Historical Review on the Identification of Mesifurane, 2,5-Dimethyl-4-methoxy-3(2H)-furanone, and Its Occurrence in Berries and Fruits

Heikki P. Kallio*

Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland
The Kevo Subarctic Research Institute, University of Turku, FI-20014 Turku, Finland

ABSTRACT: Mesifurane, 2,5-dimethyl-4-methoxy-3(2H)-furanone, is a natural compound used a worldwide as a flavoring for foods, beverages, and cosmetics. Global sales of mesifurane are around $100 million. Its significance as a flavor-impact compound in some Nordic berries was discovered in the early 1970s in Finland. Synthesized mesifurane was used as a key compound in aroma mixes exploited in a Finnish patent. Mesifurane is a significant flavorant in arctic brambles, mangoes, strawberries, and many other fruits and berries and is an enzymatic methylation product of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. Because of the obscurity of the information on the history of the commonly used trivial name, mesifurane, it is time to lift the veil and reveal the background of the present situation. The key player was a northern berry, arctic bramble (Rubus arcticus), the Finnish name of which is mesimarja. Forty years ago, aroma research was limited by technical factors, but nowadays there is a surplus of information.

KEYWORDS: arctic bramble, DMME, mesifurane, 2,5-dimethyl-4-methoxy-3(2H)-furanone

ARCTIC BRAMBLE, RUBUS ARCTICUS L., AND RELATED BERRIES

Arctic bramble (Rubus arcticus L.) is a boreal Eurasian berry and one of the Finnish wild natural treasures. It has become more and more rare because of changes in agriculture and forestry practices, but the berry is still commonly used for traditional liqueur production and for special delicacies. In addition being present in middle and south-east Finland, arctic bramble is a natural resource even in Finnish Lapland. The species exists especially in the deep valleys of the Teno, Kevojoki, and Pulmankijoki rivers as far north as 70 °N, which was thoroughly investigated by Mäkinen et al. (Figure 1). Its rich existence in these northern subarctic areas is due to the local climatic conditions in the river valleys.

According to Tammisola, natural populations of arctic bramble, which are richly fruiting, contain several incompatibility classes of the self-incompatible natural plant. This fact, together with the reduced natural growth areas, may be one reason for the dramatically decreased numbers of fruit crops of wild arctic bramble berries in Finland. In order to secure the maintenance and management of arctic bramble cultivations, molecular and morphological methods have been developed to monitor the relative densities of different R. arcticus cultivars in the field.

The aroma of arctic bramble is fruity, fragrant, and highly unique among Rubus berries. The biology, breeding, and cultivation of arctic bramble have been widely investigated in Finland and Sweden. Also, hybrids of R. arcticus and R. stellatus as well as of R. arcticus and R. idaeus have been developed for commercial cultivation. The material for aroma studies were mainly donations of Drs. Annikki Rynnänen and Erkki Huokuna (Agricultural Research Centre of Finland, South-Savo Experiment Station) and of Dr. Gunny Larsson (the Swedish University of Agricultural Sciences, Sweden).

DISCOVERY, ISOLATION, AND IDENTIFICATION OF THE MAJOR AROMA-IMPACT COMPOUND OF ARCTIC BRAMBLE

The research on arctic bramble started with the isolation of carbonyl compounds and by fractionating them as 2,4-dinitrophenylhydrazones in various classes by TLC. Analysis of the derivatives was carried out with packed column GC by using reference compounds. Acetone, diacetyl, methyl butanal, 2-butenal, and pentenal were shown to decrease during the ripening of the berry. However, it was immediately recognized that the ultimate goal could not be reached without GC-MS analysis of the volatiles with proper open tubular glass capillary columns, which were not commercially available at the time. Thus, the columns had to be prepared by the research group. Briefly, after drawing the glass tubing into the tunnel furnace, the inside surface of the capillary tubing was etched with CH₃Cl pyrolysis at a high temperature, which was followed by a static coating procedure with an FFAP liquid phase. The columns were used for GC-FID and GC-MS analyses of arctic bramble aroma compounds and later for many other purposes.

In addition, to maximize the number of compounds identified, interest was also focused on searching for the most relevant fractions and compounds with the typical arctic bramble aroma. The limitations of the packed GC columns were understood, and the only option was to carry out a sniffing procedure from the column outlet with the instrumentation available. As such, the sniffing sessions were highly preliminary and rough, and the results were not sufficient to be published in a quality journal. However, the interesting

Received: January 28, 2018
Revised: February 28, 2018
Accepted: February 28, 2018
Published: February 28, 2018
area framed on the chromatogram in Figure 2, labeled “sweet, pungent (arctic bramble),” gave a clear guideline on how to proceed. The volatile fraction obtained from the juice by vacuum distillation retained the typical natural aroma, and the descriptions of the compounds representing the marked peak area in Figure 2 were not far away from those of the juice.

The chromatogram obtained with a wall-coated, open tubular glass capillary column of the same arctic bramble volatile fraction is presented in Figure 3, which has three mass spectra inserted. Peak 137 was the most abundant compound in the distillate, covering one-third of all the volatiles, and represented the framed major peak in Figure 2. Sniffing the GC exit revealed the arctic bramble aroma characteristics of the compound. Isolation of this unknown compound from the extract of the distillate was carried out with preparative gas chromatography (Figure 4a), and a purity of >98% was reached (Figure 4b), which was sufficient for further analysis. Returning the compound back to the dearomatized juice restored the natural aroma surprisingly well. Among any berry-aroma profiles, it is unusual that one character-impact compound is this dominant.

The major compound of the concentrated arctic bramble distillate (Figure 3) was found to be an unknown compound with a molecular weight of 142. Two raspberry × arctic bramble hybrids were also found to contain this compound. In order to reveal the secret of the character-impact unknown, it was isolated and analyzed with capillary-GC-EI-MS, HR-MS, 1H NMR, UV, IR, and sensory methods, and the proper reference compounds were synthesized.

In the end, this seemingly simple protocol to identify a small molecule with a molecular weight of 142 was not trivial 45 years ago, a time when glass capillary columns were not yet even commonly used. A research group including several doctoral students in the area of berry and mushroom aromas was guided by Dr. Erkki Honkanen, the actual “founder” of scientific aroma chemistry of foods in Finland.

HR-MS analysis of the isolated compound gave a more accurate estimate of the molar mass, 142.0629 (C12H10O3). No elements other than 12C, 13C, O, and H were found. The unknown was DNPH-reactive (it contained a carbonyl moiety) and showed keto-enol tautomerism with KOH treatment. NMR revealed without a doubt the existence of a –O–CH3 substituent. The possibility of a –C(==O)–CH3 group, based on the NMR analysis, was, however, highly misleading misinformation. Thus, the first conclusions were that the structure was 4-methoxy-4-hexene-2,3-dione (Figure 5). Because the research group had no protocol to synthesize this methoxylated vicinal dione, they proceeded with the synthesis of a nonmethoxylated reference compound, 4-hexene-2,3-dione. Later, it was shown that the compound synthesized was the correct reference compound for the unknown but did not have the expected structure.

Information on the discovery of 4-methoxy-4-hexene-2,3-dione as “a key aroma compound in arctic bramble” was sent in spring 1974 to the organizers of the Fourth International Congress of Food Science and Technology, which was held in Madrid, Spain, September 23–27, 1974.

The summary of the analysis was accepted in the abstract book as abstract 1a.13 and denoted as an oral presentation. The congress was a forum where in addition to the aroma analysis, the progress in the preparation and use of capillary GC-columns was presented. At the congress, according to Mans Boelens, the dione compound presented had evidently a very short half-life, and after a later re-evaluation of the IR-analyses and the NMR data, it became clear that the unknown compound was 2,5-dimethyl-4-methoxy-3(2H)-furanone (MW 142.154, monoisotopic mass 142.063, CAS 4077-47-8). The new submission ended up in the final publication. The compound synthesized earlier as the reference to the dione-structured unknown was later proved not to have an open structure as expected. Instead, it was 2,5-dimethyl-3(2H)-furanone and, in the end, the “correct” reference compound for the unknown, 2,5-Dimethyl-4-methoxy-3(2H)-furanone (DMMF) became public as mesifuran for the first time in the Congress Proceedings according to the Finnish name of arctic bramble, “mesimarja” (nectar berry).

The NMR and UV analyses (Figure 6a,b) matched the confirmed structure, and the UV spectra in Figure 6b are clear signs of the keto-enol tautomerism of the compound. Also, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF, furaneol) was identified in the arctic bramble distillate (compound 219, Figure 3).

As a late consolation, an open-chain dione compound, 3,4-dihydroxy-3-hexene-2,5-dione, has been shown by Engel, Hofmann, and Schieberle to have a caramel flavor similar to that of 4-hydroxy-2,5-dimethyl-3(2H)-furanone. NMR analyses proved that the odor-active open-chain tautomer was present only in lipophilic solvents. The cyclic form (diacetylformmoin) was odorless and existed in aqueous solutions.
MESIFURANE IN OTHER BERRIES AND FRUITS

In addition to *Rubus arcticus*, the berries of a Pacific type of arctic bramble native at the Aleutian Islands (*Rubus arcticus* subsp. *stellatus* Sm.) have a weak aroma of artic bramble *R. arcticus*. The berries contain significant amounts of mesifurane but less than the Finnish arctic bramble. Hybrids of arctic brambles and raspberries (*R. idaeus* L.) developed at the Agricultural Research Centre of Finland were also quite rich in the compound, and raspberries were also later found to contain low amounts of mesifurane. The research group of Dr. Honkanen further verified the existence of the compound both in wild strawberries (*Fragaria vesca* L.) and in the strawberry (*Fragaria x ananassa*) cultivar ‘Senga Senga’. They defined mesifurane as a key aroma compound in strawberries, as later verified also by Larssen and Poll and numerous other research groups. In a thorough comparison of ten strawberry varieties, the contents of mesifurane were found to vary between 0.3 and 1.7 mg per kilogram of fresh berries.

Frozen storage of strawberries cause the loss of DMMF. Stability of DMMF and DMHF were further studied by the research group of Honkanen, and the half-lives of the compounds at room temperature at pH 4 were 320 and 100 days, respectively. Roscher, Schwab, and Schreier have also verified that mesifurane is more stable than DMHF for a wide range of pHs.

Already in 1974, Hunter, Bucek, and Radford found 2,5-dimethyl-4-methoxy-3(2H)-furanone in Alphonso mangoes by GC-MS using a stainless-steel capillary column coated with a 20 M Carbowax liquid phase. They also displayed the IR spectrum of the synthesized reference compound. Since the early studies of arctic bramble, mango, and strawberry, many other fruits, such as bacuri, cape gooseberry, capuaca, champa, guava, kiwifruit, lychee, passion fruit, pepino, and pineapple, are now known to contain 2,5-dimethyl-4-methoxy-3(2H)-furanone to various extents as a natural aroma compound. In most cases, its hydroxyl counterpart, DMHF, has also been coidentified, which is an indication of their close relation in the metabolome.

The research group of Cruz-Rus et al. has recently introduced methods for screening for mesifurane in strawberries based on optimized PCR analyses of the key gene *FaOMT*. This may be a starting point for fast-screening tools in the

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Figure 2. Packed-column GC-FID sniffing analysis of aroma compounds of arctic bramble (*Rubus arcticus* L.) isolated from pressed juice by vacuum—steam distillation with a climbing-film evaporator at 22 °C at a pressure of 10 Torr. The distillate was collected in a 2 L receiving flask and chilled in a mixture of NaCl and ice. The flask was connected to a vacuum pump by two cold traps chilled with liquid nitrogen. The combined distillate was saturated with purified NaCl and extracted for 24 h with pentane—diethyl ether (1:2, v/v) in a Kutscher—Steudel continuous extractor. Dichloromethane (5 mL) was added to remove the water as an azeotrope. The aroma extract was concentrated with a Widmer column at 35 °C to a final volume of 250 μL. The column used was a PerkinElmer F-11 GC 180 cm steel column (6.5 mm i.d.) filled with Chromosorb W (60—80 mesh, acid washed, DMCD treated) and coated with an 11% (w/w) GE-SF-96 liquid phase (glass-liner injector: 220 °C; oven program: 50—210 °C, 4 °C/min). After the column, the flow between the FID and the sniffing port was 1:80.
breeding of strawberries and maybe also other fruits and berries with superior flavor properties.

**BIOSYNTHESIS AND PROPERTIES OF MESIFURANE**

The biosynthesis of mesifurane in arctic bramble berries has not been investigated, but it is hypothesized in the Ph.D. Thesis of Kallio\(^\text{22}\) to be a ripening-related enzymatic methylation of the corresponding 2,5-dimethyl-4-hydroxy-3(2\(^H\))-furanone, which is evidently synthesized from fructose (Figure 7a). D-Glucose, D-fructose, D-fructose 6-phosphate, and D-fructose 1,6-diphosphate have been confirmed as intermediates of the precursor pathway of DMHF formation in strawberries.\(^\text{48−50}\) It has further been shown that in ripening strawberries, DMHF is a methylation substrate, and \(S\)-methyl-\(^{14}\)C-adenosyl-L-methionine is the methyl donor in the biosynthesis of mesifurane (DMMF).\(^\text{51}\) The locus required for the production of mesifurane in strawberry is the *Fragaria x ananassa* O-methyltransferase gene (*FaOMT*), and the partially purified enzyme has a molar mass of 80 kDa.\(^\text{52−55}\) Repression of *FaOMT* results in the loss of DMMF.\(^\text{56}\) An enoneoxidoreductase gene, *FaQR* is also required for DMHF accumulation during the ripening of strawberries.\(^\text{57}\) Zabetakis and Holden suggested already in 1996 that the glucose of DMHF is the evident precursor of the free aglycone on the basis of studies of strawberry-callus cultures.\(^\text{58}\) A detailed review of the topic was published in 1999.\(^\text{59}\) The glycosidic forms of both DMHF and DMMF are known to exist in strawberries.\(^\text{60}\) In 2017, Fu et al. revealed more details on several genes related to aroma formation in strawberries and in postharvest ripening.\(^\text{61}\) Development of free DMHF and DMMF was much higher at 25 °C than at 15 °C, and light also had a significant effect on the progress.\(^\text{61}\)

Chidley et al. found that the corresponding methyltransferase in Alphonso mango, *MiOMTS*, one of the early known sources of DMMF, shows substrate specificity toward furaneol, analogous to that of strawberries.\(^\text{62}\) Quantitative real-time PCR displayed ripening-related expression of *MiOMTS* in the skin and soft parts of mango, and this was in accordance with the development rate of DMMF. No analogous investigations on arctic bramble have been carried out so far, and the details were not even proposed in 1975.

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**Figure 3.** Analysis of aroma compounds of arctic bramble (Rubus arcticus L.) isolated from pressed juice by vacuum–steam distillation (see Figure 1). A Varian Aerograph 20100-20 was used with an FFAP glass capillary column (140 m, 0.32 mm i.d.) prepared with a static coating procedure at 23 °C after a CH\(_2\)Cl\(_2\) pyrolysis treatment for 2 × 30 s at 720 °C (oven temperature: 60–230 °C, 2 °C/min; injector split: 1:100; nitrogen carrier: 0.7 mL/min; injector: 245 °C; FID 255 °C). The mass spectra (El, 70 eV) were obtained with the same column with a LKB-900 instrument with a Ryhage-type jet separator (sweep time: 1.5 s/decade).\(^\text{15}\) Reprinted with permission from ref 15. Copyright 1976 Wiley Global.
In arctic bramble, the content of mesifurane increased exponentially during ripening (Figure 7b). The behavior of DMHF could not be defined reliably because of its extremely low contents in distillates. According to Pérez et al., unripe strawberries did not contain mesifurane at all, but at the final ripening stage, an intense increase was observed like in the studies of Vandendriessche et al. Ubeda et al. showed that the active enzymatic liberation of mesifurane from its glycosidic precursors in strawberry was more effective than that by acidic hydrolysis. Already in 1991, Sen, Schieberle, and Grosch had recognized the effective enzymatic liberation of DMHF from strawberries, but the increase of DMMF was not determined.

It is worth noticing that both furaneol and mesifurane are chiral compounds, and their isomers have been verified in strawberries, pineapples, and grape wines. According to Pérez et al., unripe strawberries did not contain mesifurane at all, but at the final ripening stage, an intense increase was observed like in the studies of Vandendriessche et al. Ubeda et al. showed that the active enzymatic liberation of mesifurane from its glycosidic precursors in strawberry was more effective than that by acidic hydrolysis. Already in 1991, Sen, Schieberle, and Grosch had recognized the effective enzymatic liberation of DMHF from strawberries, but the increase of DMMF was not determined.

It is worth noticing that both furaneol and mesifurane are chiral compounds, and their isomers have been verified in strawberries, pineapples, and grape wines. After the biosynthesis of DMMF in ripening berries, pH-dependent racemization starts, and the progression toward equilibrium between the two enantiomers, (+)-(2R)-2,5-dimethyl-4-methoxy-3(2H)-furanone (R-mesifurane) and (−)-(2S)-2,5-dimethyl-4-methoxy-3(2H)-furanone (S-mesifurane), depends on the time and physical conditions. Isomerization occurs via the enol structure, but the rate is unknown (Figure 6b). It is possible that in the isolated aroma fractions, enantiomers of mesifurane may not typically be in the ratio existing in the berries, even though natural racemization takes place in the intact fruits. The most proper way to mimic the situation in berries or juice for sensory analyses, both orthonasal and retronasal, might be direct headspace analysis of DMMF. Also, the solvent-assisted...
An elegant method for the quantitative analysis of both DMMF and DMHF in strawberries, for example, is the stable-isotope-dilution assay (SIDAs) introduced by Sen, Schieberle, and Grosh in 1991. The procedure has been applied in berries, juices, jams, and candies. The method was later used successfully for the quantitation of enantiomers of DMMF with deuterated racemic reference compounds with chiral gas chromatography. A thorough review of the GC analysis of enantiomeric aroma compounds was compiled by Mosandl in 1992, and the article has been a guideline for the next generations.

Bruche et al. showed clear differences between the aroma properties of the enantiomers of both mesifurane and furaneol by GC–sniffing the resolved enantiomers from pineapples, strawberries, and reference compounds using HPLC and cyclodextrin-based GC columns. They described the racemic mesifurane as sweet, spicy, cherrylike, and earthy. After the chromatographic separation of the antipodes, the first enantiomer had a sweet, almost odorless note, and the one that eluted later had the same characteristics as the racemate. In a chiral-column-GC–sniffing analysis, Fischer and Hammerschmidt described the first enantiomer of DMMF as weak, sweet, malty, and roasty and the second as strong, fruity, sweet, malty, earthy, and roasty. They revealed a 1000-fold intensity difference between the enantiomers. In all these investigations, the DMMF in the fruits was a racemic mixture of enantiomers, practically in 1:1 ratio, and Bruche et al. proposed this to be due to racemization via keto–enol tautomerism.

Emura et al. synthesized DMMF for his research on arctic bramble and strawberry using the method of Willhalm and Thomas, a patent (Kuulutusjulkaisu 51890) was applied for in Finland on September 19, 1974, and granted on May 10, 1977. The patent’s translated title was “A method to give an arctic bramble-like aroma to foods or drinks, especially to liqueurs, by adding flavorings that give the products the aroma and taste of arctic bramble”. In Finland, addings to berry liqueurs was not allowed, but in some other countries, it was and still is. Despite this, the patent application was sold to a traditional Finnish liqueur company. Whether the company ever used the information for anything remains unknown. More than 20 international patents have been granted on the topic of DMMF since 1989. Our goal in the future is to investigate the kinetics of the racemization of mesifurane in vivo in Nordic arctic bramble during ripening and storage.

### COMMERCIAL USE AND INTERNATIONAL TRADE OF MESIFURANE

There are several market analyses of mesifurane available in electronic publications from 2016 and 2017. According to PubChem on October 22, 2017, at least 38 companies from 6 countries are vendors of this flavor ingredient. More than half of them are Chinese, one-fourth are from the United States, and the rest are mostly from Europe and Japan. The price level of mesifurane (DMMF) has commonly been $1–2 per gram. It was estimated that the global mesifurane market size was 6000 tons in 2015, of which the United States produced 23%. The growth is rated at around 5% a year until 2025, reaching a value $154 million. Food and beverages cover 65% of consumption, and pharmaceuticals compose around one-fourth. Also, animal feeds seem to form an expanding market segment.
AUTHOR INFORMATION

Corresponding Author
*Tel.: +358 40 5033024. Fax: +358 29 450 5040. E-mail: helikki.kallio@utu.fi.

ORCID

Helikki P. Kallio: 0000-0001-6579-7063

Notes

The author declares no competing financial interest.

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