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Review

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one and its derivatives: analysis, synthesis and biosynthesis—a review

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Abstract

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one is believed to be a key flavour constituent in many fruits and baked foods. The analytical and organic methods applied to the analysis of DMHF and its derivatives, synthesis and biosynthesis are reviewed. Possibilities for further elucidation of biosynthetic pathways and the biotechnological production of DMHF are considered. © 1999 Elsevier Science Ltd. All rights reserved.

1. The analysis of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one and its derivatives

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one (DMHF) is widely distributed in nature and has a very low flavour threshold value in water (4×10^{-5} mg/kg); hence, its effect on food aroma is considerable (Latrasse, 1991). 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one occurs in nature in four forms (Fig. 1): 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one glucoside (DMHF glucoside), 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one 6′-O-malonyl-β-D-glucopyranoside (DMHF malonyl-glucoside), 2,5-dimethyl-4-methoxy-2*H*-furan-3-one (mesifuran) as well as the free aglycone (Latrasse, 1991; Mayerl, Naf, & Thomas, 1989; Roscher, Herderich, Steffen, Schreier, & Schwab, 1996a).

Recently, DMHF has attracted the interest of many researchers working in the field of flavour analysis (Pickenhagen, Velluz, Passerat, & Ohloff, 1981), aroma generation through chemical (Schieberle, 1992) and enzymatic processes (Roscher, Schreier, & Schwab, 1997c), organic synthesis using enzymes as catalysts (Wong & Whitesides, 1985) and the sensory evaluation of complex flavour mixtures (Larsen, Poll, & Olsen, 1992). Analytical techniques such as HPLC with either UV (Lee & Nagy, 1987) or Photo Diode Array (PDA) detection (Zabetakis, Gramshaw, & Robinson, 1996), GLC-FID (Williams & Mottram, 1981) and GLC-MS (Withopf, Richling, Roscher, Schwab, & Schreier, 1997) have been used for the analysis of DMHF and derivatives. Recently, the first studies on the biosynthesis of DMHF have taken place using either strawberry fruits (Hong, Huang, Reineccius, Harlander, & Labuza, 1990; Roscher et al., 1997c) or callus cultures (Zabetakis & Holden, 1996). Plant tissue culture techniques may provide useful tools for the elucidation of the biosynthetic pathway of strawberry flavour molecules, including manipulation of culture conditions to increase the net yield and further genetic manipulation (Zabetakis & Holden, 1997).

In this review are considered first the analysis of sources where the furanones occur naturally (e.g. fruits and other unprocessed material) and, second foodstuffs where occurrence results from exogenous processes, including the baking of bread, cooking of meat and manufacture of preserves.

1.1. Naturally occurring furanones

Free DMHF was the first furanone isolated and identified, simultaneously in pineapple (Rodin, Himel, Silverstein, Leeper, & Gortner, 1965) and strawberry (Willhalm, Stoll, & Thomas, 1965). The aroma of DMHF has been described as having a rotten note (Rodin et al.), a caramel-like note (Shaw, Tatum, & Berry, 1969; Tonsbeek, Plancken, & Weerdhof, 1968), as being sweet, floral and fruity (Miller, Libbey, & Yang, 1973) and becoming fruity and strawberry-like at low concentrations (Re, Mauren, & Ohloff, 1973).

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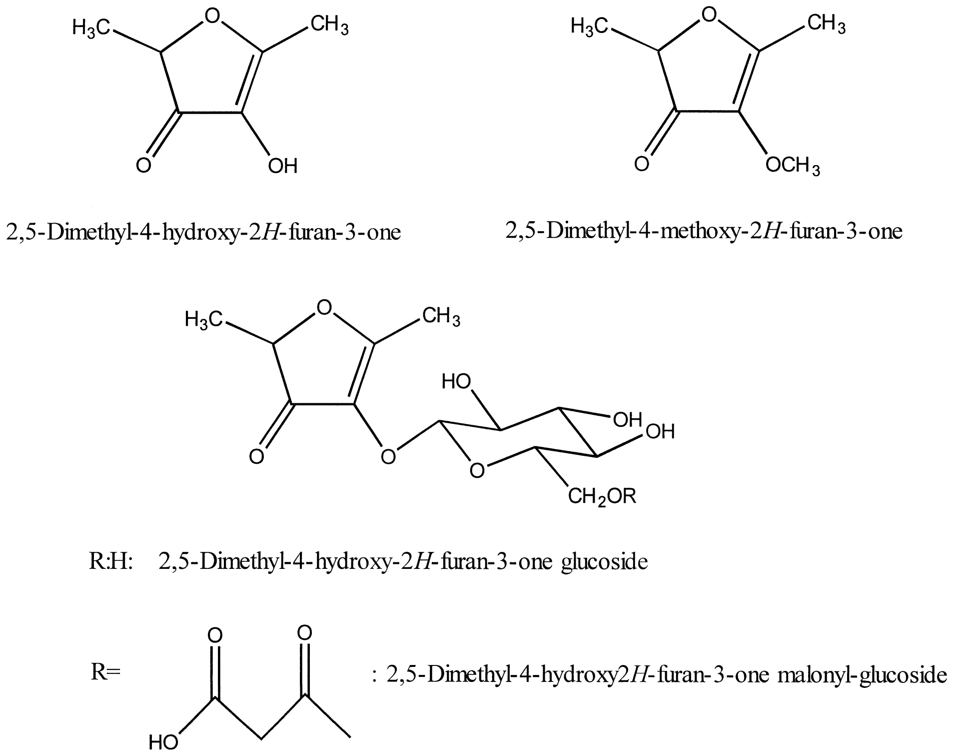

Fig. 1. The structures of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one, 2,5-dimethyl-4-methoxy-2*H*-furan-3-one, 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one glucoside and 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one malonyl-glucoside.

Later, DMHF glucoside was identified, firstly in strawberry (Mayerl et al., 1989); since then it has been regarded as the precursor of DMHF in all the fresh sources (eg fruits) where both compounds exist. It has also been detected in pineapple (Wu et al., 1990) and tomatoes (Krammer, Takeoka, & Buttery, 1994). The amounts of DMHF glucoside in pineapple (Wu, Kuo, Hartman, Rosen, & Ho, 1991) and strawberry (Wintoch, Krammer, & Schreier, 1991; Sanz, Perez, & Richardson, 1994) have been measured (Table 1). Recently, 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one 6′O-malonyl-β-D-glucopyranoside (DMHF malonyl-glucoside) has been identified in strawberry fruit (*Fragaria* × *ananassa*, cv. Senga Sengana) (Roscher et al., 1996a) with a ratio of the malonylated glucoside to its non-acylated glucoconjugate of 1:1 in the ripe fruit. Its presence suggests that the glucosides may be important derivatives of free DMHF and that their biosynthesis is therefore worthy of further study.

The synthesis of phenyl 6′-O-malonyl-β-D-glucopyranoside has been reported (Roscher, Steffen, Herderich, Schwab, & Schreier, 1996b). This compound together with the synthesized 6′-O-malonylated-β-D-glucopyranosides of benzyl alcohol, 2-phenylethanol, geraniol, citronellol and DMHF have been used as references for the spectroscopic identification of malonylated glucosides in plants (Withopf et al., 1997). This report indicated that malonylation of the glucoconjugates may be a common pathway in plant secondary metabolism. DMHF malonyl-glucoside was identified in raspberry and strawberry.

Table 1
The amounts of DMHF, DMHF glucoside and mesifuran in some fruits (mg of analyte kg^{-1} of fruit)

Fruit	DMHF	DMHF glucoside	Mesifuran
Strawberry (cv. Elsanta)[a]	20.9	4.3	79.5
Wild strawberry[a]	16.7	10.9	12.1
Wild raspberries[b]	0.1	nd[j]	tr[j]
Arctic bramble[c]	nd[j]	nd[j]	18
Alphonso mango[d]	2	nd[j]	nd[j]
Pineapple (from Ivory Coast)[e]	7.4	nd[j]	0.2
Pineapple (from Costa Rica)[f]	0.7	0.4	nd[j]
Tomato (cv. Ace)[g]	0.7	nd[j]	nd[j]
Tomato (var. 6203)[h]	nd[j]	tr[j]	nd[j]
Wine (various grape cultivars)[i]	0.1–3.5	nd	nd

[a] Zabetakis and Holden, 1995.
[b] Honkanen et al., 1980.
[c] Kallio, 1976b.
[d] Idstein and Schreier, 1985.
[e] Pickenhagen et al., 1981.
[f] Wu et al., 1991.
[g] Krammer et al., 1994.
[h] Buttery et al., 1995.
[i] Guedes de Pinho and Bertrand, 1995.
[j] tr: traces, nd: not detected.

Mesifuran, described elsewhere as having a Xeres wine-like note (Hunter, Busek, & Radford, 1974), has been claimed to be the major flavour-active component of the arctic bramble (Pyysalo, Suihko, & Honkanen, 1977). The odour value of mesifuran was estimated to be extremely high (600,000), whereas the odour value reported for DMHF was less than 100. Previously it was claimed, for arctic bramble, that the contribution of mesifuran to the total volatiles increased from 0.2 to 20% of the volatiles during ripening in the field (Kallio, 1976a).

Many of these furanones occur as a racemate in natural products (strawberry, pineapple, grape and wine) (Mosandl, Bruche, Askari, & Schmarr, 1990) and therefore stereospecific analysis is not useful for assessment of the authenticity of these commodities, or their derived products (Brueche et al., 1991). DMHF was identified in fresh and processed tomato and its contribution to aroma assessed (Buttery, Takeoka, Krammer, & Ling, 1994). Calculations of the concentration/threshold ratios have shown that DMHF occurs at a concentration well above its threshold and it is, thus, among the 10 compounds with the highest probability of contributing to both fresh and processed tomato flavour (Buttery, Takeoka, & Ling, 1995).

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one was also identified as one of 54 constituents of chempedak fruit (Wong, Lim, & Wong, 1992). A commercial lovage (*Levisticum officinale* Koch.) extract was found to contain six compounds of high sensory relevance, one of them being DMHF (Blank & Schieberle, 1993). Both DMHF and mesifuran were identified in the Finnish wild raspberry by Honkanen, Pyysalo, and Hirvi (1980) using GC–MS. In this study, more than 40 compounds not reported previously as raspberry volatiles were detected and included 11 terpenes as well as DMHF and mesifuran. Studies on mango aroma by high resolution GC/MS have revealed that it contained at least 152 aroma compounds and that DMHF is a major constituent (Idstein & Schreier, 1985). However, when the importance of some lactones and DMHF to mango aroma was assessed by Wilson, Shaw, and Knight (1990), it was claimed that DMHF did not make a positive contribution to the flavour.

A comparison of the amounts of DMHF and mesifuran in strawberries, pineapples and mangoes was carried out by GC after ethyl ether extraction (Pickenhagen et al., 1981). The contribution of DMHF to pineapple flavour was also reported in a review of the flavour of this fruit by Karg (1983), who claimed that the two essential flavour compounds of pineapple flavour were DMHF and 3-methyl mercaptopropionic acid. In a more recent study of pineapple flavour using capillary GC coupled to Fourier transform IR spectroscopy, Fehl and Marcott (1989) identified DMHF and phenylacetaldehyde, an unwanted off-flavour compound. A convenient reversed-phase HPLC method for the analysis of DMHF in pineapple and grapefruit juices was developed by Lee and Nagy (1987). This method is fast and convenient and avoids heating problems associated with the GC analysis of unstable compounds, such as DMHF (Williams & Mottram, 1981).

Use of GC-MS to identify DMHF and mesifuran (compounds responsible for a strawberry-like flavour note) together with nasal appraisal by sniffing the GC eluate led to the identification of these furanones for the first time in berries and wines of interspecific grapevine breeding (Rapp, Knipser, & Engel, 1980). This report indicates that an objective diagnosis method could be developed in order to recognize the furanones at the seedling stage. Rapp, Knipser, Engel, and Hastrich (1983) have identified DMHF, in various grape varieties, as the compound responsible for a strawberry aroma which can be troublesome for some *Vitis vinifera* × *labrusca* (European × American) hybrids. In a comparative study of various cultivars of grapes, mesifuran was claimed to be responsible for the caramel-like undertone in the aroma fraction obtained from Concord and Niagara grapes, where it was detected in substantial amounts (0.15 and 0.18 mg/kg, respectively) but only found in trace amounts in Elvira (*V. labrusca*, L. × *V. riparia*, M.) grapes (Schreier & Paroschy, 1981).

However, analysis of the red grape juice of the Isabella (*V. vinifera* × *V. labrusca*) cultivar showed that this juice contained all the characteristic compounds of *V. labrusca* grapes, except for DMHF (Cesare & Nani, 1992). A recent paper describes the analytical determination of DMHF in wines by GC–MS and the possible application of this analysis to differentiate white wines from hybrids and various *V. vinifera* cultivars (Guedes de Pinho & Bertrand, 1995). Sixty-five wines of various hybrids and more than an hundred wines have been analysed using this method. It has been reported that vinification with grape skin contact leads to a decrease in DMHF levels and suggests that DMHF concentration can be increased by using pectolytic enzymes with -glucosidic secondary activities. In a study on the aroma of strawberry wine, Schreier and Drawert (1981) proposed that concentrations of fruit-derived aroma compounds such as mesifuran, methyl cinnamate and β-decalactone could be measured and used as a reference in order to detect adulteration of wines with aroma compounds.

Larsen et al. (1992), who considered the concentrations of aroma components in the light of threshold values, found DMHF, together with linalool and ethyl hexanoate to be important strawberry aroma compounds. In a complementary study, the odour thresholds of 24 aroma compounds in the strawberry cultivar Senga Sengana were determined using duo-trio tests; DMHF was found to be one of the 3 most important compounds (Larsen & Poll, 1992). Since, in most of the reports, the analysis of DMHF and derivatives was carried out by GC, a very useful study of the gas chromatographic analysis of DMHF has been carried out by Williams and Mottram (1981). They compared five types of capillary columns and illustrated the advantage of fused silica capillary columns for the analysis of potentially unstable compounds, such as DMHF.

Characterisation of the aroma of six strawberry cultivars and quantification of DMHF and mesifuran has been reported by Douillard and Guichard (1990). The volatiles of the strawberries were isolated by direct solvent extraction using dichloromethane. Sixty compounds were identified and quantified by GC–MS. In this study, hept-2-en-3-ol was used as the internal standard for further analysis by GC–MS. The recovery factors of the analytes were assumed, however, to be equal to that of the internal standard—which could lead to incorrect quantitative estimations and partly explain the quite different results obtained by these authors when compared to those of Larsen et al. (1992). Supercritical Fluid Extraction (SFE) has been studied as a preparative tool for strawberry aroma analysis and compared to traditional solvent extraction using dichloromethane (Polesello, Lovati, Rizzolo, & Rovida, 1993). It was found that, using SFE, it was possible to recover the majority of the aroma compounds including the character impact compound of wild strawberries (mesifuran). However, this extraction method was more selective (i.e. fewer aromatic compounds were recovered) and therefore less efficient for recovering of the whole aroma profile, than the traditional solvent extraction. The quantitative determination of DMHF and mesifuran in fresh and processed fruits can also be carried out using a stable isotope dilution assay (Sen, Schieberle, & Grosch, 1991), which provides a high degree of sensitivity and accuracy. In a comparison of the industrial recovery of strawberry flavour by fractional distillation and plate condensation, 38 volatile indicator compounds were identified (Kollmannsberger & Berger, 1994) including mesifuran but not DMHF. However conventional fractional distillation gave a higher yield of the sensorially and analytically preferred aroma concentrate (26% as opposed to 15%).

Probably the best method so far reported for the analysis of DMHF, DMHF glucoside and mesifuran in strawberries is that reported by Sanz et al. (1994) where DMHF, DMHF glucoside and mesifuran were determined simultaneously by an HLPC–UV method after clarification of the juice with Celite and Carrez I and II solutions. The great advantage of this method is its simplicity and speed; the whole analysis can be completed in less than 90 min. Different amounts of the three furanones during the ripening of strawberries have been found in seven cultivars (Sanz, Richardson, & Perez, 1995): the relation of the DMHF and mesifuran levels to the overall aroma evaluations and preferences showed that the best correlation values between DMHF content and strawberry aroma were for Parker and Benton strawberry cultivars.

1.2. Furanones induced by exogenous processes

In a study of "sweet" aroma components for three types of jam, Sugawara, Ito, and Odagiri (1982) showed that the presence of DMHF in the aroma concentrate of strawberry jam explains why this jam has a sweeter odour than grape or blueberry jam. Given that DMHF is present in the starting slurries of all these fruits, the sweeter odour of strawberry jam could be explained by the major contribution of Maillard reactions that most probably take place during the distillation of the fruit slurries. This proposal was supported by the fact that the aroma concentrate of each jam has a sweeter aroma than that of the corresponding slurry in organoleptic tests.

Several heterocyclic compounds, including DMHF, have been identified in cognac where the amount increased with the age of the brandy (Pisarnitskii, Egorov, Egofarova, & Erygin, 1979). The concentrations of the heterocyclic compounds were correlated with the concentrations of ascorbic acid, which was proposed as the most probable precursor of carbocyclic and heterocyclic compounds accumulating during the ageing of brandy. Schieberle (1992) demonstrated the formation of DMHF from sugar phosphates by heating 0.2 mmol buffered solutions of sugar phosphates at 100 or 150°C for 60 or 45 min, respectively and proposed a possible role for ascorbic acid by showing that addition of ascorbic acid enhanced by a factor of 2 the amounts of DMHF formed from fructose-1,6-diphosphate and fructose-6-phosphate.

DMHF has been claimed to be a predominant odour compound in pastry made with margarine as determined by comparison of flavour profiles of puff-pastries using aroma extraction dilution analysis (AEDA) (Gassenmeier & Schieberle, 1994). Numerous heterocyclic flavour compounds are formed during roasting of raw foods and many investigations have been devoted to gaining an understanding of this process (Cerny & Grosch, 1992; Semmelroch, Laskawy, Blank, & Grosch, 1995; Ziegleder, 1991). Thirteen compounds, including DMHF, were identified by GC–MS in an aroma concentrate prepared from sponge cake by Takei (1977) who used several model systems to study the conditions necessary to produce DMHF; different combinations of glucose, arginine, egg lecithin and sodium carbonate were tested. Roasted almond volatiles were separated on the basis of carbonyl and non-carbonyl fractions and each fraction was analysed by GC–MS (Takei & Yamanishi, 1974). Twenty five compounds were identified and DMHF seemed to make the largest contribution to the sweet aroma of roasted almond. In an analogous study of the flavour extracts of raw and roasted cocoas by GC–MS, 20 new flavour compounds were identified for the first time as cocoa volatiles, among them DMHF and several other heterocyclic compounds (Ziegleder, 1991). Also, sugar degradation products, DMHF included, which are formed during drying and roasting of cocoa, important for the flavour development, are discussed. In similar studies with different varieties of roasted coffees, the profiles of volatile compounds were determined and compared for

Robusta and Arabica type coffees (Semmelroch et al., 1995; Tressl, Bahri, Koeppler, & Jensen, 1978). Tressl et al. found 11 diphenols and 7 caramel compounds which were extracted from roasted coffee with pentane–ether, whereas Semmelroch et al. determined 14 important aroma compounds in roasted coffee. Both studies reported higher DMHF levels in Arabica type coffee.

Cerny and Grosch (1993) have studied the effect of the material of cooking utensils on the profile of the flavour formed during roasting. They compared beef flavour roasted in glass to that cooked in stainless steel. The latter material resulted in a higher cooking temperature which led to the formation of a more caramellized flavour. Further studies on the aroma of processed beef by AEDA have shown that DMHF as well as two pyrazines and a thiazoline are particularly important compounds for the roasted, caramel-like and earthy odour notes of roasted and stewed beef (Cerny & Grosch, 1992, 1993; Guth & Grosch, 1994).

Kallio (1988) compared the levels of DMHF in two maple syrups and one birch syrup and concluded that the DMHF concentration was higher in the birch syrup. The difference between the two types of syrups may be related to the larger amounts of fructose and glucose in the birch syrup. Kallio, Rine, Pangborn, and Jennings (1987) have demonstrated that heating birch syrup caused the reduction of the aroma intensity of vanillin but had little effect on the amounts of DMHF and increased the development of burnt aromas. Kallio, Leino, and Salorinne (1989) also reported that the identification of DMHF in the headspace of birch syrup was possible by using headspace GC–MS analysis; DMHF was also found to be one of the major flavour components of Finnish birch syrup (0.8–2.4 mg/kg) (Kallio, 1989).

For orange juice, storage at 35°C for 12 weeks resulted in the formation of DMHF above its taste threshold level (Tatum, Nagy, & Berry, 1975). Walsh, Rouseff, Naim, and Zehavi (1995) have shown that the addition of L-cysteine to the juice reduced the levels of DMHF formed at all temperatures and time-periods tested. Naim et al. (1993) found that small amounts (25 mM) of L-cysteine and N-acetyl-L-cysteine reduced browning and DMHF formation in orange juice stored at 45°C for up to 14 days due to inhibition of Maillard-type reactions by thiols. In the case of aseptically concentrated orange juice, processing at three holding temperatures (84, 87 and 90°C) for 72 s did not affect the amount of DMHF (Rassis and Saguy, 1995). However, DMHF concentration decreased during the storage of the juice at 32°C for 7 weeks.

1.3. Studies on the thermally induced formation of DMHF

For the synthesis of DMHF (Mazenod, Dealy, & Naf, 1992; Wong, Mazenod, & Whitesides, 1983), 6-deoxy-ketohexoses (6-deoxy-D-fructose) and methyl α-D-glucopyranoside, respectively, were used. Given the potential of the Maillard reaction to generate heterocyclic compounds (including pyrans and furans), it is not surprising that DMHF formation due to heat has also been studied (Baltes, Kunert-Kirchhoff, & Reese, 1989) and a mechanism for formation of oxygen-containing heterocyclic compounds from D-glucose has been suggested by Kroh (1994). The reaction proceeds principally via 1,2- or 2,3-enolisation of the sugar and β-elimination of water leading to the formation of the 1-, 3- and 4-hexosuloses. Subsequent intramolecular cyclisation and further dehydration gives rise to hydroxymethylfurfural, hydroxyacetylfuran or DMHF (Fig. 2).

An in-depth study of the formation of DMHF in heat-processed foods was reported by Schieberle (1992) who elucidated the effect of the nature of the heated sugar on the amounts of DMHF formed and suggested a possible thermal pathway for the formation of DMHF. The key precursor of DMHF was fructose-1,6-diphosphate (FDP) which gives rise to acetylformoine and subsequent dehydration of the latter to form DMHF. However, similar amounts of the furanone were obtained when fructose-6-phosphate was used and therefore fructose-6-phosphate appears equally important.

Several studies have focused on the reactions between different amino acids and sugars (Silwar, 1992; Kunert-Kirchhoff & Baltes, 1990; Eiserich, Macku, & Shibamoto, 1992). The aroma produced by the reaction between glucose and proline at 200°C has been reported to be due primarily to five compounds: diacetyl, 2-acetyl-1,4,5,6-tetrahydropyridine, 2-acetyl-1-pyrroline, 2-acetyl-3,4,5,6-tetrahydropyridine and DMHF (Roberts & Acree, 1994). In a study of the reaction of cysteine with glucose (Eiserich et al., 1992), the antioxidative activity of microwave-induced volatile compounds was evaluated; DMHF was the strongest antioxidant. In a similar study on the reaction of cysteine with rhamnose (Silwar, 1992), it was found that the model system cysteine/rhamnose roasted at 200–220°C produced a complex mixture of compounds (180 compounds were detected of which DMHF and 5-methyl furfural were judged to be the most important odorants). This study also revealed that, under roasting conditions, the furan oxygen was substituted by sulphur from H_2S released from cysteine and gave rise to thiophenes. The formation of DMHF by the reaction between pentose sugars and amino acids was investigated by Blank and Fay (1996). When either xylose, ribose or arabinose was heated with glycine or L-alanine at 90°C for 1 h, DMHF was formed as well as directly from pentoses. Experiments using ^{13}C-labeled glycine and alanine suggest the incorporation of the Strecker degradation product, formaldehyde, into the pentose moiety thus forming DMHF. The proposed mechanism of DMHF formation

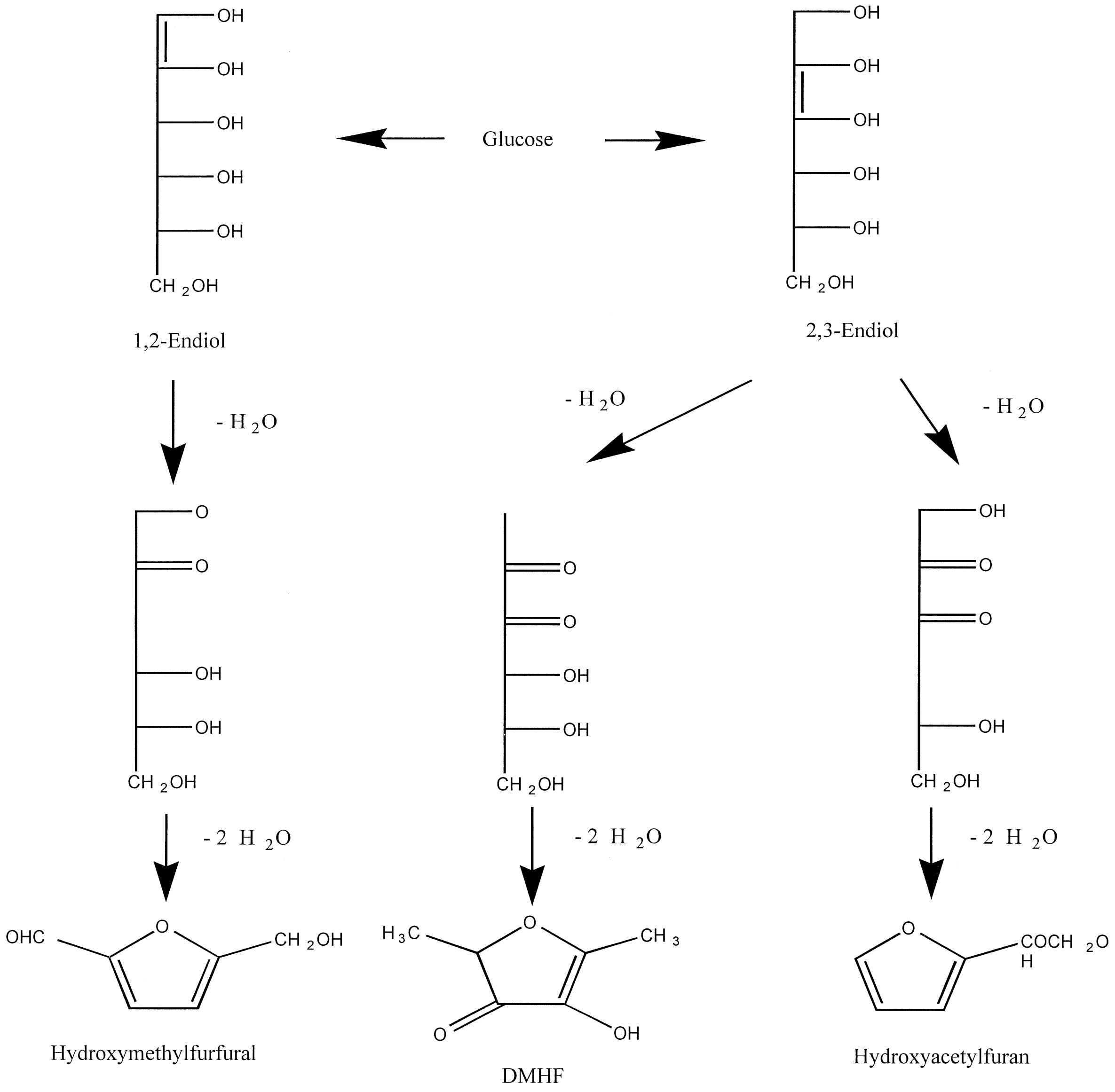

Fig. 2. 1,2- and 2,3-enolisation of D-glucose and formation of hydroxymethylfurfural, DMHF and hydroxyacetylfuran from D-glucose via 3- and 4-deoxyhexosulose.

is based on decomposition of the Amadori compound via 2,3-enolization (see Fig. 2), chain elongation by reaction with formaldehyde, and reduction of the resulting acetylformoine intermediate to DMHF.

Thermal degradation of DMHF gives rise to volatile components and this reaction has been studied in model systems where DMHF was heated alone (Shu, Mookherjee, & Ho, 1985), in the presence of phenylalanine (Kunert-Kirchhoff & Baltes, 1990) or cystine (Shu & Ho, 1989). Shaw and Berry (1977) studied the thermal degradations of mixtures of hexoses and amino acids and showed that DMHF is one of the furans formed when rhamnose reacts with alanine at pH 3.5. Shu and Ho (1989) described the effect of reaction parameters (i.e. medium, duration, water content, temperature, pH and O_2 availability) on the yield of volatiles from the thermal reaction of cystine with DMHF and concluded that the reaction at pH 2.2 generated the best meat-like flavour in terms of the roasted and meaty notes evaluated.

A comparison of microwave and thermally-induced Maillard reactions and the effect of amino acids (aliphatic, aromatic, basic, acidic or sulphur-containing) was carried out by Yaylayan, Forage, and Mandeville (1994). The presence of amino acids with alkyl side chains was found to be essential for the generation of caramel notes, sulfur-containing amino acids for meaty notes and basic amino acids for nutty and baked notes. Also, Shibamoto and Yeo (1994) compared the flavour generated by the cysteine–glucose model system when heated in microwave oven with that resulting from use of a conventional oven and showed that heterocyclic compounds were formed in considerably higher amounts in the microwaved samples, although DMHF concentrations were six-fold higher in the conventionally heated sample.

Apart from these studies with model systems, Nagy, Rouseff, and Lee (1989) have studied the effect of thermal processing and prolonged product storage on the formation of DMHF in citrus juice products. The heat-induced changes (100°C for 30 min) for the most odour-active volatiles in strawberries (DMHF included) were reported by Schieberle (1994), who found by AEDA that the concentrations of nine compounds increased so dramatically that they appeared as new odorants in the heated fruit material: of these compounds (E,E)-2,4-decadienal and (E)-β-damascenone were the most odour-active. The results implied that these compounds were liberated from precursors during the heating process. The concentration of DMHF in the fresh and heated strawberry juice was found to be 16.2 and 29.4 (mg/kg), respectively. It was concluded that the decrease in the amounts of the grass-like (Z)-3-hexenal and the fruity-smelling esters, ethyl- and methyl-butanoate, 2-methyl- and 3-methyl-butanoate, in combination with the increase in concentrations of (E)-β-damascenone, (E,E)-2,4-decadienal and the caramel-like smelling DMHF, are the components mainly responsible for the flavour produced during the heating of strawberries.

The stability of both DMHF and mesifuran has been studied in order to assess whether there was any loss of these furanones during processing and canning of fruits (Hirvi, Honkanen, & Pyysalo, 1980). It was found that the decomposition of both furanones followed first-order kinetics and is pH-dependent, with optimum stability at pH 4. Saccharose and ethyl alcohol had no effect on the stability of the furanones over a concentration range (0–20%). In an additional study of the stability of naturally occuring DMHF derivatives (Roscher et al., 1997d), the half lives of all the DMHF derivatives were determined in aqueous buffer solutions at different pH values (from pH 2.0 to 8.0 at intervals of 1.5 pH units). It was found that the greatest stability of all the DMHF derivatives occurred at pH 3.5–5.0 (the natural pH value of strawberries is in the range 3.5–4.0). 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one glucoside was stable from pH 2.0–8.0 and might be unaffected in juices or concentrates over a long storage period. Hydrolysis of malonyl-glucoside cannot take place directly by a β-glucosidase but requires an initial malonylesterase activity with the subsequent release of free DMHF in a second glycolytic step.

2. The synthesis of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one and its precursors

The chemical synthesis of DMHF has been studied because of its high commercial value as a food flavouring.

The first report of a chemo-enzymatic synthesis of 6-deoxy-D-fructose and 6-deoxy-L-sorbose was that of Hough and Jones (1952). Racemic lactaldehyde was reacted with dihydroxyacetone phosphate (DHAP) to give the two methyl pentoses. The chemical synthesis of DMHF from 6-deoxy-ketohexoses starting with threonine and FDP has been reported (Wong et al., 1983). Threonine was reacted with ninhydrin at 100°C to form lactaldehyde (Zagalak, Frey, Karabatsos, & Abeles, 1966) and the enzymes biphosphate aldolase and triose phosphate isomerase were used to liberate DHAP in situ from FDP. The generated DHAP was then allowed to react with lactaldehyde, via an aldol condensation to produce 6-deoxy-D-fructose (in the case of D-lactaldehyde) or 6-deoxy-L-sorbose (in the case of L-lactaldehyde) (Fig. 3). Conversion of these deoxy sugars to DMHF was achieved by heating at 80°C for 20 h with piperidine. The crude yield of DMHF for this reaction was 78% (Wong et al.). However, the disadvantage of this procedure means that it is almost impossible to control the concentration of lactaldehyde (the immediate precursor of the 6-deoxy-hexoses) which is a very reactive compound that readily dimerises (Chrysochoou, 1973).

In order to carry out in vivo metabolic studies of DMHF, ^{14}C-monolabelled DMHF was synthesized by applying the procedure outlined in the previous paragraph (i.e. reaction of a 6-deoxy hexose with piperidine, Wong et al., 1983) to fucose L-[1-^{14}C]. The yield of this reaction was 40% and the monolabelled DMHF obtained had a radiochemical purity of 95% which decreased, however, to 30% within 3 days of storage in aqueous solution at −20°C (Roscher, Schreier, & Schwab, 1997b).

Given the significance of 6-deoxy-ketohexoses as DMHF precursors, other studies on their synthesis have been reported (Drueckhammer et al., 1991; Durrwachter, Druekhammer, Nozaki, Sweers, & Wong, 1986a; Durrwachter, Sweers, Nozaki, & Wong, 1986b; Fessner, Badia, Eyrisch, Schneider, & Sinerius, 1992; Hecquet, Bolte, & Demuynck, 1994; Toone, Simon, Bednowski, & Whitesides, 1989; Wong & Whitesides, 1985) but all have followed the same synthetic approach despite its main deficiency—i.e. the amount of lactaldehyde, prior to its aldol condensation with DHAP, was not measured. Therefore, only the overall yield can be determined and not the yield of each individual step.

A different biochemical synthetic approach has been used for the preparation of 6-deoxy-hexoses, the immediate DMHF precursors; transketolase has been used for synthesing carbohydrates (Kobori, Myles, & Whitesides, 1992) and, in particular, 6-deoxy-D-fructose and 6-deoxy-L-sorbose (Hecquet et al., 1994). In this approach, (R,R) and (R,S)-2,3-dihydroxybutyraldehyde were separately reacted with hydroxypyruvate using spinach leaf transketolase as catalyst to give 6-deoxy-D-fructose

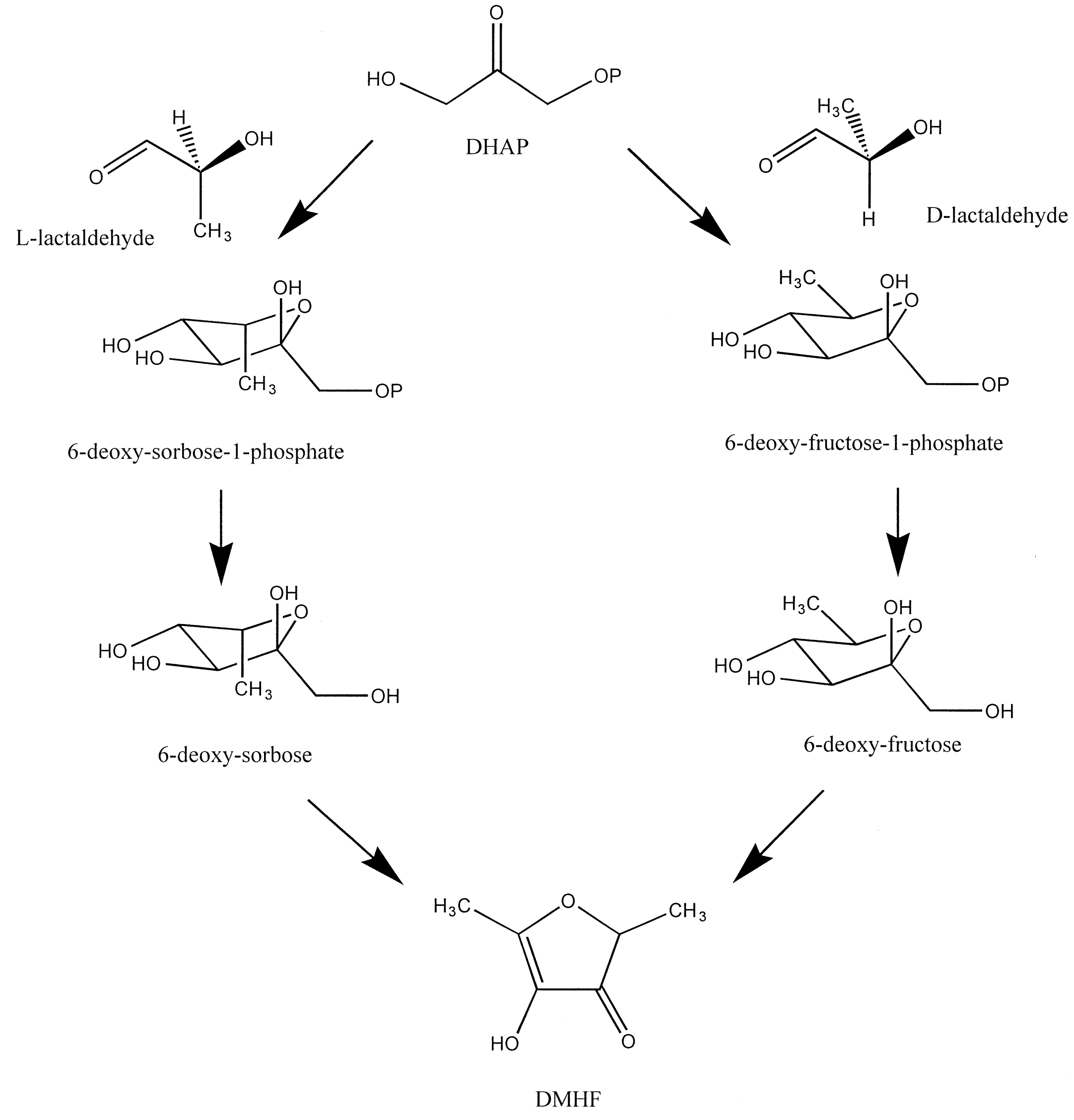

Fig. 3. Synthesis of 6-deoxyhexoses and DMHF.

and 6-deoxy-L-sorbose with an overall combined yield of 24%. Another interesting chemical route to DMHF uses methyl-D-glucopyranoside as the starting material (Mazenod et al., 1992). The advantage of this approach is that the starting reagent is readily available, inexpensive and the yield of each synthetic step can be determined; the overall yield was 56%. However rather hazardous chemicals (phosgene and toluene) are needed. 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one synthesis can also be achieved by the conversion of dihydroxydiketones using mild basic reagents (Buchi, Demde, & Thomas, 1973). Three approaches have been used and include the bromination of 2,5-dimethyl-2,5-dimethoxy-2,5-dihydrofuran and the subsequent oxidation of the latter (overall yield 51%), the hydrodimerization of methylglyoxal with zinc in aqueous acetic acid solution which results in the formation of DMHF derivatives, but not of DMHF, and the conversion of hexane-3,4-dione to DMHF (overall yield 46%). Another synthetic route to DMHF is the electrohalogenation of propargyl acetate and amide; Inokuchi, Matsumoto, Tsuji, and Torii (1992) have reported the electrosynthesis of 2,5-diacetoxy-4,4-dibromo-3-hexanone and conversion to DMHF (overall yield 39%).

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one has also been synthesized by the conversion of (2R,3R)-tartaric acid through a five-step sequence with an overall yield of 18.5% (Briggs, Haines, & Jones, 1985). The key intermediate in this synthesis, (4R,5R)-4,5-diacetyl-2,2-dimethyl-1,3-dioxolane, was produced either by reaction of methylmagnesium chloride with the corresponding 4,5-bis(dimethylamide) or via a related reaction sequence involving a Grignard reaction on the corresponding dinitrile. Another approach to DMHF synthesis is that of Fellous and George (1992) who reacted 2,5-dimethyl-3(2*H*)-furanone with hydrogen peroxide in the presence of a base.

3. The biosynthesis of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one

Linking the presence of DMHF to that of DMHF glucoside could be a useful step in the search for the biosynthetic pathway of DMHF, as it is suggested that the glucoside is the probable precursor of the free aglycone (Zabetakis & Holden, 1996). DMHF is a component of the male sex pheromone of the insect *Eurycotis floridana* (walker), where the male calling behaviour is associated with the sex pheromone released by the anterior part of tergites 2, 7 and 8. Glandular extracts of tergite 7 possess a characteristic odour of caramel, which is attractive at a distance for the females; DMHF and 4-hydroxy-5-methyl-3(2*H*)-furanone have been identified as the compounds responsible for this odour (Farine, Le Quere, Duffy, Semon, & Brossut, 1993). It is suggested that these insects and many plant species synthesize DMHF for their propagation by attracting either insects of the other sex (in the case of walker flies) or insects for pollination of plants.

The biosynthesis of DMHF has been investigated in *Zygosaccharomyces rouxii* and strawberry callus cultures. In the former case, the quantitative effect of different precursor sugars on the yield of DMHF from the yeast cultures was evaluated and it was proposed that 6-deoxyketoses and FDP are the main precursors of DMHF in this system (Hecquet, Sancelme, Bolte, & Demuynck, 1996) but a biosynthetic pathway to DMHF was not proposed.

In an analogous study with strawberry callus cultures by Zabetakis and Holden (1996), it was indicated the intrinsic potential of strawberry cells to produce DMHF glucoside when the cells were provided with a suitable precursor (6-deoxy-D-fructose). This study also showed the possible important role of 6-deoxyketoses in DMHF biosynthesis. Therefore, further investigations were required concerning the factors controlling the bioformation and bioavailability of these deoxy sugars. In such a study, it was revealed that the deoxy-hexoses, 6-deoxy-L-mannose (rhamnose), 6-deoxy-D-galactose (D-fucose) and 6-deoxy-L-galactose (L-fucose) could be probable precursors of the furan ring of DMHF since, when these hexoses were fed to strawberry calli, the production of DMHF-glucoside was observed, by HPLC-PDA. Of the four hexoses tested, 6-deoxy-D-fructose supplementation resulted in the highest amount of DMHF-glucoside and this may be explained on the basis that it is the aldose or ketose nature of the deoxy sugar, and not the configuration (D- or L-) which mainly governs product formation (Zabetakis et al., 1996).

Recently, Roscher et al. (1997a) have claimed using the ratios [^{13}C]/[^{12}C] of rhamnose, that there is a similar distribution of these isotopes in DMHF formed by *Pichia capsulata* and thus rhamnose may be the precursor of DMHF.

Lactaldehyde has been proposed as the precursor of 6-deoxy-fructose-1-phosphate, and, thereafter, DMHF in strawberries (Zabetakis & Holden, 1995) and therefore 1,2-propanediol has been considered as a possible important precursor in a DMHF biosynthetic pathway. 1,2-Propanediol has been identified in strawberry and its role as a flavour precursor discussed (Zabetakis & Gramshaw, 1997); exogenous 1,2-propanediol enhanced the formation of DMHF-glucoside in strawberry callus cultures. As alcohol dehydrogenases occur in strawberries (Mitchell & Jelenkovic, 1995; Yamashita, Iino, & Yoshikawa, 1978), it is possible for 1,2-propanediol to be oxidised to lactaldehyde which could react with DHAP, an omnipresent key metabolic compound, to produce 6-deoxy-fructose-1-phosphate (Zabetakis & Gramshaw, 1997). This deoxy sugar may in turn be converted to DMHF-glucoside, as suggested by Zabetakis and Holden (1995). The final steps of a putative DMHF biosynthetic pathway are outlined in Fig. 4.

Interestingly, when lactaldehyde was supplemented in the culture medium of strawberry calli, free DMHF, but not the glucoside, was formed. It can, thus, be proposed that there may be two, possibly parallel, biosynthetic pathways to this furanone; one leading to the free aglycone and one to DMHF glucoside (Zabetakis, Moutevelis-Minakakis, & Gramshaw, 1998). Given the presence of 1,2-propanediol in strawberry (Zabetakis & Gramshaw, 1997), the effect of *Methylobacterium extorquens*, a bacterial strain with the capability to oxidise primary and secondary alcohols to the corresponding carbonyl compounds, on the DMHF bioformation in strawberry calli was investigated by treating strawberry cell cutures with these bacteria. It was found that this treatment resulted in the formation of DMHF and mesifuran by strawberry callus cultures (Zabetakis, 1997). In this study, it was also found, using HPLC–PDA on the dinitrophenylhydrazones, that when bacterial suspension cultures were supplemented with 1,2-propanediol, lactaldehyde was formed; this microbially-derived aldehyde might then be used by the plant cells for the biosynthesis of the furanones.

Roscher et al. (1997c) have suggested that DMHF is the precursor of DMHF glucoside based on a 0.7% incorporation of ^{14}C-DMHF to ^{14}C-DMHF glucoside. These authors suggested that DMHF is the precursor of DMHF-glucoside and not vice versa, a statement which might be true in the case of strawberry fruit. However, since the glucosidation and the glycolysis reactions are readily reversible, DMHF-glucoside could be the precursor of DMHF in strawberry calli (Zabetakis & Holden, 1996). In strawberry calli, the endogenous

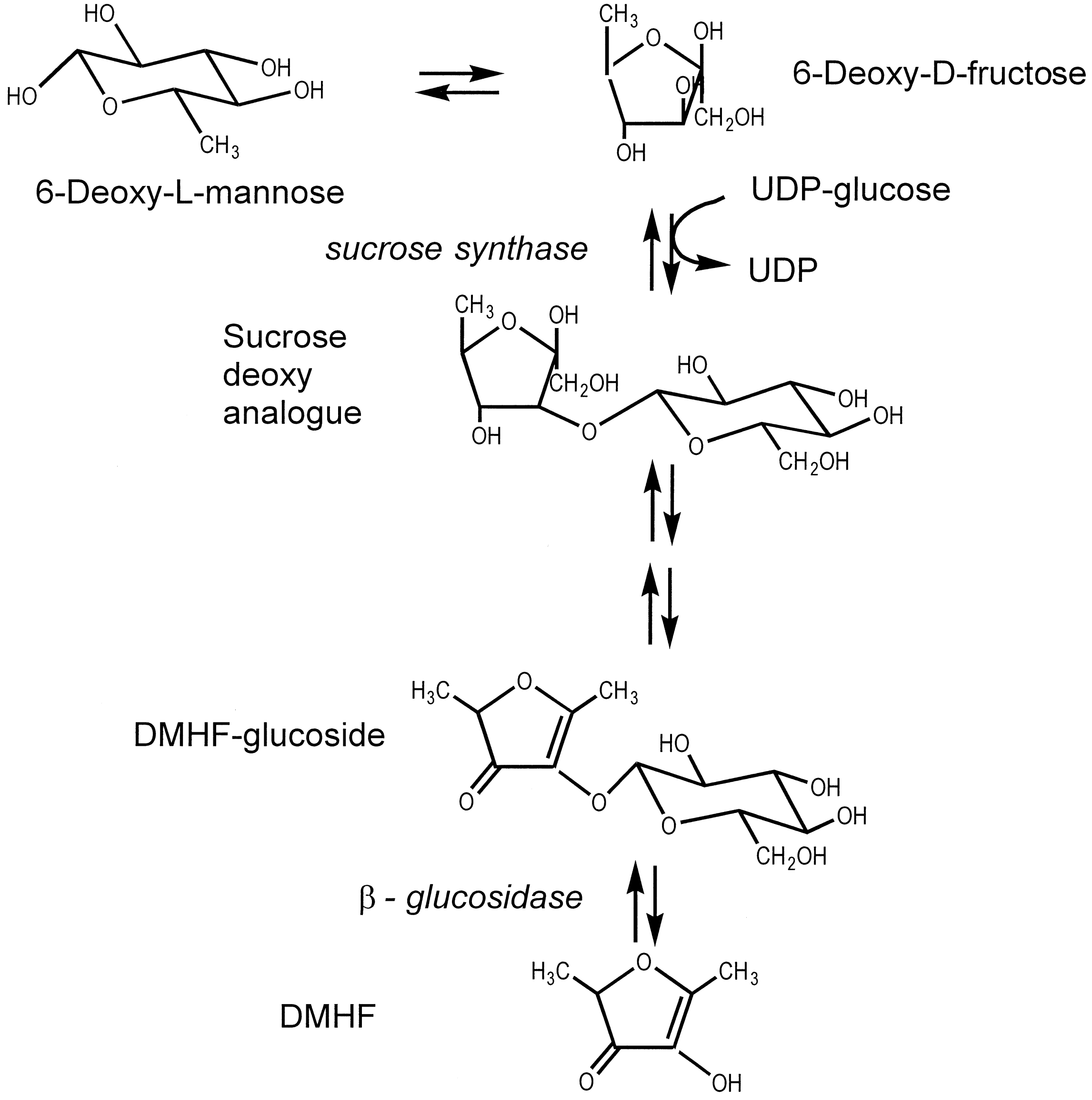

Fig. 4. Proposed outline for the biosynthesis of DMHF (adapted from Zabetakis et al., 1996).

amounts of DMHF and derivatives are zero and the size of the metabolic pool of each furanone should be thus defined only by the metabolism of exogenously supplied precursors. The callus cultures might therefore be a better biological system for the study of glucosidation and glycolysis reactions involving DMHF and derivatives. In view of that, further experiments with strawberry callus cultures and labelled precursors are needed in order to elucidate the mechanism of the formation of DMHF glucoside.

In summary, in this review, the analytical methods used for the analysis of DMHF in various sources have been evaluated. Given that DMHF is thermolabile, HPLC–UV is probably the most appropriate method for reliable quantitative results. The different synthetic routes to DMHF were also reviewed in order to explore possibilities for the efficient synthesis of isotopically labelled DMHF and potential precursors of it. These labelled compounds should be of great significance in elucidating the chemical/biochemical pathways of DMHF formation in any system where DMHF is a key flavour component. Lastly, the studies on DMHF biogenesis were also critically presented and points of interest, where future work should be focused, have been suggested.

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