Cool without Menthol & Cooler than Menthol and Cooling Compounds as Insect Repellents

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Cool without Menthol

Over the last 30 years a considerable number of compounds have been synthesized and evaluated for the physiological sensation of "cooling".

In the 1970's Wilkinson Sword Ltd. conducted an extensive research program under the company leadership of Roy Randolph (who interestingly was my Aunt Joyce's husband until his recent death). During this period Hugh R. Watson and co-workers designed and evaluated about 1200 compounds for their cooling activity. The interest in such compounds relates to cooling sensation without the minty and volatile side effects of menthol such as eye irritation from aftershave lotions, etc.

Of these original Wilkinson Sword compounds, three have now been successfully commercialized - WS-23 (2-Isopropyl-N,2,3-trimethylbutyramide), WS-3 (N-Ethyl-p-menthane-3-carboxamide) and WS-5 [Ethyl 3-(p-menthane-3-carboxamido)acetate].

In the case of WS-3, the suppliers (Millennium Specialty Chemicals, Givaudan, Frutarom [as Framidice 3], Natural-Advantage & Qarôma, ) do not specify optical purity (as like menthol, this material can exist in 4 configurational isomers and 8 enantiomeric forms), but Watson et al. indicate, in general, that compounds in the all equitorial form (like menthol) are preferred and that WS-3 with the1R,3R,4S form (like (-)-menthol) should be optimal in cooling aspects. However, we are unaware of comparative studies that provide specific cooling properties of the various enantiomers. As WS-23 has no chiral center this compound (supplied by Millennium Specialty Chemicals, Penta, Rhodia & Qarôma) has only one form. WS-5, which is the "coldest" of commercially available "coolants" recently received GRAS approval, and is available from Millennium Specialty Chemicals & Qarôma.

Millennium describes WS-23 as an almost odorless white powder. It is characterized by a high cooling activity with no side effects such as burning, stinging or tingling sensations. Typical applications include use as a coolant in medicinal preparations, oral care products and confectionery products.

Millennium describes WS-3 as white crystalline and almost odorless. Its chief use is as a coolant in medicinal preparations, oral care products and confectionery products. Givaudan indicates this chemical is effective in combination with mint oils. It gives more impact, freshness and long-lasting flavour. The taste threshold is 200 ppb.
(by placing impregnated paper strip in the mouth). Mosciano, et. al. (2000) describes the Odor @ 10% as "Virtually odorless being slightly alcoholic and cooling" and the Taste @ 10-100 PPM as "Intense lingering cooling Trigeminal effect. The cooling sensation slowly but steadily grows to a lingering cooling mouth feel with a slightly camphoraceous and minty character."

WS-5 is known to be significantly cooler than WS-3. In US Patent Application No. 20050222256 by Mark Erman, et al. (October 6, 2005) they indicate that, for "highly purified (1R,3R,4S)-WS-5", the perceived cooling is about 2.5-3.0 times stronger than WS-3. See also Mark Erman, Progress in Physiological Cooling Agents, Perfumer & Flavorist, Vol. 29, No. 8, pp. 34-50 (2004).

In the recent US Patent 7,030,273 by Hong Sun, entitled Novel Compounds with Physiological Cooling Effect (April, 18, 2006) assigned to Qarôma, a new series of compounds, many with cooling intensity equal to or greater than WS-23 have been disclosed.

Other commercial cooling chemicals include the Menthone glycerol ketal$^{19}$ (sold as Frescolat® MGA by Haarmann & Reimer). Both the racemic and leavo-forms appear on the FEMA GRAS list but the leavo-form appears to be the item of commerce. (-)-Menthyl lactate (sold as Frescolat® ML by Haarmann & Reimer ) is faintly minty in odor and virtually tasteless, with a pleasant, long-lasting cooling effect.

(-)-Menthoxyp propane-1,2-diol$^{20}$ (sold as Coolant Agent 10 by Takasago International) is another commercial cooling agent. Takasago reports that the threshold (in mouth) is 1 ppm (about 20-100% that of menthol) and that the time of cold feeling maintenance is 20-25 minutes for a 100 ppm solution (about twice that of menthol). While the cooling strength of Cooling agent 10 is accepted as being about 20-25% that of menthol, it is also noted that "in a Vaseline ointment, 3-(1-menthoxyp)propane-1,2-diol shows a cool feeling 2.0 to 2.5 times stronger than that of l-menthol".\textsuperscript{25} It should also be noted that the cool-feeling intensity of the (2S) isomer is from 2 to 3 times superior to that of the (2R) isomer and is from 1.5 to 2 times superior to that of the racemic modification.\textsuperscript{28}
A related material, 3-((L-Menthoxy)-2-methylpropane-1,2-diol, appears on the FEMA GRAS list.

Although Isopulegol has previously been mentioned in the literature as having a cooling sensation, normally associated with a Minty, herbaceous, bitter-sweet odor and taste, Takeshi Yamamoto of Takasago has recently found\(^1\) that highly purified (\(-\))-Isopulegol (in excess of 99.7\% enantiomeric purity) is odorless and that the optically active form imparts a feeling of freshness, crispness, and coolness to citrus type fragrances. Sensate mixtures containing (\(-\))-Isopulegol, menthyl lactate and menthoxypropanediol have been patented\(^2\) for cosmetics. (\(-\))-Isopulegol is sold under the name "Coolact P\(^\text{®}\)" by Takasago International.

Recently, Takasago has patented the cis & trans p-Menthane-3,8-diol\(^2\) (PMD38 or Coolact 38D\(^\text{®}\)) as cooling agents\(^3\).

Although numerous other compounds (e.g., 2,3-dihydroxy-p-menthan, 3,3,5-trimethylcyclohexanone glycerol ketal) are mentioned in the literature and patents as having intrinsic cooling properties, the ones described here are those that possess very little or no odor and are sold in commerce. In the case of Questice\(^\text{®}\) (menthyl pyrrolidone carboxylate), the cooling property is considered not to be intrinsic but arises from enzymatic hydrolysis to menthol. Questice received FEMA GRAS status in 2005.

In March 2002, a Firmenich patent discloses the use of (1R,3R,4S)-3-menthyl-3,6-dioxaheptanoate, (1R,2S,5R)-3-menthyl methoxyacetate, (1R,2S,5R)-3-menthyl 3,6,9-trioxadecanoate, (1R,2S,5R)-3-menthyl 3,6,9-trioxadecanoate, (1R,2S,5R)-3-menthyl (2-hydroxyethoxy)acetate & (1R,2S,5R)-menthyl 11-hydroxy-3,6,9-trioxaundecanoate as cooling sensate compounds.
In 2001, Firmenich also patented Cubebol as a cooling and refreshing agent.\textsuperscript{27}

Further, in early 2004, T. Hasegawa Co., Ltd. patented a new series of cooling compounds based on alkyloxy amides in the p-menthane series\textsuperscript{29}.

In 2005, Givaudan patented a series of new p-menthane carboxamides - one of which [N-(4-cyanomethylphenyl) p-menthanecarboxamide] is claimed to be 10 times cooler than menthol\textsuperscript{31}.

Also in 2005, IFF patented\textsuperscript{32} a series of methyl half acid ester derivatives, one of which is N,N-Dimethyl menthyl succinamide (or more correctly 2-isopropyl-5-methylocyclohexyl 4-(dimethylamino)-4-oxobutanoate). This compound also received FEMA GRAS status (FEMA No. 4230) in 2005. Sensory evaluation indicated: Cooling and refreshing on tongue, palate and front gums; fruity flavor with estery topnotes and sour undertones. Cooling onset time 25 seconds. Cooling duration time 11.25 minutes. 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.

In November 2005, Qarōma announced the development of a new liquid cooling sensate called ICE 1500 (patents pending). This cooling sensate is a Eutectic mixture of ICE 1000 (WS-23) and ICE 3000 (WS-3), both of which are GRAS cooling compounds. The ICE 1500 remains liquid even at temperatures below -20\degree C. Further, ICE 1500 combines the instant cooling sensations of ICE 1000 with the gradual and smooth cooling sensation of ICE 3000. It also eliminates the dust problem associated with the handling of both the ICE 1000 and ICE 3000. In flavor...
applications, ICE1500 provides saltiness and/or flavor enhancement of about 20-30% in a wide variety of foodstuffs such as salsas, salad dressings and marinades, margarine, soups & bouillons, as well as alcoholic beverages, when used at levels where the cooling sensation is imperceptible or barely perceptible. To download the full press release Click HERE. See also the United States Patent Application 20050265930 by Erman, et. al., (December 1, 2005) entitled Physiological cooling compositions which describes similar liquid cooling compositions.

Icilin also known as AG-3-5, chemical name 1-[2-hydroxyphenyl]-4-[2-nitrophenyl]-1,2,3,6-tetrahydropyrimidine-2-one) was discovered in 1983 to produced sensations of coldness when in contact with mucous membranes (nostrils, lips and eyelids) of the researchers, and also when ingested (see Wei et al, J. Pharm. Pharmacol. 1983, 35:110-112). It is recognized that Icilin is a considerably more potent coolant than menthol. It is a super agonist that is 2.5-fold more efficacious and nearly 200-fold more potent than the reference cold thermosensory agonist, l-menthol

In April of 2004, Unilever Home & Personal Care USA, A Division of Conopco, Inc., disclosed a series of Icilin analogs that possess cooling potency similar to menthol (using cellular fluorescence)!

A number of patents using these materials (often in combination) in flavoring, perfume, cosmetic and oral care products have been issued. Some of these are listed below.

**Cooling Compounds as Insect Repellents**

Recently, it has been discovered that a number of cooling agents also have potent Insect repelling activity. In particular, Gautschi & Blondeau of Givaudan (United States Patent Application 20040028714, February 12, 2004) have discovered that WS-3 (N-Ethyl-p-menthane-3-carboxamide) and related N-substituted p-menthane carboxamides have insect repelling activity equal to or exceeding that of DEET (Diethyl-m-toluamide). The following example compares the repellency of WS-3 vs. DEET.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after Treatment</th>
<th>Treated Section % Present</th>
<th>Untreated Section % Present</th>
<th>Dead % Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS-3</td>
<td>1</td>
<td>1.5</td>
<td>98.0</td>
<td>0.5</td>
</tr>
<tr>
<td>WS-3</td>
<td>2</td>
<td>0.6</td>
<td>99.0</td>
<td>0.5</td>
</tr>
<tr>
<td>WS-3</td>
<td>3</td>
<td>4.5</td>
<td>95.0</td>
<td>0.5</td>
</tr>
<tr>
<td>WS-3</td>
<td>7</td>
<td>20.0</td>
<td>79.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Barnard, et. al., compared the mean percent repellency for Merck's IR3535® (a component in Avon's SKIN-SO-SOFT Bug Guard PLUS IR3535® Insect Repellent), Bayer's KBR3023 (Bayrepel®), para-Menthane-3,8-diol (PMD), and DEET against black salt marsh mosquitoes. In *J Med Entomol. 2002 Nov;39(6):895-9*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean % Repellency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck's IR3535®</td>
<td>88.6</td>
</tr>
<tr>
<td>Bayer's KBR3023 (Bayrepel®)</td>
<td>97.5</td>
</tr>
<tr>
<td>para-Menthane-3,8-diol (PMD)</td>
<td>89.2</td>
</tr>
<tr>
<td>DEET</td>
<td>94.8</td>
</tr>
</tbody>
</table>

Questice® (menthyl pyrrolidone carboxylate) has also been patented as an insect repellent (Watkins, et al., *United States Patent 6,451,844, September 17, 2002*) and Kalbe and Nentwig in *German Patent No. 19840321* describe the use of menthyl lactate or menthol glycerol acetal for repelling mites and other insects. In addition Watkins, et al., compared the repellent activity of Questice® vs. DEET and Menthol against mosquitoes.

<table>
<thead>
<tr>
<th>Comparative Repellency of Questice®, DEET &amp; Menthol Against Mesquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Menthyl 2-pyrrolidone-5-carboxylate</td>
</tr>
<tr>
<td>DEET</td>
</tr>
<tr>
<td>Menthol</td>
</tr>
</tbody>
</table>

Cooler Than Menthol ??
In November 2001, T. Hoffmann and co-workers in Germany reported a compound that occurs naturally in Malt with much greater cooling power than menthol. They say the most active compound, 4-methyl-3-(1-pyrrolidinyl)-2[5H]-furanone, which is from a family called cyclic alpha-keto enamines, is 35 times more powerful in the mouth, and 512 times more powerful on the skin, than menthol, the active ingredient in mint (based on threshold value ratios). The cooling effect also lasts twice as long. The three most active compounds synthesized are shown below.

![Chemical structures](image)

- **5-methyl-4-(1-pyrrolidinyl)-3-[2H]-furanone**
  - Odorless
  - Cooling Threshold - 1.5-3.0 ppm

- **4,5-dimethyl-3-(1-pyrrolidinyl)-2[5H]-furanone**
  - Faintly mint-like
  - Cooling Threshold - 2.0-4.0 ppm

- **4-methyl-3-(1-pyrrolidinyl)-2[5H]-furanone**
  - Odorless
  - Cooling Threshold - 0.02-0.06 ppm

This research received considerable press coverage [Today's Chemist (February 2002), Science News (Dec. 15, 2001), AAAS Eureka Alert (November 6, 2001), Irish Examiner (December 13, 2001), ScienceBase.com - Elemental Discoveries (December 2001), Deutsche Forschungsanstalt für Lebensmittelchemie Annual Report Summaries, 2001, The Hindu (Nov 22, 2001), ABC-Australia (Great Moments in Science), etc.], several of which quote the researchers as saying: "We've found the world's most powerful natural cooling agents without a mint odour."

However, at the December 4, 2003 meeting of the Society of Flavor Chemists meeting, industry sources revealed that in "actual practice" the cooling power of 4-methyl-3-(1-pyrrolidinyl)-2[5H]-furanone was very much lower than expected and this material was of little interest.

This chart shows the approximate cooling threshold ratios relative to menthol:

![Cooling threshold chart](chart)

Thus, it appears that the use of reported cooling threshold levels to estimate "actual cooling strength" can give erroneous results. At this same meeting, Mark Erman of Millennium Specialty Chemicals provided extensive information on the relative cooling strength of a number of cooling compounds. With permission, we have posted Dr. Erman's presentation on our site. This cooling strength information is provided in chart form below. Structures of the various WS compounds may be found in Dr. Erman's presentation.
Physiology of Cooling from materials such as menthol

The action of menthol and similar coolant compounds on "thermoreceptors" provides the "cool" sensation via cold receptors. In the case of menthol and certain other coolant compounds one can also get a "hot" or stinging "pain" sensation. Menthol can act at high concentrations in much the same way as capsaicin to produce a hot sensation, but in this case, it stimulates the fibers that register both cold temperatures as well as those that respond to warmth.

Recently (2001), Gordon Reid & Maria-Luiza Flonta at the University of Bucharest have discovered an inward ionic current that is activated by moderate cooling in a small number of rat sensory neurons. This current has features that are found in intact cold receptors, including sensitization by menthol, adaptation upon sustained cooling, and modulation by calcium, and is likely to be important in cold sensing. Early models indicated that menthol stimulates cold receptors by blocking voltage-dependent Ca2+ channels, leading to a reduction in intracellular Ca2+ and
inhibition of Ca2+-dependent K+ channels. However, Reid and others have since discovered that menthol stimulates entry of Ca2+ and increases intracellular Ca2+ concentration in cold-sensitive neurons; thus stimulation of cold receptors by menthol can be explained more simply by sensitization of the cold-induced inward Ca2+ current.

In the March 7, 2002 issue of Nature, McKemy, Neuhausser & Julius have characterized and cloned a menthol receptor from trigeminal sensory neurons that is also activated by thermal stimuli in the cool to cold range. This cold- and menthol-sensitive receptor, CMR1, is a member of the TRP family of excitatory ion channels, and they propose that it functions as a transducer of cold stimuli in the somatosensory system. These findings, together with their previous identification of the heat-sensitive channels VR1 and VRL-1, demonstrate that TRP channels detect temperatures over a wide range and are the principal sensors of thermal stimuli in the mammalian peripheral nervous system. In Nature 416, 52–58 (7 March 2002).

In the same issue of Nature, Charles Zucker explains how the discovery of a cold-sensitive ion channel will help dissect how the nervous system encodes and decodes the temperature spectrum. In Nature 416, 27–28 (7 March 2002)

Similarly, Andrea Peier et. al. described the cloning and characterization of TRPM8, a receptor activated by cold temperatures and by the cooling agent, menthol. In Cell, 108(5):705-15 (Mar 8, 2002)

Also in March 2002, Viana & co-workers results suggest that cold sensitivity is not associated to a specific transduction molecule but instead results from a favorable blend of ionic channels expressed in a small subset of sensory neurons. In Nat Neurosci 2002 Mar;5(3):254-60


In February 2004, H-J Behrendt, et. al., published a study on the effects of 70 odorants and menthol-related substances on recombinant cold-menthol receptor TRPM8 (mTRPM8), expressed in HEK293 cells. These were examined using a fluorometric imaging plate reader (FLIPR®) assay. In all, 10 substances (linalool, geraniol, hydroxycitronellal, WS-3, WS-23, FrescolatMGA, FrescolatML, PMD38, CoolactP and Cooling Agent 10) were found to be agonists. A summary of the potencies and efficacies are shown below (as adapted from their data). With a few exceptions, the data is in good agreement with the sensory data shown in the graphs above. This work gives a new approach for screening cooling compounds. In British Journal of Pharmacology 141:737-745, 2004
Relative Potency of TRPM8 agonists based on EC$_{50}$ values (mean) with (-)-Menthol = 100

Note - Icilin (EC$_{50}$ = 0.2±0.1 μM) not shown
(Relative icilin potency vs (-)-Menthol at 100 is 2050) -

For those interested see Physiology of Cooling references below.

### Coolant References:


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22. Suares; Alan Joseph; Znaiden; Alexander Paul; Feliciano; Donald Carl; Carrabotta; Michele, Cosmetic compositions with sensate mixtures based on isopulegol, United States Patent 6,267,974, July 31, 2001. Assigned to Unilever Home & Personal Care USA, Division of Conopco, Inc.


27. Velazco; Maria Ines; Wuensche; Laurent; Deladoey; Patrice, Use of cubebol as a flavoring ingredient, United States Patent 6,214,788, April 10, 2001. Assigned to Firmenich SA.


32. Dewis; Mark L.; Huber; Michelle E.; Cossette; Michael V., Menthyl half acid ester derivatives, processes for preparing same, and uses thereof for their cooling/refreshing effect in consumable materials, United States Patent 6,884,906, April 26, 2005. Assigned to International Flavors & Fragrances Inc.

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18. Eccles R, Role of cold receptors and menthol in thirst, the drive to breathe and arousal, *Appetite* 2000 Feb;34(1):29-35
22. Andrea M. Peier, Aziz Moqrich, Anne C. Hergarden, Alison J. Reeve, David A. Andersson, Gina M. Story,


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