani chord in mouse was observed.[244] Very recently, it was found that the addition of a nearly tasteless aqueous extract isolated from edible beans (*Phaseolus vulgaris* L.) to a model chicken broth enhanced the savory taste sensation.[245] The key molecules inducing the kokumi were identified as γ-L-glutamyl-L-valine, γ-L-glutamyl-L-leucine, and γ-L-glutamyl-L-cysteinyl-β--alanine (homoglutathione). It is expected that further progress in understanding the neurophysiology and molecular biology of the kokumi receptor may clarify this phenomenon at a molecular level.



Kokumi-inducing natural products

4.16.7 Sour and Salty Tastes

4.16.7.1 Sour Taste Receptors

The sour taste is one of the five basic tastes and is elicited by acids. However, it is unclear how taste cells transduce a sour taste because acids (specifically protons) have diverse effects on cell membranes. It has been shown that acids in a single receptor cell may block ion channels, permeate ion channels, change intracellular pH, and alter transporter function. Although the variety of effects and potential targets are well recognized, until recently there has been little success in characterizing the molecular species involved in the transduction machinery. Very recently, two groups independently revealed that the acid receptor consisted of the molecule PKD2L1. Zuker *et al.*[9] investigated the acid receptor using bioinformatics based on the genome data of mice and found that PKD2L1 was expressed in the taste bud. This molecule belongs to the TRP family (transient receptor potential). It was revealed that a transmembrane ion channel protein encoded by the gene PKD2L1 is a

taste receptor protein of the sour taste receptor system. At almost the same time, Matsunami *et al.*[10] showed that two TRP channel members, PKD1L3 and PKD2L1, are coexpressed in a subset of taste receptor cells in specific taste areas, and the PKD1L3 and PKD2L1 heterodimer may function as a sour taste receptor.

4.16.7.2 Sour-Tasting Natural Products

Sour-tasting compounds are called as acidulants giving a sharp taste to foods. They also act as preservatives. Many natural foods are acidic. For example, oranges, lemons, apples, and yogurt contain natural acids, such as citric acid, that give them their characteristic taste. Acids have been used for centuries as important contributors to flavor and the acid environment they produce prevents the growth of many microorganisms. Organic acids employed as food additives are listed below.[13]

4.16.7.2.1 Citric acid

Citric acid is a sour principle of citrus fruits such as orange and lemon and exhibits a mild and refreshing sour taste. It is widely used to add an acidic (sour) taste to soft drinks, jams, candies, and so on. It is also used as a natural preservative. By taking advantage of the buffer action of citric acid, sodium citrate is used in seasonings, and as a pH controller and emulsifier for processed cheese. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It also serves as an environmentally benign cleaning agent and acts as an antioxidant. Citric acid is produced by the fermentation of glucose. Approximately, 35 000 tons are consumed annually in Japan.

4.16.7.2.2 Malic acid

In nature, malic acid is found in the L-form in many fruits such as apple, and indeed it is sometimes called apple acid, and contributes to the sour taste of green apples. The chemically synthesized product is racemic, but there appears to be no difference in the quality of taste or sour intensity. Racemic malic acid has been approved as a food additive in Japan. While it is almost as sour as citric acid, it gives a slightly stimulating and continuous sour taste quality. It is used as a single dose, normally along with other organic acids in soft drinks, lactobacillus beverages, sherbet, jams, and pickles. It is produced industrially from maleic acid by hydration.

4.16.7.2.3 Tartaric acid

L-Tartaric acid is an abundant constituent of many fruits such as grapes and bananas and exhibits a slightly astringent and refreshing sour taste. It is one of the main acids found in wine. It is added to other foods to give a sour taste and is normally used with other acids such as citric acid and malic acid as an additive in soft drinks, candies, and so on. It is produced by acid hydrolysis of calcium tartrate, which is prepared from potassium tartrate obtained as a by-product during wine production. Optically active tartaric acid is used for the chiral resolution of amines and also as an asymmetric catalyst.

4.16.7.2.4 Lactic acid

Lactic acid is a sour principle of yogurt and lactobacillus beverages. It exhibits a soft and thick sour taste quality with slight astringency. It is a syrupy liquid produced by fermentation with a lactobacillus and is formed in the body by the metabolism of sugars. Due to its pH-controlling effect, it is used in soft drinks, pickles, Japanese sakes, sherbets, and so on. It is industrially produced by the fermentation of glucose and chemical synthesis. Approximately 12 000 tons are consumed annually as a food additive in Japan.

4.16.7.2.5 Succinic acid

Succinic acid is an umami-tasting constituent of shellfish, as well as a kokumi-tasting substance in Japanese sake. It is sometimes added to Japanese sake and soy sauce to improve the taste quality. It is industrially produced from maleic acid by hydrogenation and subsequent purification. It is also approved as a food additive in Japan. Recently, an efficient fermentation method has also been studied.[246]

4.16.7.2.6 Fumaric acid

Fumaric acid is a naturally occurring sour-tasting compound found in many plants such as *Fumaria officinalis* L. (Fumariaceae), *Boletus scaber* Bull. (Boletaceae), and *Fomes igniaries* (Fries) Kickx. (Pluporaceae). It is an essential component for respiration in plant and animal tissues. It is produced by fermentation with mold, such as *Rhizopus nigricans*, or by chemical synthesis. It is also used in soft drinks and ice cream and as an acidulant along with citric acid.

4.16.7.3 Salty Taste

It is believed that saltiness is induced by compounds passing directly through ion channels in the tongue, which leads to an action potential. Recently, it was reported[247] that both amiloride-sensitive and amiloride-insensitive mechanisms contribute to NaCl taste transduction. The amiloride-sensitive mechanism relies on the ENaC, which is widely expressed on the apical membrane of fungiform taste cells. The amiloride-insensitive mechanism, which predominates in circumvallated and foliate taste buds, was also reported to involve a variant of the nonselective cation channel transient receptor potential vanilloid receptor subtype 1 (TRPV1).[248] It has been suggested that additional mechanisms must contribute to the amiloride-insensitive NaCl response. Unfortunately, no naturally occurring product is known to exhibit a salty taste, despite several attempts to identify or synthesize one.

4.16.8 Conclusion

As reflected in this chapter, nature creates great diversity in plants and animals, even in terms of taste quality. However, we still do not have a thorough understanding of taste sensation. Recently, there are accumulated evidences for fatty substance receptors using rodents.[249] It is speculated that humans may also have the same receptors. Fat has occasionally been proposed as a possible basic taste. It is highly expected that recent progress in our understanding of neurophysiology with molecular-biological tools will help to clarify the nature of all of the taste sensations and the results should be useful for addressing several issues that bear on human health, such as low-calorie sweeteners for obesity, salt substitutes for patients with hypertension, and novel taste-modifying compounds for drug delivery systems.

References

1. H. Henning, *Z. Psychol. Physiol. Sinnesorgane* **1915**, *74*, 203–219.
2. K. Ikeda, *J. Tokyo Chem. Soc.* **1909**, *30*, 820–836.
3. L. J. Filer, Jr.; S. Garattini; M. R. Kare; W. A. Reynolds; R. J. Wurtman, Eds., *Glutamic Acid: Advances in Biochemistry and Physiology*; Raven Press: New York, 1979; 400pp.
4. Y. Kawamura; M. R. Kare, Eds., *Umami: A Basic Taste*; Marcel Dekker, Inc.: New York, 1987; 649pp.
5. S. Yamaguchi; K. Ninomiya, *Food Rev. Int.* **1998**, *14*, 123–138.
6. G. Nelson; M. A. Hoon; J. Chandrashekar; Y. Zhang; N. J. Ryba; C. S. Zuker, *Cell* **2001**, *106*, 381–390.
7. K. L. Mueller; M. A. Hoon; I. Erlenbach; J. Chandrashekar; C. S. Zuker; N. J. P. Ryba, *Nature* **2005**, *434*, 225–229.
8. J. G. Brand, *J. Nutr.* **2000**, *130*, 942S–945S.
9. A. L. Huang; X. Chen; M. A. Hoon; J. Chandrashekar; W. Guo; D. Traenkner; N. J. P. Ryba; C. S. Zuker, *Nature* **2006**, *442*, 934–938.
10. Y. Ishimaru; H. Inada; M. Kubota; H. Zhuang; M. Tominaga; H. Matsunami, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12569–12574.
11. M.-R. Kim; Y. Kusakabe; H. Miura; Y. Shindo; Y. Ninomiya; A. Hino, *Jpn. J. Taste Smell Res.* **2002**, *9*, 659–660.
12. Y. Kurihara, *Kikan Kagaku Sosetsu* **1999**, *40*, 61–69.
13. R. S. Shallenberger, *Taste Chemistry*; Blackie Academic & Professional, an imprint of Chapman & Hall: Glasgow, UK, 1993; 613pp.
14. T. Hofmann; C.-T. Ho; W. Pickenhagen, Challenges in Taste Research: Present Knowledge and Future Implications. In *Challenges in Taste Chemistry and Biology*; T. Hofmann, C.-T. Ho, W. Pickenhagen, Eds.; ACS Symposium Series 867; American Chemical Society: Washington, DC, USA, 2004; pp 1–24.
15. Y. Ariyoshi; S. Ito; Y. Izumi; T. Shiba; S. Suzuki; I. Chibata; M. Fujimaki, *Kagaku Sosetsu* **1976**, *14*, 217.
16. Y. Kurihara; A. Kobayashi, *Kikan Kagaku Sosetsu* **1999**, *40*, 230.
17. R. S. Shallenberger; T. E. Acree; C. Y. Lee, *Nature* **1969**, *221*, 555–556.
18. L. B. Kier, *J. Pharm. Sci.* **1972**, *61*, 1394–1397.

19. J. M. Tinti; C. Nofre, Design of Sweeteners. A Rational Approach. In *Sweeteners*; D. E. Walters, F. T. Orthoefer, G. E. DuBois, Eds.; ACS Symposium Series 450; American Chemical Society: Washington, DC, USA, 1991; pp 88–99.
20. G. Morini; A. Bassoli; P. A. Temussi, *J. Med. Chem.* **2005**, *48*, 5520–5529.
21. B. Lythgoe; S. Trippett, *J. Chem. Soc.* **1950**, 1983–1990.
22. K. Mizutani; T. Kuramoto; Y. Tamura; N. Ohtake; S. Doi; M. Nakamura; O. Tanaka, *Biosci. Biotechnol. Biochem.* **1994**, *58*, 554–555.
23. E. Mosettig; U. Beglinger; F. Dolder; H. Lichiti; P. Quitt; J. A. Waters, *J. Am. Chem. Soc.* **1963**, *85*, 2305–2309.
24. H. Kohda; R. Kasai; K. Yamasaki; O. Tanaka, *Phytochemistry* **1976**, *15*, 981–983.
25. I. Sakamoto; K. Yamasaki; O. Tanaka, *Chem. Pharm. Bull.* **1977**, *25*, 844–846.
26. I. Sakamoto; K. Yamasaki; O. Tanaka, *Chem. Pharm. Bull.* **1977**, *25*, 3437–3439.
27. M. Kobayashi; S. Horikawa; I. H. Degrandi; J. Ueno; H. Mitsuhashi, *Phytochemistry* **1977**, *16*, 1405–1408.
28. T. Tanaka; H. Kohda; O. Tanaka; F. H. Chen; W. H. Chou; J. L. Leu, *Agric. Biol. Chem.* **1981**, *45*, 2165–2166.
29. A. D. Kinghorn; C. D. Wu; D. D. Soejarto; C. M. Compadre, Stevioside. In *Alternative Sweeteners,* 3rd ed.; L. O. Nabors, Ed.; Marcel Dekker, Inc: New York, USA, 2001; pp 167–183.
30. T. Takemoto; S. Arihara; T. Nakajima; M. Okuhira, *Yakugaku Zasshi* **1983**, *103*, 1167–1173.
31. K. Matsumoto; R. Kasai; K. Ohtani; O. Tanaka, *Chem. Pharm. Bull.* **1990**, *38*, 2030–2032.
32. T. Tanaka; O. Tanaka; Z. Lin; J. Zhou, *Chem. Pharm. Bull.* **1985**, *33*, 4275–4280.
33. H. Yamada; M. Nishizawa, *Tetrahedron* **1992**, *48*, 3021–3044.
34. F. Fullas; R. A. Fusain; E. Bordas; J. M. Pezzuto; D. D. Soejarto; A. D. Kinghorn, *Tetrahedron* **1991**, *47*, 8515–8522.
35. F. Fullus; D. D. Soejarto; A. D. Kinghorn, *Phytochemistry* **1992**, *31*, 2543–2545.
36. H. Yamada; M. Nishizawa, *J. Org. Chem.* **1995**, *60*, 386–397.
37. J. Kim; J. M. Pezzuto; D. D. Soejarto; F. A. Lang; A. D. Kinghorn, *J. Nat. Prod.* **1988**, *51*, 1166–1172.
38. J. Kim; A. D. Kinghorn, *Phytochemistry* **1989**, *28*, 1225–1228.
39. M. Nishizawa; H. Yamada; Y. Yamaguchi; S. Hatakeyama; I. S. Lee; E. J. Kennelly; J. Kim; A. D. Kinghorn, *Chem. Lett.* **1994**, 1555–1558.
40. E. J. Kennelly; L. Cai; L. Long; L. Shamon; K. Zaw; B. Zhou; J. M. Pezzuto; A. D. Kinghorn, *J. Agric. Food Chem.* **1995**, *43*, 2602–2607.
41. D. J. Yang; Z. C. Zhong; Z. M. Xie, *Acta Pharmacol. Sin.* **1992**, *27*, 841–844.
42. H. Arakawa; M. Nakazaki, *Chem. Ind.* **1959**, 671.
43. M. Yoshikawa; T. Murakami; T. Ueda; H. Shimoda; J. Yamahara; H. Matsuda, *Heterocycles* **1999**, *50*, 411–418.
44. A. D. Kinghorn; D. D. Soejarto, *CRC Crit. Rev. Plant Sci.* **1986**, *4*, 79–120.
45. A. D. Kinghorn; D. D. Soejarto, *Med. Res. Rev.* **1989**, *9*, 91–115.
46. N. Kaneda; I.-S. Lee; M. P. Gupta; D. D. Soejarrto; A. D. Kinghorn, *J. Nat. Prod.* **1992**, *55*, 1136–1141.
47. K. Mori; M. Kato, *Tetrahedron* **1986**, *42*, 5895–5900.
48. L. O. Nabors; G. E. Inglett, A Review of Various Other Alternative Sweeteners. In *Alternative Sweeteners*; L. O. Nabors, R. C. Gelardi, Eds.; Marcel Dekker, Inc.: New York, 1986; pp 309–323.
49. A. D. Kinghorn; C. M. Compadre, Less Common High-Potency Sweeteners. In *Alternative Sweeteners, 3rd ed. Revised and Expanded*; L. O. Nabors, Ed., Marcel Dekker, Inc.: New York, 2001; pp 209–233.
50. G. G. Birch; S. E. Kemp, *Chem. Senses* **1989**, *14*, 249–258.
51. R. J. Haefeli; D. Glaser, *Lebensm. Wiss. Technol.* **1990**, *23*, 523–527.
52. H. Wieser; H. Jugel; H.-D. Belitz, *Z. Lebensm. Unters. Forsch.* **1977**, *164*, 277–282.
53. R. Vleggaar; L. G. J. Ackerman; P. S. Steyn, *J. Chem. Soc. Perkin Trans. I* **1992**, 3095–3098.
54. Y. Amino; K. Yuzawa; K. Mori; T. Takemoto, Crystals of Non-Natural Stereoisomer Salt of Monatin as Sweeteners. PCT Int. Appl. WO 2003045914, 5 June 2003.
55. P. J. Van Wyk; L. G. J. Ackerman, 3-(1-Amino-1,3-dicarboxy-3-hydroxybut-4-yl)-Indole Compounds. U.S. Patent 4,975,298, 4 December 1990.
56. R. S. Shallenberger, *Taste Chemistry*; Blackie Academic & Professional, an imprint of Chapman & Hall: Glasgow, UK, 1993; Chapters 4–6, pp 141–212.
57. H. van der Wel; K. Loeve, *Eur. J. Biochem.* **1972**, *31*, 221–225.
58. R. B. Iyengar; P. Smits; F. van der Ouderaa; H. van der Wel; J. van Brouwershaven; P. Raves-tein; G. Richters; P. D. van Wassenaar, *Eur. J. Biochem.* **1979**, *96*, 193–204.
59. L. Edens; L. Heslinga; R. Klok; A. M. Ledeboer; J. Maat; M. Y. Toonen; C. Visser; C. T. Verrips, *Gene* **1982**, *18*, 1–12.
60. J.-H. Lee; J. L. Weickmann; R. K. Koduri; P. Ghosh-Dastidar; K. Saito; L. C. Blair; T. Date; J. S. Lai; S. M. Hollenberg; R. L. Kendall, *Biochemistry* **1988**, *27*, 5101–5107.
61. A. M. de Vos; M. Hatada; H. van der Wel; H. Krabbendam; A. F. Peerdeman; S.-H. Kim, *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1406–1409.
62. C. M. Ogata; P. F. Cordon; A. M. de Vos; S.-H. Kim, *J. Mol. Biol.* **1992**, *228*, 893–908.
63. H. van der Wel, *Trends Biochem. Sci.* **1980**, *5*, 122–123.
64. K. Tonosaki; K. Miwa; F. Kanemura, *Brain Res.* **1997**, *748*, 234–236.
65. J. A. Morris; R. H. Cagan, *Biochim. Biophys. Acta* **1972**, *261*, 114–122.
66. H. van der Wel, *FEBS Lett.* **1972**, *21*, 88–90.
67. Z. Bohak; S.-L. Li, *Biochim. Biophys. Acta* **1976**, *427*, 153–170.
68. G. Frank; H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 585–592.
69. G. Hudson; K. Biemann, *Biochem. Biophys. Res. Commun.* **1976**, *71*, 212–220.
70. M. Kohmura; N. Nio; Y. Ariyoshi, *Agric. Biol. Chem.* **1990**, *54*, 2219–2224.
71. J. A. Morris; R. H. Cagan, *J. Agric. Food Chem.* **1976**, *24*, 1075–1077.
72. M. Kohmura; N. Nio; Y. Ariyoshi, *Agric. Biol. Chem.* **1990**, *54*, 1521–1530.
73. C. Ogata; M. Hatada; G. Tomlinson; W. C. Shin; S.-H. Kim, *Nature* **1987**, *328*, 739–742.
74. J. R. Somoza; F. Jiang; L. Tong; C.-H. Rang; J. M. Cho; S.-H. Kim, *J. Mol. Biol.* **1993**, *234*, 390–404.

75. M. Kohmura; N. Nio; Y. Ariyoshi, *Agric. Biol. Chem.* **1990**, *54*, 3157–3162.
76. M. Kohmura; N. Nio; Y. Ariyoshi, *Agric. Biol. Chem.* **1991**, *55*, 1831–1838.
77. M. Kohmura; T. Mizukoshi; N. Nio; E. Suzuki; Y. Ariyoshi, *Pure Appl. Chem.* **2002**, *74*, 1235–1242.
78. S.-H. Kim; C.-H. Kang; R. Kim; J. M. Cho; Y. B. Lee; T.-K. Lee, *Protein Eng.* **1989**, *2*, 571–575.
79. Z. Hu; M. He, *Yunnan Zhi Wu Yan Jiu* **1983**, *5*, 207–212.
80. X. Liu; S. Maeda; Z. Hu; T. Aiuchi; K. Nakaya; Y. Kurihara, *Eur. J. Biochem.* **1993**, *211*, 281–287.
81. S. Nirasawa; X. Liu; T. Nishino; Y. Kurihara, *Biochim. Biophys. Acta* **1993**, *1202*, 277–280.
82. S. Nirasawa; T. Nishino; M. Katahira; S. Uesugi; Z. Hu; Y. Kurihara, *Eur. J. Biochem.* **1994**, *223*, 989–995.
83. S. Nirasawa; Y. Masuda; K. Nakaya; Y. Kurihara, *Gene* **1996**, *181*, 225–227.
84. D. Ming; G. Hellekant, *FEBS Lett.* **1994**, *355*, 106–108.
85. M. Kohmura; M. Ota; H. Izawa; D. Ming; G. Hellekant; Y. Ariyoshi, *Biopolymers* **1996**, *38*, 553–556.
86. H. Izawa; M. Ota; M. Kohmura; Y. Ariyoshi, *Biopolymers* **1996**, *39*, 95–101.
87. F. M. Assadi-Porter; D. J. Aceti; H. Cheng; J. L. Markley, *Arch. Biochem. Biophys.* **2000**, *376*, 252–258.
88. K. Ishikawa; M. Ota; Y. Ariyoshi; H. Sasaki; M. Tanokura; D. Ming; J. Caldwell; F. Abildgaad, *Acta Crystallogr. D Biol. Crystallogr.* **1996**, *52*, 577–578.
89. J. E. Caldwell; F. Abildgaard; Z. Dzakula; D. Ming; G. Hellekant; J. L. Markley, *Nat. Struct. Biol.* **1998**, *5*, 427–431.
90. F. M. Assadi-Porter; D. J. Aceti; J. L. Markley, *Arch. Biochem. Biophys.* **2000**, *376*, 259–265.
91. H. van der Wel; G. Larson; A. Hladik; C. M. Hladik; G. Hellekant; D. Glaser, *Chem. Senses* **1989**, *14*, 75–79.
92. S. Theerasilp; Y. Kurihara, *J. Biol. Chem.* **1988**, *263*, 11536–11539.
93. S. Theerasilp; H. Hitotsuya; S. Nakajo; K. Nakaya; Y. Nakamura; Y. Kurihara, *J. Biol. Chem.* **1989**, *264*, 6655–6659.
94. H. Igeta; Y. Tamura; K. Nakaya; Y. Nakamura; Y. Kurihara, *Biochim. Biophys. Acta* **1991**, *1079*, 303–307.
95. N. Takahashi; H. Hitotsuya; H. Hanzawa; Y. Kurihara, *J. Biol. Chem.* **1990**, *265*, 7793–7798.
96. Y. Masuda; S. Nirasawa; K. Nakaya; Y. Kurihara, *Gene* **1995**, *161*, 175–177.
97. Y. Kurihara; S. Nirasawa, *Trends Food Sci. Technol.* **1994**, *5*, 37–42.
98. H. Yamashita; S. Theerasilp; T. Aiuchi; K. Nakaya; Y. Nakamura; Y. Kurihara, *J. Biol. Chem.* **1990**, *265*, 15770–15775.
99. S. Harada; H. Ohtani; S. Maeda; Y. Kai; N. Kasai; Y. Kurihara, *J. Mol. Biol.* **1994**, *238*, 286–287.
100. S. Nakajo; T. Akabane; K. Nakaya; Y. Nakamura; Y. Kurihara, *Biochim. Biophys. Acta* **1992**, *1118*, 293–297.
101. H. Yamashita; T. Akabane; Y. Kurihara, *Chem. Senses* **1995**, *20*, 239–243.
102. K. Kurihara; L. M. Beidler, *Nature,* **1969**, *222*, 1176–1179.
103. K. Abe; H. Yamashita; S. Arai; Y. Kurihara, *Biochim. Biophys. Acta* **1992**, *1130*, 232–234.
104. Y. Shirasuka; K. Nakajima; T. Asakura; H. Yamashita; A. Yamamoto; S. Hata; S. Nagata; M. Abo; H. Sorimachi; K. Abe, *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1403–1407.
105. M. Suzuki; E. Kurimoto; S. Nirasawa; Y. Masuda; K. Hori; Y. Kurihara; N. Shimba; M. Kawai; E. Suzuki; K. Kato, *FEBS Lett.* **2004**, *573*, 135–138.
106. A. Hiura; T. Akabane; K. Ohtani; R. Kasai; K. Yamasaki; Y. Kurihara, *Phytochemistry* **1996**, *43*, 1023.
107. D. Sugita; R. Inoue; Y. Kurihara, *Chem. Senses* **1998**, *23*, 93–97.
108. K. Yoshikawa; K. Amimoto; S. Arihara; K. Matsuura, *Tetrahedron Lett.* **1989**, *30*, 1103–1106.
109. K. Yoshikawa; K. Amimoto; S. Arihara; K. Matsuura, *Chem. Pharm Bull.* **1989**, *37*, 852–854.
110. M. Maeda; T. Iwashita; Y. Kurihara, *Tetrahedron Lett.* **1989**, *30*, 1547–1550.
111. Y. Kurihara; K. Ookubo; H. Tasaki; H. Kodama; Y. Akiyama; A. Yagi; B. Halpern, *Tetrahedron* **1988**, *44*, 61–66.
112. K. Yoshikawa; N. Shimono; S. Arihara, *Tetrahedron Lett.* **1991**, *32*, 7059–7062.
113. L. M. Kennedy; L. R. Saul; R. Sefecka; D. A. Stevens, *Chem. Senses* **1988**, *13*, 529–543.
114. K. Yoshikawa; S. Tumura; K. Yamada; S. Arihara, *Chem. Pharm. Bull.* **1992**, *40*, 2287–2291.
115. T. Shiba, *Kagaku Sosetsu* **1976**, *14*, 129–156.
116. Y. Yamada; N. Nakatani, *Kikan Kagaku Sosetsu* **1999**, *40*, 72–83.
117. Y. Niiho; T. Yamazaki; Y. Nakajima; T. Yamamoto; H. Ando; Y. Hirai; K. Toriizuka; Y. Ida, *J. Nat. Med.* **2006**, *60*, 82–88.
118. M. Asao; H. Iwamura; M. Akamatsu; T. Fujita, *J. Med. Chem.* **1987**, *30*, 1873–1879.
119. K. L. Mueller; M. A. Hoon; I. Erlenbach; J. Chandrashekar; C. S. Zuker; N. J. P. Ryba, *Nature* **2005**, *434*, 225–229.
120. R. B. Woodward; W. J. Brehm, *J. Am. Chem. Soc.* **1948**, *70*, 2107–2115.
121. R. M. Harris, Strychnine. In *Molecules of Death*; R. H. Waring, G. B. Steventon, S. C. Mitchell, Eds.; Imperial College Press: London, UK, 2002; pp 259–275.
122. H. L. Holmes, The Strychnos Alkaloids. In *The Alkaloids*; R. H. F. Manske, H. L. Holmes, Eds.; Academic Press: New York, 1950; Vol. I, pp 375–496.
123. M. H. Malone; K. M. St. John-Allan; E. Bejar, *J. Ethnopharmacol.* **1992**, *35*, 295–297.
124. R. B. Woodward; W. E. Doering, *J. Am. Chem. Soc.* **1944**, *66*, 849; **1945**, *67*, 860–874.
125. M. H. Ibrahim; M. I. Elbashir; A. Naser; I. A. Aelbasit; M. M. Kheir; I. Adam, *Ann. Trop. Med. Parasitol.* **2004**, *98*, 441–445.
126. S. Hatakeyama, *Kagaku to Kogyo (Tokyo, Japan)* **2004**, *57*, 716–719.
127. H. M. R. Hoffmann; J. Frackenpohl, *Eur. J. Org. Chem.* **2004**, *21*, 4293–4312.
128. J. W. Mason; L. M. Hondeghem, *Ann. N. Y. Acad. Sci.* **1984**, *432*, 162–176.
129. A. Smith, Caffeine. In *Nutritional Neuroscience*; H. R. Lieberman, R. B. Kanarek, C. Prasad, Eds.; CRC Press LLC: Boca Raton, FL, 2005; pp 341–361.
130. T. K. Leonard; R. R. Watson; M. E. Mohs, *J. Am. Diet. Assoc.* **1987**, *87*, 1048–1053.
131. I. Iancu; A. Olmer; R. D. Strous, Caffeinism: History, Clinical Features, Diagnosis, and Treatment. In *Caffeine and Activation Theory*; B. D. Smith, U. Gupta, B. S. Gupta, Eds.; CRC Press LLC: Boca Raton, FL, 2007; pp 331–347.
132. E. Fischer, *Ber. Dtsch. Chem. Ges.* **1897**, *30*, 1839–1845. From: *J. Chem. Soc. Abstr.* **1897**, *72*, I, 641–642.
133. M. F. Stidworthy; J. S. Bleakley; M. T. Cheeseman; D. F. Kelly, *Vet. Rec.* **1997**, *141*, 28.
134. N. L. Benowitz, *Annu. Rev. Med.* **1986**, *37*, 21–32.
135. F. A. Lazutka; A. Vasilauskiene; Sh.G. Gefen, *Gig. Sanit.* **1969**, *34*, 30–33.
136. L. Grinspoon; J. B. Bakalar, *J. Ethnopharmacol.* **1981**, *3*, 149–159.

137. J. L. Christie, *J. Forensic Sci.* **1976**, *21*, 671–679.
138. A. J. Jenkins; T. Llosa; I. Montoya; E. J. Cone, *Forensic Sci. Int.* **1996**, *77*, 179–189.
139. R. Kuhn; I. Low, *Angew. Chem.* **1954**, *66*, 639–640.
140. S. C. Morris; T. H. Lee, *Food Technol. Aust.* **1984**, *36*, 118–124.
141. R. M. D. Machado; M. C. F. Toledo; L. C. Garcia, *Food Control* **2006**, *18*, 503–508.
142. S. Okuda; M. Yoshimoto; K. Tsuda; N. Utzugi, *Chem. Pharm. Bull.* **1966**, *14*, 314–318.
143. W. H. Perkin, Jr.; R. Robinson, *J. Chem. Soc. Trans.* **1910**, *97*, 305–323.
144. T. Kubota; I. Kubo, *Nature (London, UK)* **1969**, *223*, 97–99.
145. M. G. J. Beets, The Sweet and Bitter Modalities: Carbohydrtates, Analogues and Derivatives.In *Structure–Activity Relationships in Human Chemoreception;* Applied Science Publishers: Barking, UK, 1978; pp 259–303.
146. S. Hasegawa, Biochemistry of Limonoids in Citrus. In *Citrus Limonoids, Functional Chemicals in Agriculture and Food*; M. A. Berhow, S. Hasegawa, G. D. Manners, Eds.; ACS Symposium Series 758; Oxford University Press, American Chemical Society: Washington, DC, USA, 2000; pp 9–30.
147. E. G. Miller; S. E. Taylor; C. W. Berry; J. A. Zimmerman; S. Hasegawa, Citrus Limonoids: Increasing Importance as Anticancer Agents. In *Citrus Limonoids, Functional Chemicals in Agriculture and Food,* M. A. Berhow, S. Hasegawa, G. D. Manners, Eds.; ACS Symposium Series 758; Oxford University Press, American Chemical Society: Washington, DC, USA, 2000; pp 132–144.
148. S. Hasegawa; M. Miyake, *Food Rev. Int.* **1996**, *12*, 413–435.
149. Y. Kano, *Acta Hortic.* **2000**, *517*, 369–374.
150. M. Yasuda; M. Iwamoto; H. Okabe; T. Yamauchi, *Chem. Pharm. Bull.* **1984**, *32*, 2044–2047.
151. B. Jayaprakasam; N. P. Seeram; M. G. Nair, *Cancer Lett.* **2003**, *189*, 11–16.
152. F. W. Bachelor; S. Ito, *Can. J. Chem.* **1973**, *51*, 3626–3630.
153. A. Wesolowska; A. Nikiforuk; K. Michalska; W. Kisiel; E. Chojnacka-Wojcik, *J. Ethnopharmacol.* **2006**, *107*, 254–258.
154. J. Fritsche; C. M. Beindorff; M. Dachtler; H. Zhang; J. G. Lammers, *Eur. Food Res. Technol.* **2002**, *215*, 149–157.
155. L. O. Spetsig, *Acta Chem. Scand.* **1958**, *12*, 592–593.
156. Z. Valenta; A. H. Gray; D. E. Orr; S. Papadopoulos; C. Podesva, *Tetrahedron* **1962**, *18*, 1433–1441.
157. L. Novotny; V. Herout; F. Sorm, *Collect. Czech. Chem. Commun.* **1960**, *25*, 1492–1499.
158. K. Ishizaki; T. Imada; M. Tsurufuji, *Hepatology Res.* **2005**, *33*, 174–177.
159. H. Hikino; Y. Kiso; M. Kubota; M. Hattori; T. Namba, *Shoyakugaku Zasshi* **1984**, *38*, 359–360.
160. H. Inouye; Y. Nakamura, Tetrahedron Lett. **1968**, 4919–4924.
161. Y. Asahina; M. Inubuse, *Yakugaku Zasshi* **1929**, *49*, 128–134.
162. R. M. Horowitz; B. Gentili, *Tetrahedron* **1963**, *19*, 773–782.
163. D. W. Thomas; C. V. Smythe; M. D. Labbee, *Food Res.* **1958**, *23*, 591–598.
164. M. S. Arayne; N. Sultana; Z. Bibi, *Pak. J. Pharm. Sci.* **2005**, *18*, 45–57.
165. A. Petritschek; F. Lynen; H. D. Belitz, *Lebensm. Wiss. Technol.* **1972**, *5*, 47–50.
166. T. K. Murray; B. E. Baker, *J. Sci. Food Agric.* **1952**, *3*, 470–475.
167. B. C. Richardson; L. K. Creamer, *NZ J. Dairy Sci. Technol.* **1973**, *8*, 46–51.
168. M. Yamashita; S. Arai; J. Matsuyama; M. Gonda; H. Kato; M. Fujimaki, *Agric. Biol. Chem.* **1970**, *34*, 1484–1491.
169. T. Shiba; K. Nunami, *Tetrahedron Lett.* **1974**, 509–512.
170. W. Pickenhagen; P. Dietrich; B. Keil; J. Polonsky; F. Nouaille; E. Lederer, *Helv. Chim. Acta* **1975**, *58*, 1078–1086.
171. Y. Katsuragi; Y. Mitsui; T. Umeda; K. Otsuji; S. Yamasawa; K. Kurihara, *Pharm. Res.* **1997**, *14*, 720–724.
172. W. L. Scoville, *J. Am. Pharm. Assoc.* **1912**, *1*, 453–454.
173. F. Korel; N. Bagdatlioglu; M. O. Balaban; Y. Hisil, *J. Agric. Food Chem.* **2002**, *50*, 3257–3261.
174. S. Kosuge; M. Furuta, *Agric. Biol. Chem.* **1970**, *34*, 248–256.
175. S. Kati-Coulibaly; V. Coxam; J.-P. Barlet, *Med. Nutr.* **1998**, *34*, 236–245.
176. L. Guo; Q. Wang; L. Zhu, *Hefei Gongye Daxue Xuebao: Ziran Kexue Ban* **2006**, *29*, 117–121.
177. T. Masuda; N. Nakatani, *Agric. Biol. Chem.* **1991**, *55*, 2337–2340.
178. S. Yazawa; N. Suetome; K. Okamoto; T. Namiki, *J. Jpn. Soc. Hortic. Sci.* **1989**, *58*, 601–607.
179. K. Kobata; T. Todo; S. Yazawa; K. Iwai; T. Watanabe, *J. Agric. Food Chem.* **1998**, *46*, 1695–1697.
180. M. L. Lopez-Rodriguez; A. Viso; S. Ortega-Gutierrez, *Mini Rev. Med. Chem.* **2003**, *3*, 729–748.
181. H. Hamada; S. Ohiwa; T. Nishida; H. Katsuragi; T. Takeda; H. Hamada; N. Nakajima; K. Ishihara, *Plant Biotechnol. (Tokyo, Japan)* **2003**, *20*, 253–255.
182. Y. Tani; T. Fujioka; M. Sumioka; Y. Furuichi; H. Hamada; T. Watanabe, *J. Nutr. Sci. Vitaminol.* **2004**, *50*, 351–355.
183. R. De Cleyn; M. Verzele, *Bull. Soc. Chim. Belg.* **1975**, *84*, 435–438.
184. R. De Cleyn; M. Verzele, *Chromatographia* **1975**, *8*, 342–344.
185. K. Hashimoto; T. Yaoi; H. Koshiba; T. Yoshida; T. Maoka; Y. Fujiwara; Y. Yamamoto; K. Mori, *Food Sci. Technol. Int. (Tsukuba, Japan)* **1996**, *2*, 24–29.
186. G. Sharma; B. Mishra, *J. Pharm. Res.* **2007**, *6*, 129–133.
187. F. Kiuchi; N. Nakamura; Y. Tsuda; K. Kondo; H. Yoshimura, *Chem. Pharm. Bull.* **1988**, *36*, 2452–2465.
188. T. Aihara, *Yakugaku Zasshi* **1950**, *70*, 405–409.
189. E. Sugai; Y. Morimitsu; K. Kubota, *Biosci. Biotechnol. Biochem.* **2005**, *69*, 1958–1962.
190. M. Jacobson, Chem. Ind. (London, UK) **1957**, 50–51.
191. D. W. Connell; M. D. Sutherland, *Aust. J. Chem.* **1969**, *22*, 1033–1043.
192. E. Steinegger; K. Stucki, *Pharm. Acta Helv.* **1982**, *57*, 66–71.
193. S. Lee; C. Khoo; C. W. Halstead; T. Huynh; A. Bensoussan, *J. AOAC Int.* **2007**, *90*, 1219–1226.
194. K. Ina; J. S. Wu; H. Etoh; A. Yagi; I. Kishima, *Nippon Shokuhin Kogyo Gakkai Shi* **1993**, *40*, 859–862.
195. M. Hara; T. Kuboi; H. Etoh, *Aroma Res.* **2001**, *2*, 265–269.
196. M. Matsuo; M. Yamazaki, *Biochem. Biophys. Res. Commun.* **1966**, *25*, 269–274.
197. K. Okano; J. Asano; G. Ishii, *J. Jpn. Soc. Hortic. Sci.* **1990**, *59*, 551–558.
198. M. G. Ettlinger; A. J. Lundeen, *J. Am. Chem. Soc.* **1956**, *78*, 4172–4173.

199. H. P. Koch; W. Jaeger, *Dtsch. Apoth. Ztg.* **1989**, *129*, 273–276.
200. A. Stoll; E. Seebeck, *Helv. Chim. Acta* **1949**, *32*, 197–205.
201. H. Schulz; H. Krueger; J. Liebmann; H. Peterka, *Lebensmittelchem.* **1999**, *53*, 2–3.
202. S. Taniguchi; T. Hatano; K. Yazaki, *Mokuzai Gakkaishi* **2006**, *52*, 67–76.
203. J. F. Prinz; P. W. Lucas, *J. Oral Rehabil.* **2000**, *27*, 991–994.
204. M. Kusumoto; F. Yoshimura, *J. Jpn. Soc. Hortic. Sci.* **1976**, *45*, 46–80.
205. R. Saijo; Y. Takeda, *Nippon Shokuhin Kagaku Kogaku Kaishi* **1999**, *46*, 138–147.
206. D. G. Nagle; D. Ferreira; Y.-D. Zhou, *Phytochemistry* **2006**, *67*, 1849–1855.
207. N. Subramanian; P. Venkatesh; S. Ganguli; V. P. Sinkar, *J. Agric. Food Chem.* **1999**, *47*, 2571–2578.
208. C.-T. Ho; S. Sang; J. W. Jhoo, Chemistry of Theaflavins: The Astringent Taste Compounds of Black Tea. In *Challenges in Taste Chemistry and Biology,* T. Hofmann, C.-T. Ho, W. Pickenhagen, Eds.; ACS Symposium Series 867; American Chemical Society; Washington, DC, USA, 2004; pp 125–138.
209. T. Ozawa; M. Kataoka; K. Morikawa; O. Negishi, *Biosci. Biotechnol. Biochem.* **1996**, *60*, 2023–2027.
210. E. Sondheimer, *Arch. Biochem. Biophys.* **1958**, *74*, 131–138.
211. J. Cheel; G. Schmeda-Hirschmann; M. Jordan; C. Theoduloz; J. A. Rodriguez; A. Gerth; D. Wilken, *Z. Naturforsch. C: J. Biosci.* **2007**, *62*, 555–562.
212. V. Cheynier; M. Duenas-Paton; E. Salas; C. Maury; J.-M. Souquet; P. Sarni-Manchado; H. Fulcrand, *Am. J. Enol. Vitic.* **2006**, *57*, 298–305.
213. K. Igarashi, *Foods & Food Ingred. J. Jpn.* **2000**, *187*, 17–29.
214. S. D. Roper, *Forum Nutr.* **2003**, *56*, 87–89.
215. G. Nelson; J. Chandrashekar; M. A. Hoon; L. Feng; G. Zhao; N. J. P. Ryba; C. S. Zuker, *Nature* **2002**, *416*, 199–202.
216. M. Ozeck; P. Brust; H. Xu; G. Servant, *Eur. J. Pharmacol.* **2004**, *489*, 139–149.
217. S. Arai; M. Yamashita; M. Noguchi; M. Fujimaki, *Agric. Biol. Chem.* **1973**, *37*, 151–156.
218. S. Yamaguchi; S. Kanno; T. Haga, *Jpn. J. Taste Smell Res.* **1995**, *2*, 467–470.
219. A. Kuninaka, *Adv. Chem. Ser.* **1966**, *56*, 261–274.
220. S. Yamaguchi, *J. Food Sci.* **1967**, *32*, 473–487.
221. K. Kurihara; M. Kashiwayanagi, *Ann. N. Y. Acad. Sci.* **1998**, *855*, 393–397.
222. H. Furukawa, *Nippon Nogeikagaku Kaishi* **1991**, *65*, 163–169.
223. Y. Hayakawa; M. Kawai, *Jpn. J. Taste Smell Res.* **2003**, *10*, 463–466.
224. M. Kawai; A. Okiyama; Y. Ueda, *Chem. Senses* **2002**, *27*, 739–745.
225. S. Otsuka; H. Eguchi, Seasonings Containing Amino Acids, Nucleotides, Inorganic Salts, and Glycogen or Amylopectin with Similar Taste with Adductor Muscle of Scallops (*Patinopecten yessoensis).* JP Patent Appl. 63,063,362, 1988.
226. T. Saigo; K. Watanabe; M. Morita; T. Nakano, *Chagyo Kenkyu Hokoku* **1979**, *50*, 31–41.
227. T. Takemoto; T. Nakajima, *Yakugaku Zasshi* **1964**, *84*, 1183–1186.
228. T. Takemoto; T. Nakajima; T. Yokobe, *Yakugaku Zasshi* **1964**, *84*, 1232–1233.
229. T. Takahashi; T. Asai, *Proc. Imp. Acad. (Japan)* **1927**, *3*, 86–89.
230. K. Aoki, *Nippon Nogeikagaku Kaishi* **1932**, *8*, 867–868.
231. S. Kodama, *J. Tokyo Chem. Soc.* **1913**, *34*, 751–757.
232. A. Kuninaka, *Nippon Nogeikagaku Kaishi* **1960**, *34*, 489–492.
233. N. Nakajima; K. Ichikawa; M. Kamada; E. Fujita, *Nippon Nogeikagaku Kaishi* **1961**, *35*, 797–803.
234. M. Honjo; K. Imai; Y. Furukawa; Y. Kanai; R. Marumoto; H. Honda; H. Aoki; T. Hirata, *Takeda Kenkyusho Nenpo* **1966**, *25*, 74–81.
235. S. Yamaguchi; T. Yoshikawa; S. Ikeda; T. Ninomiya, *J. Food Sci.* **1971**, *36*, 846–849.
236. S. Arai; M. Yamashita; M. Fujimaki, *Agric. Biol. Chem.* **1972**, *36*, 1253–1256.
237. E. Frerot; S. D. Escher, Flavored Products and Method for Preparing Same. PCT Int. Appl. WO 97/04667,1997.
238. M. Kuroda; T. Yamanaka; N. Miyamura, *Jpn. J. Taste Smell Res.* **2004**, *11*, 175–180.
239. Y. Ueda; M. Sakaguchi; K. Hirayama; R. Miyajimai; A. Kimizuka, *Agric. Biol. Chem.* **1990**, *54*, 163–169.
240. Y. Ueda; T. Tsubuku; R. Miyajima, *Biosci. Biotechnol. Biochem.* **1994**, *58*, 108–110.
241. K. Shima; N. Yamada; E. Suzuki; T. Harada, *J. Agric. Food Chem.* **1998**, *46*, 1465–1468.
242. K. Shima, *Meat Sci.* **2002**, *43*, 3–9.
243. K. Shima; K. Ishikawa; K. Izawa; E. Suzuki, *Anal. Sci.* **1998**, *14*, 1185–1186.
244. U. Watanabe; T. Yamamoto, *Jpn. J. Taste Smell Res.* **2003**, *10*, 523–526.
245. A. Dunkel; J. Koester; T. Hofmann, *J. Agric. Food Chem.* **2007**, *55*, 6712–6719.
246. R. Aoyama; M. Murase; K. Yamagishi; K. Nishi; H. Kojima, Development of Fermentation Method for Producing Non-Amino Organic Acids by Using Recombinant Coryneform Bacteria. PCT Int. Appl. WO 2005026349, 25 March 2005.
247. T. Miyamoto, *Jpn. J. Taste Smell Res.* **2004**, *11*, 17–28.
248. C. Ruiz; S. Gutknecht; E. Delay; S. Kinnamon, *Chem. Senses* **2006**, *31*, 813–820.
249. T. A. Gilbertson; I. Kim, Chemoreception of Fat. In *Chemistry of Taste: Mechanisms, Behaviors, and Mimics*, P. Given, D. Paredes, Eds.; ACS Symposium Series 825; American Chemical Society: Washington, DC, USA, 2002; pp 180–191.

Biographical Sketches

Kunisuke Izawa was born in Hyogo, Japan, in 1945, and received his B.A. in 1968 and Ph.D. in 1973 from Osaka University under the direction of Professor Takayuki Fueno. He then joined the Central Research Laboratories of Ajinomoto Co., Inc., where he studied the cobalt-catalyzed amidocarbonylation (Wakamatsu) reaction. After studying a natural product synthesis as a postdoctoral fellow at MIT (with Professor George H. Buchi) for 2 years, he returned in 1981 to Basic Research Laboratories in the same company aiming at the discovery of new methodology for pharmaceuticals. In 1990, he moved to the Process Research Laboratories as a general manager. Since then, he has been engaged in the process development of pharmaceutical fine chemicals in Ajinomoto. In 2006, he became an advisor at AminoScience Laboratories in the same company, after serving as a corporate executive fellow for 7 years. He is also serving as regional president in the Society of Synthetic Organic Chemistry, Japan, from 2007. His research interest is in the field of organic synthesis utilizing amino acids, nucleosides, and carbohydrates.

Yusuke Amino was born in Japan in 1958. He received his master degree in 1983 and Ph.D. in 1991 from Kyoto University under the direction of Professor Takeo Saegusa and Professor Yoshihiko Ito. In 1983, he joined the Central Research Laboratories of Ajinomoto Co., Inc. He studied a natural product synthesis at Colorado State University (with Professor R. M. Williams) from 1991 to 1993. After studying the chemistry of sweet peptides at UCSD (with Professor M. Goodman) in 1994, he returned to Ajinomoto Co., Inc. Since then, he has been working on the structure–activity relationships of taste compounds.

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