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65 Cooling Ingredients and Their Mechanism of Action

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INTRODUCTION

The use of "purified" cooling agents in pharmaceutical and cosmetic preparations only dates back to the late 1880s with the commercial production of menthol from Japanese peppermint (*Mentha arvensis*) oil in Japan (1). The cultivation of peppermint in Japan before the Christian era appears to predate any other country, and menthol is reputed to have been used medicinally for almost as long (2). In the Western world, it was about 1770 that the Dutch botanist, H. David Gaubius, first isolated menthol from the oil of *Mentha piperita* in Utrecht (2,3). Prior to the commercial availability of menthol, the essential oils of peppermint varieties (primarily *M.* and *M. arvensis*) were the sole source for use as cooling minty ingredients. It is significant that at the end of the 18th century only about 900 to 1400 kg of peppermint oils (both *piperita* and *arvensis*) were consumed worldwide (1). By the late 1890s, production had increased to about 175,000 kg (2). In 2007, total peppermint oil production was estimated at more than 26,000,000 kg, with about 21,500,000 kg being the oil of *M. arvensis* (commonly referred to as cornmint oil), which is used mostly for the production of natural *leavo*-menthol (4).

This chapter reviews the use of menthol and new classes of cooling agents that have been discovered since the 1970s. In addition, we briefly touch upon the efficacy of cooling agents as insect repellents. Finally, recent findings on the physiological mechanisms of cold receptors are presented.

COOLING INGREDIENTS

Menthol Background

Before World War II, production of *leavo*-menthol [hereafter referred to as (–)-menthol] was controlled exclusively by Japan and China. In 1939, Japan exported 268,920 kg of menthol, while China's exports in 1940 were 190,909 kg (1). With the advent of war, shipments to the allied countries ceased and major shortages ensued. While synthetic (–)-menthol could be produced from high citronellal feed stocks (e.g., citronella oil and citronella-type eucalyptus oils), this also was no longer an option. However, Japanese and Chinese immigrants in Brazil rapidly began planting *M. arvensis* for menthol production. In 1941, Brazil produced 5000 kg of menthol, rising to 1,200,000 kg by 1945 (1). By the 1960s, Brazil's production peaked at about 3,000,000 kg, while about the same time China began supplying menthol again.

During the 1960s, an oversupply of menthol caused the price to fall to as low as $7.70 to $8.80 per kilogram, and processors reduced production levels. This ultimately led to worldwide shortages and a price spike as high as $50 plus per kilogram in 1974 (with similar price spikes now occurring about every 10 years) (3). As menthol is a commodity, it is sometimes subject to financial speculation, which exacerbates price swings.

In 1958, India began expanding plantings of *M. arvensis*, but, until the late 1980s, the quality was highly variable and often had low menthol content. In the 1980s, new strains were introduced that gave improved oil yields and had menthol contents of 75% to 85%. By 1996, India was producing 6000 metric ton of *M. arvensis* oil and had long surpassed China as the major producer of menthol (3). In 2007, it was estimated that India would produce in excess of 20,000 metric ton of this mint oil. While the bulk of current production is used for local menthol crystallization, significant amounts of oil and crude menthol fractions are exported to Brazil, Taiwan, and Japan for further purification. The residual oil left after crystallizing

menthol still contains 35% to 45% menthol as well as menthones and other typical mint components. Much of this oil (commonly referred to as dementholized cornmint oil) is rectified by distillation and sold for use where normal peppermint oil (ex *M. piperita*) is used (toothpaste, mouthwash, etc.). In addition, some of this dementholized oil is fractionated to isolate the menthones (which can be converted by reduction into (–)-menthol) and other "natural" flavor chemicals.

During the 1970s and 1980s, a number of new routes to synthetic (–)-menthol were developed, only two of which led to long-term commercial success. These processes have been reviewed by both Leffingwell (5) and Hopp and Lawrence (6). Today, the procedure developed by Haarmann and Reimer (now Symrise) on the basis of hydrogenation of thymol to racemic *dl*-menthol followed by selective crystallization of (–)-menthol (via the benzoate ester) is the major process (7).

The Takasago process uses myrcene as the raw material, which is converted to *N*,*N*-diethylgeranylamine and then asymmetrically isomerized via the chiral rhodium (S)-BINAP (or SEGPHOS) complex to the optically active enamine of citronellal. Hydrolysis yields (+)-citronellal, which is cyclized to (–)-isopulegol by classical methods. On hydrogenation, the isopulegol gives (–)-menthol in high optical purity (8,9). An alternative starting material (instead of myrcene) is isoprene, which can be dimerized to *N*,*N*-diethylnerylamine. This material can be converted to (–)-menthol in a manner analogous to the myrcene route using rhodium (R)-BINAP as the chiral catalyst (10). Reflecting on the Takasago process, Ryoji Noyori stated in his 2001 Nobel lecture, "*This resulted from a fruitful academic/industrial collaboration*. . . ." (11).

Clark estimates that 2007 worldwide consumption of menthol from all sources (i.e., peppermint oils, natural menthol, and synthetic menthol) is 32,000 metric ton, of which 19,170 metric ton is purified menthol (4).

Table 1 provides our estimate of production in producing countries (or in the case of Symrise and Takasago, company production of synthetic (–)-menthol).

Table 2 provides the 2007 estimated worldwide usage of menthol by consumer product category—on the basis of Clark's data by region (4).

Table 1 Worldwide Sources of Menthol (2007)

Source	Metric ton
India (natural)	9,700
China (natural)	2,120
Symrise (synthetic)	3,600
Takasago (synthetic)	1,500
Other synthetic[a]	1,200
Brazil (natural)[b]	450
Taiwan (natural)[b]	300
Japan (natural)[b]	300
Total[c]	19,170

[a]Other synthetic includes menthol produced from menthone as well as racemic menthol.
[b]Primarily from *Mentha arvensis* oil or crude menthol ex India (or China).
[c]Total menthol volume based on Clark's estimate (4).

Table 2 2007 Estimated Worldwide Consumption of Menthol % by Product Category

Product category	Menthol %
Oral hygiene	28.00
Pharmaceuticals	26.60
Tobacco	25.30
Confectionaries	11.00
Shaving products	7.00
Miscellaneous	2.10

Source: From Ref. 4.

Menthol Chemistry

Menthol is a $C_{10}H_{20}O$ terpenoid alcohol (MW 156.27) with three chiral centers leading to eight possible stereoisomers (4 enantiomeric pairs). The characterization of the stereoisomeric menthols was painstakingly resolved prior to the availability of modern methods by Read (12,13). The structures of the eight enantiomers, with their optical rotations (in ethanol), are shown in Figure 1.

Only the (–)-menthol enantiomer possesses the clean desirable minty odor and intense cooling properties. For example, the (+)-menthol enantiomer is less cooling and possesses a musty off-note odor that is undesirable in most applications. This musty note is also present in racemic menthol (15). The organoleptics and cooling strengths of all of the enantiomers have been reviewed (5,6). Figure 2 provides the cooling thresholds in ppm.

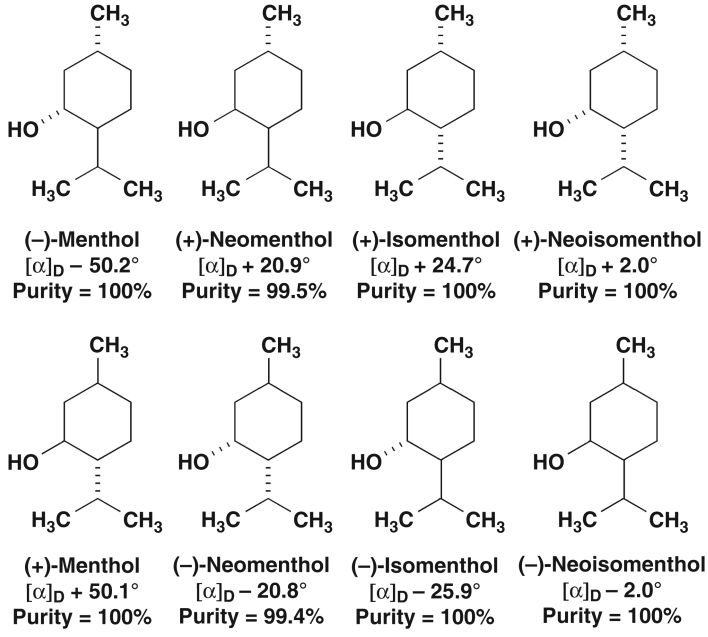

Figure 1 Stereoisomers of menthol. *Source*: From Ref. 14.

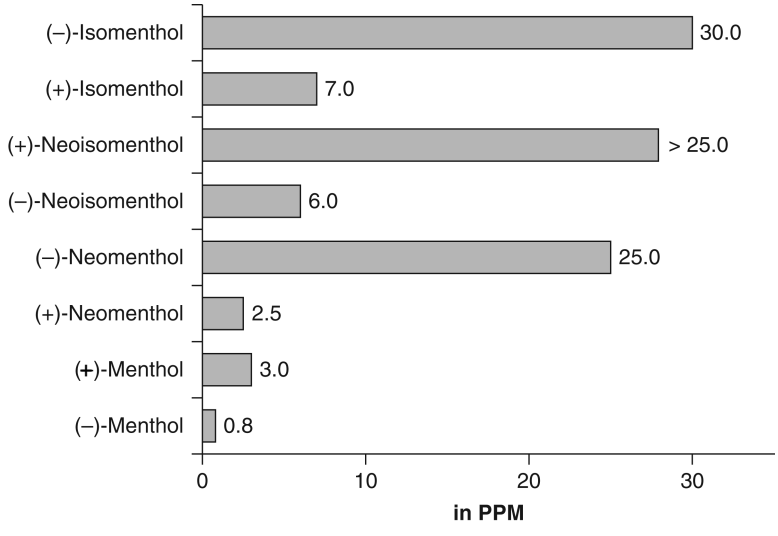

Figure 2 Cooling thresholds (in ppm) (by taste dilution). *Source*: From Ref. 14.

Table 3 Major Impurities in Synthetic and Natural Menthols

Major impurities	Synthetic %	Brazil 1 %	Brazil 2 %	China 1 %	China 2 %	India %
Menthone	0.0069	0.0258	0.0258	0.0135	0.0350	0.0295
Isomenthone	0.0000	0.0069	0.0172	0.0052	0.0123	0.0155
Menthyl acetate	0.0000	0.0100	0.0148	0.0014	0.0128	0.0048
Isopulegol	0.0022	0.1868	0.1651	0.1374	0.1914	0.1789
Neomenthol	0.0032	0.0689	0.1339	0.0951	0.0882	0.1079
Neoisomenthol	0.0000	0.0075	0.0459	0.0352	0.0177	0.0368
Isomenthol	0.0299	0.0099	0.0442	0.0296	0.0248	0.0322
Piperitone	0.0000	0.0053	0.0046	0.0018	0.0031	0.0024
Totals	0.0422	0.3211	0.4515	0.3192	0.3853	0.4080

Source: From Ref. 16.

Natural menthol ex *M. arvensis* oil is normally about 99.0% to 99.6% pure, with the remaining impurities being other constituents found in the cornmint oil. While, in most cases, the mint oil impurities contribute a pleasant peppermint aroma, certain impurities, such as mint sulfide, can also impart less desirable and harsh notes. Thus, odor discrepancies often arise when comparing samples from different companies or countries. To overcome such differences, the skilled technician can add a small percentage (e.g., 0.2–0.4%) of terpeneless peppermint oil ex *M. piperita* (or redistilled dementholized cornmint oil), which adds the desirable sweet peppermint top note. Table 3 compares the major impurities present in synthetic menthol and natural menthol samples from major producing areas (16).

Although not generally commercially available, menthol produced from *M. piperita* oil has a sweeter peppermint top note than that produced from cornmint oil (JC Leffingwell, unpublished observations).

Synthetic (–)-menthol is normally about plus 99.8% pure and has less of the minty top note present in natural menthol. Again, this can be adjusted to increase the mint character, if desired, by the addition of a small amount of terpeneless peppermint oils.

Menthol-Related Cooling Agents

Interest in menthol-related cooling agents began in the late 1950s to 1960s when several tobacco companies began to develop various esters as potential menthol release agents (17–19), some of which now appear on the flavor extract manufacturers association's GRAS list. Among those of interest today is monomenthyl succinate (MMS) (FEMA# 3810) (18), which was later patented by Mane as a cooling agent for general use (20). In addition, menthol ethylene glycol carbonate (Frescolat® MGC), with FEMA# 3805, and menthol propylene glycol carbonate (Frescolat MPC), with FEMA# 3806, were first patented as tobacco flavorants (19), again to be later patented by Haarmann and Reimer for general cooling usages (21).

A number of other menthol-related cooling agents are commercially available: menthone glycerol ketal (Frescolat MGA) (22)—both the racemic (FEMA# 3808) and *leavo* forms (FEMA# 3807); the *leavo* form appears to be the main item of commerce. This material provides a clean cooling refreshing effect and as a partial replacement of peppermint oil has been shown to provide longer-lasting sweetness and a higher cooling sensation in chewing gum (23). (–)-Menthyl lactate (Frescolat ML) is faintly minty in odor and virtually tasteless with a pleasant, long-lasting cooling effect (24). Recently, Erman has shown that the (–)-ML of commerce has the 'S' configuration for the hydroxy moiety, indicating the fact that it is produced by the esterification of (–)-menthol with (S)-(+)-lactic acid (25). 3-(l-Menthoxy) propane-1,2-diol, known as MPD, Coolact® agent 10, TK-10, and coolant agent 10, is another important commercial cooling agent, which, in contrast to menthol, is essentially odorless (26). The cooling threshold (in mouth) is 1 ppm (about 20–100% that of menthol), and the time of cold-feeling maintenance is 20 to 25 minutes for a 100-ppm solution (about twice that of menthol). While the cooling strength of Coolact agent 10 is accepted as being about 20% to 25% that of menthol, it is also noted that "in a Vaseline ointment, 3-(l-menthoxy)propane-1,2-diol shows a cool feeling 2.0 to 2.5 times stronger than that of (–)-menthol" (27). The cool-feeling intensity of the (2S) isomer is 2 to 3 times that of the (2R) isomer and 1.5 to 2 times

superior to that of the racemic modification (28). Similarly, the related menthoxyalkanols, 3-(l-menthoxy)-2-methylpropane-1,2-diol (FEMA# 3849), 3-(l-menthoxy)ethanol (Coolact 5), FEMA# 4154, 3-(l-menthoxy)propan-1-ol, and 3-(l-menthoxy)butan-1-ol have cooling properties (29). Interestingly, cooling compounds such as 3-(l-menthoxy)propane-1,2-diol and 3-(l-menthoxy)-2-methylpropane-1,2-diol when admixed with warming sensates (e.g., vanillyl butyl ether, ginger extract, or capsicum tincture) provide increased warmth and longer-lasting warmth in cosmetic and flavor systems (27,30,31). Conversely, it has also been observed that admixtures of such cooling compounds with the warming sensate vanillin-MPD (the acetal of 3-(l-menthoxy) propane-1,2-diol and vanillin), FEMA# 3904, can increase the duration of cooling sensations (32) (Fig. 3).

(–)-Isopulegol (Coolact P), FEMA# 2962, having a chemical purity of better than 99.7% and an optical purity of not less than 99.7% ee, is odorless and gives a feeling of freshness, crispness, and coolness. The cooling strength is about 20% to 30% that of (–)-menthol (33). The *p*-menthane-3,8-diols (Coolact 38D, PMD38), FEMA# 4053, consist of a mixture of (+)-*cis* and

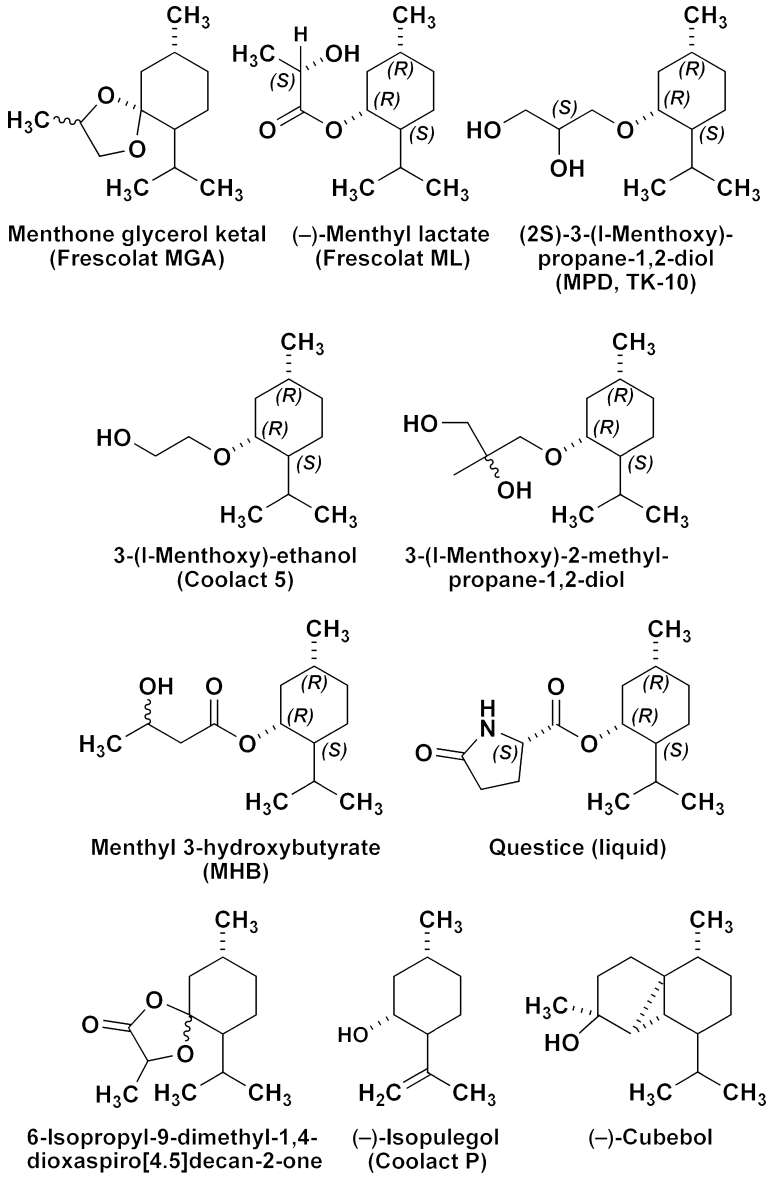

Figure 3 Menthoxy-related coolants.

(–)-*trans* PMD38 in a ratio of ~62:38 and possesses a cooling strength of about 11% that of (–)-menthol (27,34). PMD38 is a nature identical material that occurs in a number of citronellal-rich oils (e.g., *Litsea cubeba*, *Eucalyptus citriodora*) and is also effective as an insect repellant (34–36). (–)-Monomenthyl glutarate (Physcool 2, MMG), FEMA# 4006, is a nature identical cooling agent that has been found in *Litchi sinensis* accompanied by (–)-dimenthyl glutarate (37). It has been described as "probably the longest-lasting oral cooling agent that is commercially available" (38). Recently, an improved synthesis has been reported for both MMG and MMS that minimizes the amount of diester impurities (39). Similarly, (–)-MMS has been confirmed to be nature identical by its isolation from *Lycium barbarum* and *M. piperita* (37). A recent description of MMS indicates that it is virtually tasteless and has well-balanced cooling onset and length of cooling (38). Questice® (menthyl pyrrolidin-2-one 5-carboxylate) was first patented as a composition of matter that acts as a long-lasting cooling and fresh ingredient in toothpaste. The cooling properties are due to the enzymatic hydrolytic release of menthol. A liquid form was produced by reacting (–)-menthol with L-pyrrolidin-2-one carboxylic acid, while a crystalline form was produced when racemic DL-pyrrolidin-2-one carboxylic acid is employed (40). Surprisingly, it did not appear on the GRAS list until 2005 with FEMA# 2155 (41). However, it has long been employed in various cosmetics, lotions, etc. Recently, Erman has shown that the liquid form of Questice is a diastereoisomeric mixture of (–)-menthyl 5-oxopyrrolidine-2-carboxylates with a ratio of the 5S:5R configuration of ~91:8, while the solid form has a ratio of ~46:53 (25). (–)-Menthyl 3-hydroxybutyrate (MHB), FEMA# 4308, is another recent addition to the GRAS list (42). This is reported by workers at Takasago as having a long-acting excellent cooling effect and is odorless and tasteless. Potential uses include foods, drinks, cosmetics, pharmaceuticals, and cigarettes (43). Other workers indicate that the cooling effect is slightly stronger than ML (about 48% the cooling strength of menthol) (44). Firmenich workers have recently found that a diastereoisomeric mixture of the 6-isopropyl-3,9-dimethyl-1,4-dioxaspiro[4.5]decan-2-ones, prepared by reacting lactic acid with *cis* and *trans*-menthones, provides a minty, fresh, *piperita*-type flavor that is remarkable by its strength and cleanness. In combination with other cooling agents (e.g., menthyl succinate or menthol), a synergist increase in cooling strength was found. In particular, the (3S,5R,6S,9R) and (3S,5S,6S,9R) isomers are preferred (45). A patent describes the use of certain esters such as (–)-menthyl methoxyacetate and (–)-menthyl 3,6-dioxaheptanoate as cooling agents (46). In addition to the cooling properties, (–)-menthyl methoxyacetate has a head note and fruity taste resembling that of menthyl acetate, whereas (–)-menthyl 3,6-dioxaheptanoate has a bitter taste. Cubebol, a natural isolate of cubeb oil, in which it normally occurs at levels of 10% to 30% (47), is a sesquiterpenoid alcohol that has a certain stereochemical resemblance to menthol and, while not menthol derived, is included here for completeness. Cubebol has only a very weak smell and taste and provides a refreshing effect that develops in the mouth after a delay of approximately 1 to 2 minutes and lasts for approximately 30 minutes. It has applications in flavors, oral care, pharmaceutical products, etc. (48).

Carboxamide Cooling Agents

During the early 1970s, Wilkinson Sword Ltd. conducted an extensive research program in which they designed and evaluated about 1200 compounds for their cooling activity (49,50). The interest in such compounds related to cooling agents without the minty and volatile side effects of menthol, such as eye irritation, in aftershave lotions, etc. Over 25 U.S. patents were issued on these materials (51). Of these original Wilkinson Sword compounds, three were initially commercialized: WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) (52), WS-23 (2-isopropyl-*N*,2,3-trimethylbutyramide) (53), and WS-14 [*N*-([ethoxycarbonyl]methyl)-*p*-menthane-3-carboxamide] (52). WS-3 was given GRAS status (FEMA# 3455) in 1975 (54) and WS-23 (FEMA# 3804) in 1996 (55). Interestingly, WS-14 was used as a cooling agent for the Northwind cigarette introduced into test market in 1981. This test market was short lived, but it is not clear if this was because of market failure or concern that the additive testing conducted was insufficient to pass Food and Drug Administration (FDA) scrutiny (56). WS-14 is commercially available as ICE 4000 cooling sensate (57) and finds some applications as a topical cooling agent (Fig. 4).

In 2007, WS-5 [ethyl 3-(*p*-menthane-3-carboxamido)acetate], which is currently the coldest of all commercial cooling agents, was granted GRAS status as FEMA# 4309 (42). It has been found that only highly purified WS-5 is suitable for flavoring purposes (58), as less pure

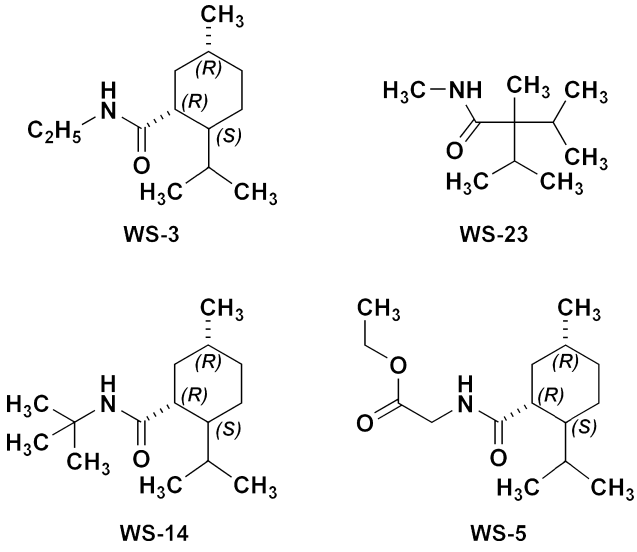

Figure 4 Successful Wilkinson sword coolants.

material exhibits a powerful bitter taste. WS-3 and WS-23 are currently the two largest volume carboxamide coolants. They are widely used in flavors, especially for chewing gum, breath fresheners, confectionaries, and oral care. They also find use in cosmetics (e.g., aftershave lotions). As both WS-3 and WS-23 are solids, there has been considerable interest in developing blends of such cooling agents that provide strong cooling but are easy to handle liquids. For example, it has been found that mixtures of ML, WS-3, and propylene glycol form stable liquid systems (59). It has also been shown that WS-3, WS-5, WS-14, and WS-23, alone or in certain combinations, when mixed with ML (or other coolants such as menthoxypropane-1,2-diol) will form stable liquid systems (60), and such mixtures often give a synergistic increase in cooling sensation. Similarly, eutectic mixtures of WS-3 and WS-23 provide liquid cooling systems (61,62), which can be used either as cooling agents or as flavor and saltiness enhancers.

Another compound that can be classified either as a carboxamide or a menthyl ester is *N*,*N*-dimethyl menthyl succinamide (FEMA# 4230 for the racemate). An International Flavors & Fragrances (IFF) patent (63) describes this as having a cooling onset time of 25 seconds with cooling duration of 11.25 minutes. The taste/sensory profile is "cooling and refreshing on tongue, palate and front gums; fruity flavor with estery top-notes and sour undertones" (at 25 ppm in water). In a chewing gum at 0.2%, it increased sweetness and exhibited a pleasant and substantive cooling effect on the tongue and roof of the mouth.

Other examples of newly discovered carboxamides coolants are a series of analogs of WS-23 [such as *N*-(2-ethoxyethyl)-2-isopropyl-2,3-dimethylbutanamide] patented by Qaroma (64) and aryl carboxamide analogs (with the reversed amide configuration) by Givaudan (65), many with cooling intensities equal to or greater than WS-23. For example, *N*-(1-isopropyl-1,2-dimethylpropyl)-1,3-benzodioxole-5-carboxamide has about 2.2 times more cooling intensity as compared with 2 ppm of menthol (Fig. 5).

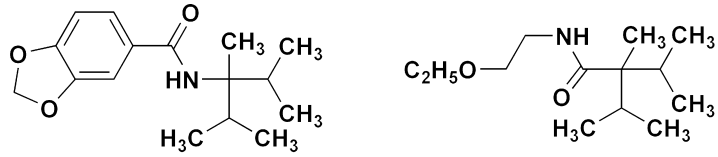

Figure 5 Recent WS-23 analogs.

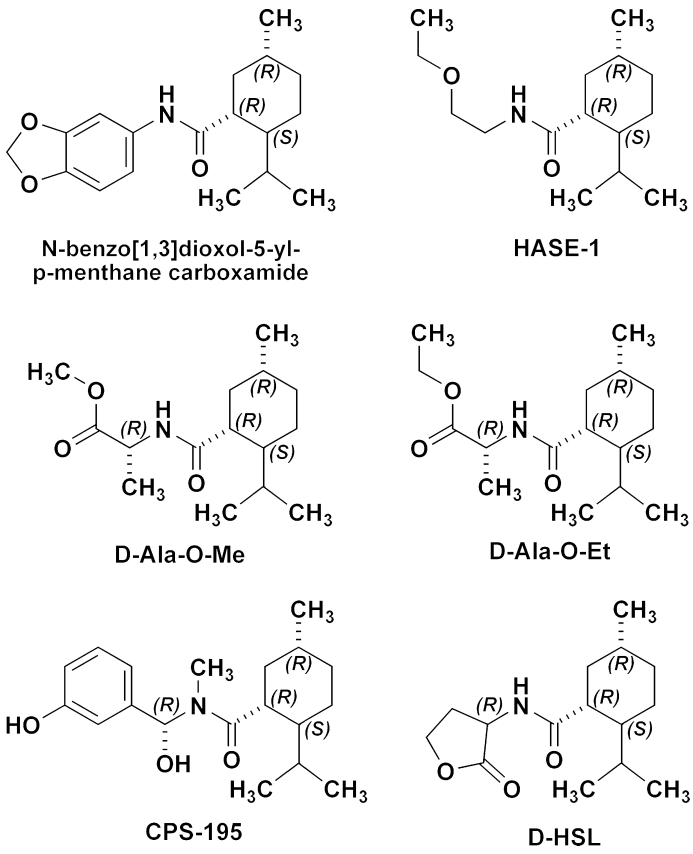

Figure 6 New *p*-menthane carboxamide coolants.

Of particular interest are various aryl *p*-menthane-3-carboxamides, such as *N*-benzo[1,3] dioxol-5-yl-3-*p*-menthanecarboxamide and *N*-benzooxazol-4-yl-3-*p*-menthanecarboxamide, which are reported to have 100 times more cooling intensity than menthol (when compared with menthol at 2 ppm) (66).

In 2004, T. Hasegawa Co. Ltd. patented a new series of strong cooling compounds on the basis of alkyloxy amides of the *p*-menthane series, which exhibit no bitterness; compound HASE-1 is an example (Fig. 6) (67).

Further, Wei (68) has shown that several materials related to WS-5 possess strong cooling with remarkable cooling longevity. For example, the methyl and ethyl ester analogs of WS-5 (referred to as D-Ala-O-Me and D-Ala-O-Et, respectively) are produced from D-alanine (rather than glycine). Similarly, when D-homoserine lactone is employed, the resultant compound is *N*-(R)-2-oxotetrahydrofuran-3-y1-(1R,2S,5R)-*p*-menthane-3-carboxamide (referred to as "D-HSL"), which also is a potent long-lasting coolant. By combining suitable sympathomimetic amine drugs that act as α-adrenergic receptor agonists to form the corresponding *p*-menthane carboxamides, Wei found certain compounds (such as L-phenylephrine *p*-menthane carboxamide, referred to as CPS-195) that were effective as long-lasting coolants and possessed additional therapeutic properties (69). The cooling duration of a number of these, applied to the skin as a 1% wt/vol in a petrolatum-based ointment, versus leading coolants is shown in Figure 7.

In the last 10 years, there has been extensive patent activity relative to physiological cooling agents. Between 1998 and 2007, more than 280 patents were issued (25,63), and from January 2005 to December 2007 more than 300 patent applications have been filed. It is beyond the scope of this article to review all of these. However, it should be noted that a recent activity trend has been the patenting of various combinations of cooling agents, both to achieve improved cooling properties and/or for liquefaction of solid coolants (59,61,70,71). For example, it has been found that blends of menthyl glutarate with low levels of

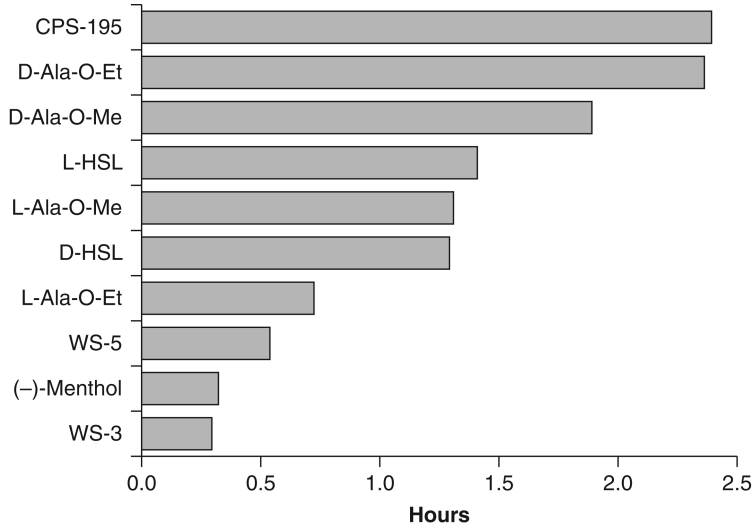

Figure 7 Topical cooling duration 1% in ointment. *Source*: From Refs. 68, 69.

(–)-isopulegol and/or PMD38 exhibit a remarkable synergistic increase in cooling in oral-care products (71).

The relative "accepted" cooling strengths of important coolants are shown in Figure 8 (72,73).

It should be noted that "accepted" cooling strengths, primarily associated with topical skin cooling, do not always agree when compared to oral sensory panel results. This is clearly shown by results obtained by Wm. Wrigley Jr. Company sensory panels comparing 5% sucrose solutions of various coolants versus 100-ppm (–)-menthol, as shown in Figure 9 (71).

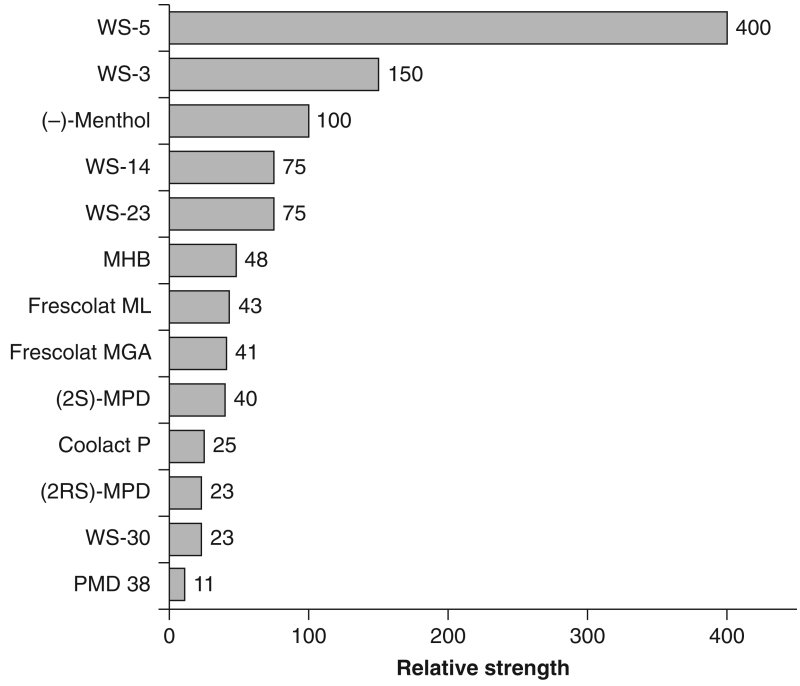

Figure 8 Approximate "accepted" cooling strengths versus menthol (as 100). *Source*: From Refs. 72, 73.

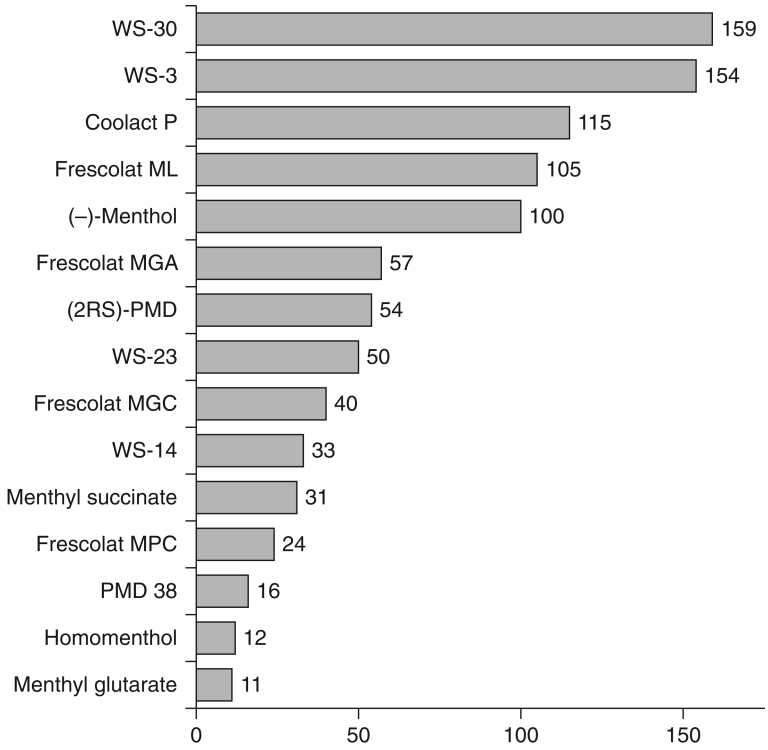

Figure 9 Relative oral cooling in 5% sucrose solutions versus 100 ppm menthol. *Source*: From Ref. 71.

COOLING COMPOUNDS AS INSECT REPELLENTS

As previously mentioned, the PMD38 have shown effectiveness as an insect repellent. Barnard has compared its efficacy against the leading insect repellants DEET, IR3535 [ethyl 3-(*N*-butyl-*N*-acetyl)-aminopropionate], and KBR3023 [sec-butyl 2-(2-hydroxyethyl)piperidine-1-carboxylate] (74,75).

Questice (menthyl pyrrolidone carboxylate) has also been patented as an insect repellent (76) and, Kalbe and Nentwig describe the use of ML or menthol glycerol acetal for repelling mites and other insects (77). Notably, Gautschi and Blondeau of Givaudan have discovered that WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) and related N-substituted *p*-menthane carboxamides have insect repelling activity against cockroaches equal to or exceeding that of DEET (diethyl-*m*-toluamide) (79). Another Givaudan patent application describes the use of a series of (–)-menthyl carbamates as insect repellents, but is silent relative to their cooling activity (80).

COLD RECEPTORS AND MECHANISM OF ACTION

The underlying process in thermoreception, whether hot or cold, is dependent on ion transport across cellular membranes. Cellular membranes consist of an oily phospholipid bilayer, which would be impermeable to ions such as K^+ or Ca^{2+}, except for receptor protein ion channels.

The flow of ions through these gated ion channels can cause rapid changes in ion concentrations, which in turn produce electrical signals that are the basis for many biological processes (80). In the case of thermoreceptors, these are activated when a thermal (or chemical) stimulus excites primary afferent sensory neurons of the dorsal or trigeminal ganglia (81).

In the last 12 years, there has been tremendous progress in determining the various receptor structural sequences. Thermoreceptors belong to the class of transient receptor potential (TRP) channels of which seven subfamilies exist (TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, and TRPN). Six members of three TRP subfamilies are involved in mammalian

Table 4 Thermoreceptor Agonists

Chemical agonist (botanical source)	ThermoTRP
Capsaicin (hot chilli peppers, e.g., Tabasco®)	TRPV1
Piperine (black pepper corns)	TRPV1
Allicin (fresh garlic)	TRPV1, TRPA1
Camphor (*Cinnamomum camphora*)	TRPV3, TRPV1
Δ-9-Tetrahydrocannabinol (*Cannabis sativa*)	TRPV2, TRPA1
2-Aminoethoxydiphenyl borate (synthetic)	TRPV1, TRPV2, TRPV3
4-α-phorbol 12,13-didecanoate (synthetic)	TRPV4
(-)-Menthol (peppermint)	TRMP8, TRPV3
1,8-Cineole, eucalyptol (eucalyptus)	TRPM8
WS-3 (synthetic)	TRPM8
Icilin (synthetic)	TRPM8, TRPA1
Cinnamaldehyde (cinnamon, cassia)	TRPA1, TRPV3
Allyl isothiocyanate (mustard, horseradish)	TRPA1
Benzyl isothiocyanate (mustard, horseradish)	TRPA1
Phenethyl isothiocyanate (mustard, horseradish)	TRPA1

Abbreviation: TRP, transient receptor potential.
Source: From Refs. 80, 84.

temperature-sensitive thermoreception. The closely related TRPV analogs are activated by heat, TRPV1 (≥43°C), TRPV2 (≥52°C), TRPV3 (22–40°C), and TRPV4 (>~27°C), while TRPM8 (<~28°C) and TRPA1 (<~18°C) are activated by cold (80). Certain types of chemical agonists activate these same thermoTRP channels. TRPV1 was the first thermoreceptor characterized and is referred to as a vanilloid receptor, as it is activated by capsaicin as well as heat. The cold and menthol receptor, TRPM8, was characterized by McKemy, Neuhausser, and Julius (82) and by Peier et al. (83) in 2002. Paradoxically, the cold receptor TRPA1, which is activated by noxious cold to produce a pain-like sensation, produces a human sensorial effect often described as "hot."

Table 4 provides examples of chemical agonists that activate these thermoTRPs.

All of these thermoTRPs are gated Ca^{2+} channels consisting of six transmembrane domains (TM1–TM6) flanked by large N- and C-terminal cytoplasmic domains (80). A schematic representation is shown in Figure 10 with the putative ion channel between TM5–TM6 in TRPM8, which is activated by menthol and other cold stimuli. In the case of TRP channels, it has been shown that they can oligomerize into tetramer assemblies, which presumably modulate the calcium ion gating processes (85–87).

Much of the knowledge gained on TRP activation by chemical stimuli has been derived by genetic expression of putative receptor domains and measurement of Ca^{2+} flux intensity by fluorometric imaging assays. Behrendt et al. used this technique to screen 70 odorants and menthol-related substances for activity on the recombinant cold-menthol receptor TRPM8

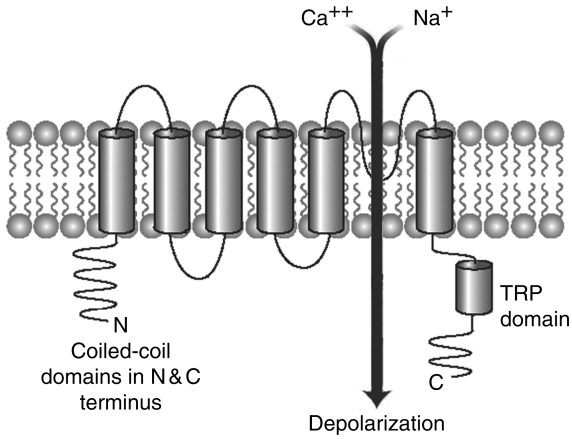

Figure 10 TRPM8 receptor channel.

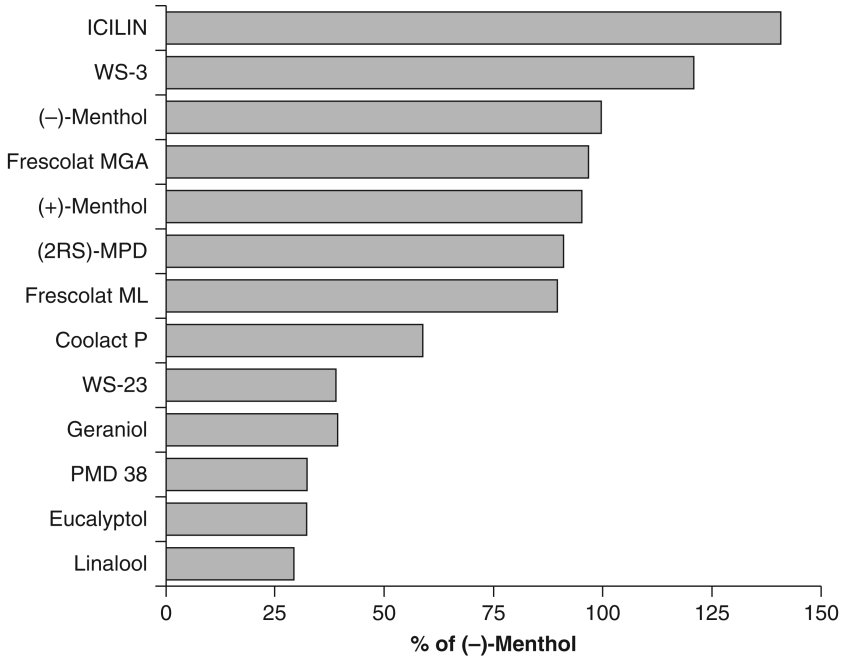

Figure 11 Efficacy of coolants on the TRPM8 receptor (by Ca^{2+} fluorometric assay). *Source*: From Refs. 72, 73, 88.

(mTRPM8), as expressed in HEK293 cells (88). The percentage efficacy of the most active candidates as compared to menthol is shown in Figure 11.

Although the fluorometric assay technique does not always translate into the human perception scale, it is already being used in industry to screen for promising new coolants (89).

In conclusion, from peppermint to menthol and to a plethora of new novel cooling compounds, we are now beginning to understand the importance of cooling substances even in the genetics of life. From early menthol-camphor-based over-the-counter (OTC) pharmaceuticals, which created famous trademarks such as Vicks® Vaporub and Mentholatum® in the early 20th century, to improved modern toothpastes, gums, breath fresheners, and cosmetic lotions, we expect this is just the beginning of even greater things for the future.

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