Food Chemistry 361 (2021) 130052

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Synthesis of 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one from maltol and its taste identification

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ARTICLE INFO

*Keywords:*
Maillard reaction
DDMP
Maltol
Taste
Bitter

ABSTRACT

2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) exists in many foods, and its effect on taste is controversial. The aim of this study was to clarify whether DDMP has bitter taste or not. For this purpose, DDMP was synthesized from maltol instead of from glucose for the first time. In contrast, DDMP derived from glucose was also prepared and further purified. Their structures were identified by NMR and MS, and considered to be the same substance. The sensory analysis showed that DDMP derived from maltol was tasteless. Further studies indicated that some impurities in Maillard reaction made DDMP derived from glucose taste bitter.

## 1. Introduction

Maillard reaction leads to large number of different reactive intermediates or final products, including 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP). DDMP was first isolated from stored orange juice powder (Tatum, Shaw, & Berry, 1967; Mills, Weisleder, & Hodge, 1970). Later, DDMP was discovered in various foods, such as garlic oil (Sun et al., 2019), *Ipomoea staphylina* (Padmashree et al., 2018), rose tea (Qin et al, 2019), heated pear (Lee et al., 2013), mango (Ali et al., 2012), prune (Čechovská et al., 2011), etc. In the Maillard reaction, DDMP is generally considered to be converted from 1-DG, and can further form furan and pyran compounds (Voigt & Glomb, 2009; Voigt, Smuda, Pfahler, & Glomb, 2010; Zhou et al., 2011; Li et al., 2019). In terms of physiological activity, although DDMP is thought to generate free radicals to damage DNA through self-oxidation (Hiramoto et al., 1997), more evidence shows that DDMP has a strong antioxidant effect (Kanzler et al., 2016; Yu et al., 2013a, 2013b; Čechovská et al., 2011; Lee et al., 2011) and other activities (Ban et al., 2007; Beppu et al., 2012; Teoh, Mashitah, & Ujang, 2011).

So far there has been considerable research on DDMP, yet DDMP is still a controversial compound. Shaw reported that DDMP is odorless (Shaw, Tatum, & Berry, 1971), but recent reports claimed that DDMP has a strong bitter taste and is one of the main bitter substances in food processing (Bin, Jiang, Cho, & Peterson, 2012; Bin & Peterson, 2016; Jiang & Peterson, 2013; Li, Wu, Tang, & Yu, 2019b; Li et al., 2019a). In these papers the DDMP as reference substance was derived from glucose through the Maillard reaction (Van Den Ouweland & Peer, 1970). It is accepted that the product mixtures derived from Maillard reaction are complex, which makes separation and purification difficult. The so-called purified DDMP may be accompanied by trace impurities, which cause deviations in taste judgment. Therefore, it is necessary to synthesize DDMP through a step-by-step reaction to clarify academic controversies. In this paper, maltol was used as the starting material to synthesize DDMP through a 5-step reaction, and its taste was assessed.

## 2. Materials and methods

### 2.1. Reagents and chemicals

Maltol (99%) and 5% Pd/C were purchased from J&K Scientific Ltd. $Pb(OAc)_4$ was purchased from Acros Organics. Lipase from *Candida rugosa*, lipase from porcine pancreas and cation exchange resin were purchased from Sigma-Aldrich. Novozym 435 was purchased from Novozymes Biologicals, Inc. $NaHCO_3$, NaCl, anhydrous $Na_2SO_4$, $Na_2CO_3$ and the solvents were of analytical grade.

TLC was performed on $GF_{254}$ precoated plates and visualized using phosphomolybdic acid dip or ultraviolet light. Column chromatography was performed on silica gel 60.

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https://doi.org/10.1016/j.foodchem.2021.130052
Received 14 March 2021; Received in revised form 2 May 2021; Accepted 4 May 2021
Available online 13 May 2021

### 2.2. Synthesis of DDMP from maltol

Maltol (10 g, 80 mmol) was dissolved in acetic anhydride (15 mL), the mixture was heated at 90 °C for 6 h, then cooled and ethyl acetate (200 mL) was added. The ethyl acetate solution was washed with saturated aqueous $NaHCO_3$ (1 × 100 mL), then aqueous NaCl (2 × 200 mL), dried over anhydrous $Na_2SO_4$ then evaporated under reduced pressure to give **2** as a yellow oil 13.2 g, yield 98%. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 7.69 (d, $J$ = 5.7 Hz, 1H, H-2), 6.40 (d, $J$ = 5.7 Hz, 1H, H-3), 2.34 (s, 3H, -OC*H*$_3$), 2.27 (s, 3H, H-7). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 172.0 (C-4), 167.6 (-O-*C*═O), 159.1 (C-2), 154.3 (C-6), 138.7(C-5), 116.8 (C-3), 20.3 (-O*C*H$_3$), 15.0 (C-7). HR-ESI-MS *m/z* calculated for $C_8H_8O_4$ $[M + H]^+$ 169.0495, found 169.0494.

**2** (9 g, 54 mmol), 5% Pd/C (1 g), and 120 mL of ethyl acetate were added to a round-bottomed flask. The mixture was stirred under a hydrogen atmosphere at room temperature for 3 h, then the solution was filtered and evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography. Elution was conducted with petroleum ether petroleum ether/EtOAc = 3/1, and 40-mL fractions were collected; fractions 40–75 (product by TLC analysis, petroleum ether/EtOAc = 2/1, $R_f$ value 0.45) were combined and the solvent was removed to give a colorless oil 6.7 g, yield 75%. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 4.50 (t, $J$ = 6.8 Hz, 2H, H-2), 2.68 (t, $J$ = 6.8 Hz, 2H, H-3), 2.27 (s, 3H, -OC*H*$_3$), 1.99 (s, 3H, H-7). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 183.9 (C-4), 168.5(-O-*C*═O), 166.9(C-6), 129.3 (C-5), 67.7(C-2), 35.6(C-3), 20.3 (-O*C*H$_3$), 16.0 (C-7). HR-ESI -MS *m/z* calculated for $C_8H_{10}O_4$ $[M + H]^+$ 171.0652, found 171.0655.

$Pb(OAc)_4$ (31 g, 70 mmol) was added to a solution of **3** (6 g, 35 mmol) in dry toluene (150 mL) under $N_2$. The reaction mixture was heated to 90 °C and stirred for 15 h, then cooled and washed with aqueous NaCl (3 × 150 mL), dried over anhydrous $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel 60 column chromatography. Elution was conducted with $CH_2Cl_2$/EtOAc = 50/1, and 30-mL fractions were collected; fractions 80–120 (product by TLC analysis, $CH_2Cl_2$/EtOAc = 20/1, $R_f$ value 0.38) were combined and the solvent was removed to give a colorless oil 2.7 g, yield 35%. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 5.43 (dd, $J$ = 8.6, 4.8 Hz, 1H, H-3), 4.52 (dd, $J$ = 12.0, 4.8 Hz, 1H, H-2a), 4.45 (dd, $J$ = 12.0, 8.6 Hz, 1H, H-2b), 2.27 (s, 3H, -OC*H*$_3$), 2.16 (s, 3H, -OC*H*$_3$), 2.04 (s, 3H, H-7). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 179.9 (C-4), 169.6(-O-*C*═O), 168.3(-O-*C*═O), 167.9 (C-6), 128.2 (C-5), 69.3(C-2), 68.0(C-3), 20.7(-O*C*H$_3$), 20.2 (-O*C*H$_3$), 16.3 (C-7). HR-ESI-MS *m/z* calculated for $C_{10}H_{12}O_6$ $[M + NH_4]^+$ 246.0972, found 246.0972.

Lipase (50 mg, derived from *Candida rugosa*) was added to a solution of **4** (2 g, 9 mmol) in water (20 mL). The mixture was stirred at room temperature for 3 h, then the aqueous phase was extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$, and then evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography. Elution was conducted with petroleum ether petroleum ether/EtOAc = 3/1, and 15-mL fractions were collected; fractions 50–80 (product by TLC analysis, petroleum ether /EtOAc = 1/1, $R_f$ value 0.42) were combined and the solvent was removed to give enantiomer (+)-**5** as a colorless oil 0.8 g, yield 48% (unreacted enantiomer (–)-**4** was recovered with a yield 49%). $[\alpha]_D$ + 74.3 (*c* 0.75, $CHCl_3$). $^1$H NMR (600 MHz, DMSO) $\delta$ 4.43 (dd, $J$ = 11.6, 4.6 Hz, 1H, H-2a), 4.20 (dd, $J$ = 11.6, 8.8 Hz, 1H, H-2b), 4.12 (dd, $J$ = 8.8, 4.5 Hz, 1H, H-3), 2.21 (s, 3H, -OC*H*$_3$), 1.95 (s, 3H, H-7). $^{13}$C NMR (150 MHz, DMSO) $\delta$ 185.7 (C-4), 168.6 (-O-*C*═O), 166.8 (C-6), 127.4 (C-5), 72.4 (C-2), 67.5 (C-3), 20.5 (-O*C*H$_3$), 16.2 (C-7). HR-ESI-MS *m/z* calcd for $C_8H_{10}O_5$ $[M + H]^+$ 187.0601, found 187.0601.

$Na_2CO_3$ (0.29 g, 2.7 mmol) was added to a solution of (+)-**5** (0.5 g, 2.7 mmol) in water (10 mL) at 0 °C. The reaction mixture was warmed up to room temperature and stirred for 4 h. When the reaction was complete, Cation exchange resin was added and stirred for another 5 min then filtered. The filtrate was removed under reduced pressure to give **1** as colorless oil 0.35 g, yield 90%. The product became solid after storage in the refrigerator. $[\alpha]_D$ + 132.3 (*c* 0.60, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 4.49 (dd, $J$ = 10.5, 5.9 Hz, 1H, H-2a), 4.44 (dd, $J$ = 12.1, 5.9 Hz, 1H, H-3), 4.06 (dd, $J$ = 12.1, 10.5 Hz, 1H, H-2b), 2.12 (s, 3H, H-7). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 188.1 (C-4), 160.3 (C-6), 131.3 (C-5), 70.9 (C-2), 67.1 (C-3), 15.8 (C-7). HR-ESI-MS *m/z* calcd for $C_6H_8O_4$ $[M + H]^+$ 144.0495 , found 145.0494.

### 2.3. Synthesis and further purification of DDMP from glucose

DDMP was synthesized *via* Maillard reaction (Van Den Ouweland & Peer, 1970; Kanzler et al., 2016) with slight modifications. A mixture of 0.2 mol D-glucose, 0.2 mol piperidine, and 150 mL ethanol was refluxed for 1.5 h. Then, 0.2 mol of acetic acid in 30 mL of ethanol were added slowly, and the mixture was heated at 75 °C for 32 h. After ethanol was evaporated under reduced pressure, the residue was taken up in 100 mL of water, and the precipitate was formed immediately. The mixture was filtered to obtain the precipitate and the filtrate, and then the filtrate was extracted with ethyl acetate (6 × 150 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$, and then evaporated under reduced pressure. The crude product was purified by polyamide 6 column chromatography (petroleum ether /EtOAc = 1/1) and silica gel 60 column chromatography ($CH_2Cl_2$/$CH_3OH$ = 50:1, v/v) in sequence, and then recrystallized from ether/light petroleum to obtain DDMP as a beige solid. In the process, a large amount of piperidinohexose reductone **8** was obtained from the precipitate.

The above beige DDMP was chromatographed on an LH-20 gel column (bed volume 60 mL). Elution was conducted with 1:1 dichloromethane/methanol, and 2-mL fractions were collected after one bed volume; fractions 1–8 were combined and the solvent was removed to give F-I fraction as brown viscous, yield 5%; Fractions 11–13 (product by TLC analysis) were combined and the solvent was removed to give F-II fraction as a white solid, yield 94%. The F-II fraction was identified as high-purity DDMP.

### 2.4. Synthesis of DDMP-5-camphorsulfonate

(+)-Camphorsulfonyl chloride (262 mg, 1.05 mmol) was added to a solution of DDMP (144 mg, 1.0 mmol) and $Et_3N$ (121 mg, 1.2 mmol) in dry $CH_2Cl_2$ (10 mL) under $N_2$. The reaction mixture was stirred at room temperature for 6 h, then cooled and washed with aqueous NaCl (3 × 150 mL), and dried over anhydrous $Na_2SO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel 60 column chromatography. Elution was conducted with petroleum ether/EtOAc = 4/1, and 6-mL fractions were collected; fractions 25–34 (product by TLC analysis, petroleum ether/EtOAc = 2/1, $R_f$ value 0.40) were combined and the solvent was removed to give a colorless solid 310 mg, yield 87%. $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 4.62 (dd, $J$ = 11.0, 6.2 Hz, 1H, H-2a), 4.41 (dd, $J$ = 13.4, 6.2 Hz, 1H, H-3), 4.16 (dd, $J$ = 13.3, 11.0 Hz, 1H, H-2b), 4.01 (d, $J$ = 15.0 Hz, 1H, -SO3-C*H*$_2$–), 3.66 (d, $J$ = 15.0 Hz, 1H, -SO$_3$-C*H*$_2$–), 2.46–2.43 (m, 1H, H-6'a), 2.42–2.40 (m, 1H, H-3'a), 2.22 (s, 3H, H-7), 2.15–2.13 (m, 1H, H-4'), 2.11–2.06 (m, 1H, H-5'a), 1.97 (d, $J$ = 18.5 Hz, 1H, H-3'b), 1.80–1.75 (m, 1H, H-6'b), 1.48–1.44 (m, 1H, H-5'b), 1.14 (s, 3H, C-7'-C*H*$_3$), 0.93 (s, 3H, C-7'-C*H*$_3$). $^{13}$C NMR (150 MHz, $CDCl_3$) $\delta$ 213.7 (C-2'), 186.7 (C-4), 173.3 (C-6), 126.9 (C-5), 70.8 (C-2), 66.9 (C-3), 58.0 (C-7'), 49.7 (-SO$_3$-C*H*$_2$–), 47.9 (C-1'), 42.7 (C-4'), 42.3 (C-3'), 26.7 (C-5'), 25.0 (C-6'), 19.6 (C-7'-*C*H$_3$), 19.5 (C-7'-*C*H$_3$), 17.3 (C-7). HR-ESI-MS *m/z* calculated for $C_{16}H_{22}O_7S$ $[M + H]^+$ 359.1159, found 359.1163.

### 2.5. Characterization

#### 2.5.1. NMR analysis

The NMR analyses were performed on a Bruker 600 MHz spectrometer with a CryoProbe 5 mm QCI probe. $D_2O$, DMSO-$d_6$, MeOH-$d_4$ and chloroform-*d* were used as solvents, and tetramethylsilane was the internal standard.

#### 2.5.2. HR-MS analysis

The HR-ESI-MS analyses were performed on an AB Sciex Triple TOF 6600. Spectra were acquired in turbospray ESI mode at a flow rate of 0.2 mL $min^{-1}$. The mass spectrometer was operated in the full-scan mode, monitoring positive ions from *m/z* 70 to 450.

#### 2.5.3. GC–MS analysis

The GC–MS analyses were performed on an Agilent 7890B–5975 N gas chromatograph–mass spectrometer. The column used was a fused silica column (DB-5MS, 30 m, 0.25 mm i.d., 0.25 μm film thickness); the carrier gas was helium at 1.2 mL/min; the temperature program was heating for 2 min at 50 °C, 50 to 280 °C at 5 °C/min; injection temperature, 280 °C; transfer line temperature of 240 °C; ion source temperature, 120 °C; ionization energy, 70 eV; mass scan range, *m/z* 30–450.

#### 2.5.4. Optical rotation analysis

The optical rotation analyses were performed on an SGWzz-1 polarimeter. The sample tube length, 20 cm; temperature, 20 °C; solvent, $CHCl_3$.

### 2.6. Sensory Analyses.

Training of the sensory panel and determination of taste followed the methods previously described (Ottinger, & Hofmann, 2001; Bin, & Peterson, 2016): Assessors were trained to evaluate bitter taste intensity using quinine hydrochloride (0.05 mmol/L). Sensory analyses were performed in a panel room at 22–25 °C in three different sessions. The detection thresholds of taste compounds were determined in a triangle test using tap water (pH 6.5) as the solvent. The samples were presented in order of increasing concentrations (serial 1:1 dilutions), and the threshold values evaluated in three different sessions by six panelists whose data were averaged.

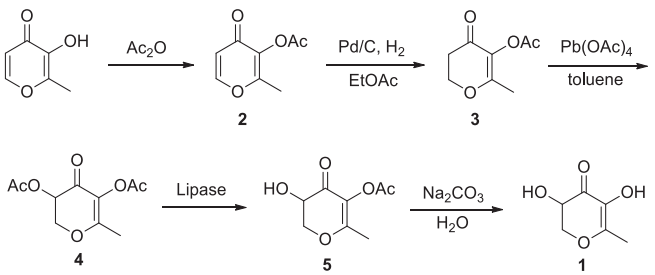
## 3. Results and discussion

### 3.1. Synthesis of DDMP

The structure of DDMP and maltol are highly similar, so it is feasible to synthesize DDMP from maltol. The conjugated structure of maltol makes its properties very stable, and it is impossible to synthesize DDMP through the hydroboration-oxidation reaction directly. Therefore, the strategy we adopted is to selectively reduce the double bond at C-2,3 positions, then perform acetylation or oxidation at the C-3 position, and finally obtain the target product through hydrolysis (Scheme 1). Although an attempt by Mills et al. to utilize the strategy was defeated, it is still worth taking advantage of new synthetic technology to try again.

#### 3.1.1. Hydrogenation of maltol acetate

Maltol acetate has a structure with two double bonds, which is difficult to maintain in the form of dihydromaltol acetate during reduction. Therefore, based on the poor selectivity, it is necessary to optimize the catalytic system and reaction conditions of the hydrogenation.




**Scheme 1.** Synthesis route of DDMP.

Firstly, the commonly used catalytic systems Pd/C, Raney nickel, tris (triphenylphosphine) rhodium chloride (Wilkinson's catalyst) and sodium borohydride-nickel chloride ($NaBH_4$-$NiCl_2$) were tested in the hydrogenation. The results indicated that Wilkinson's catalyst cannot catalyze the reduction of maltol acetate; And when Raney Nickel and $NaBH_4$-$NiCl_2$ systems were used as catalysts, the major products were not **3**, but over-reduced products; Encouragingly, the Pd/C system could convert half of the substrate into dihydromaltol acetate. Therefore, the Pd/C system was more suitable for hydrogenation of maltol acetate.

Further optimization of the reaction conditions was conducted by examining the effect of solvents and catalyst amounts. Methanol, ethanol and acetonitrile were not suitable for the reaction because they produced a lot of over-reduced products. Using acetic acid as an additive fails to inhibit the formation of over-reduction products. Ethyl acetate provided good result that overall, 80% dihydromaltol acetate was formed. Surprisingly, when the catalyst amount was reduced to 5%, the complete conversion time was extended to 5 h, and the proportion of dihydromaltol acetate was also slightly declined.

From the results obtained, it seemed that the hydrogenation of maltol acetate carried out in ethyl acetate with 10% Pd/C was optimal. Under these conditions, maltol acetate was about 80% converted into the target product, and the separation yield was about 75%.

#### 3.1.2. Acetoxylation of dihydromaltol acetate

The $^{13}C$ NMR chemical shift of carbonyl group from dihydromaltol acetate is about $\delta$ 184, which indicates that the α position is inactive and is unlikely to participate in α-oxidation or α-acetoxylation. After the failed direct α-oxidation reaction, we turned our attention to the acetoxylation reaction. Commonly used reagents for carbonyl α-position acetyl acetoxylation are manganese triacetate (Adachi, Hasegawa, Katakawa, & Kumamoto, 2017), lead tetraacetate (Teng et al., 2006) and potassium permanganate/acetic acid system (Marín-Barrios et al., 2014). Only the lead tetraacetate system was effective. However, $Pb(OAc)_4$ failed to completely convert the substrate **3**, and the target product **4** was further eliminated to produce maltol acetate **2**. For this reason, we have optimized the amount of lead tetraacetate, solvent, temperature and reaction time.

The optimization results are summarized in Table 1. At the outset we studied the acetoxylation of dihydromaltol acetate in toluene. It proceeded with 40% conversion to **4**. Cyclohexane and benzene provided neither suitable yield nor selectivity. Thus, toluene was used to test the effect of the other conditions. The 1.5 equivalents of lead tetraacetate produced the target product with highest yield in 15 h at 90 °C, and the target product **4** remained, even after reaction periods of 30 h and the use of more catalyst and higher temperature. Under these conditions, about 40% of dihydromaltol acetate was converted into **4**, and the

**Table 1**
Acetoxylation conditions.

| Entry | $Pb(OAc)_4$ (equiv.) | Solvent | Time | T (°C) | **4/3/2** [a] |
|---|---|---|---|---|---|
| 1 | 2 | toluene | 30 | 100 | 4/4/2 |
| 2 | 2 | benzene | 30 | 80 | 3/4/4 |
| 3 | 2 | cyclohexane | 30 | 100 | 0.5/9.5/0 |
| 4 | 0.5 | toluene | 30 | 100 | 2/6/2 |
| 5 | 1 | toluene | 30 | 100 | 3/5/2 |
| 6 | 1.5 | toluene | 30 | 100 | 4/4/2 |
| 7 | 2.5 | toluene | 30 | 100 | 4/4/2 |
| 8 | 3 | toluene | 30 | 100 | 4/4/2 |
| 9 | 1.5 | toluene | 25 | 100 | 4/4/2 |
| 10 | 1.5 | toluene | 20 | 100 | 4/4/2 |
| 11 | 1.5 | toluene | 15 | 100 | 4/4/2 |
| 12 | 1.5 | toluene | 10 | 100 | 3/6/1 |
| 13 | 1.5 | toluene | 15 | 80 | 3/6/1 |
| 14 | 1.5 | toluene | 15 | 90 | 4/4/2 |
| 15 | 1.5 | toluene | 15 | 110 | 4/4/2 |

*Note*: a. The ratio of compound **4**, **3** and **2** was determined by GC–MS.

isolated yield of the target product was 35%.

### 3.1.3. Hydrolysis of DDMP diacetate

Generally, sodium methoxide/methanol, potassium carbonate/methanol, sodium hydroxide/methanol–water and other alkali-catalyzed systems can hydrolyze ester groups quickly and efficiently. Unfortunately, attempts to convert the diacetate **4** into **1** by hydrolysis with base or acid were not successful (Table 2). In the catalytic system using methanol as the solvent, sodium methoxide, potassium carbonate, and DBU can quickly catalyze the ester hydrolysis. However, the target product would convert into by-product, and the complex mixture could not be separated by column chromatography. When using water as the solvent, there was almost no measurable amount of deacetylated **4** formed. It may be because the 3-acetyl group in the structure of DDMP diacetate is unstable, and it is easy to eliminate reaction under alkaline conditions and further generate various impurities.

Lipase-catalyzed ester synthesis and hydrolysis have been widely used in industrial fields, and are environmentally friendly and easy to operate. Therefore, the enzymatic hydrolysis of diacetate **4** was considered to be an effective way to replace the failed chemical methods. Among the selected enzymes, lipase from *Candida rugosa* could hydrolyze the 3-acetyl group completely, but had no effect on 5-acetyl group, while lipase from porcine pancreas had no effect on either. It is noteworthy that the lipase from *Candida rugosa* displayed high enantioselectivity, and the optically pure enantiomer (+)-**5** was obtained as with an enantiomeric excess value of up to 98%. Novozym 435 could hydrolyze both 3-acetyl group and 5-acetyl group slowly; however, the poor selectivity led to separation difficulties.

The hydrolysis of DDMP-3-acetate was carried out with sodium carbonate in water, and DDMP was obtained with a 90% yield through a simple separation.

## 3.2. Identification of DDMP from different precursors

D-Glucose has been a widely used precursor of DDMP. The $^{1}$H NMR spectrum in Fig. 1 showed signals for three coupling protons ($\delta_H$ 4.08, 4.18, 4.33), one independent methyl group ($\delta_H$ 2.04, H-7), while the $^{13}$C NMR spectrum exhibited 6 carbon signals including one unsaturated ketone carbon, two olefinic carbons. COSY correlations established the subunits from H-2 to H-3, while their connectivities were completed by detailed analysis of HSQC correlations from the protons H-2a ($\delta_H$ 4.08) and H-2b ($\delta_H$ 4.33) to the secondary carbon C-2 ($\delta_C$ 72.6), from H-3 ($\delta_H$ 4.18) to tertiary C-3 ($\delta_C$ 69.0). We compared the NMR and MS spectrum of DDMP derived from maltol with DDMP derived from glucose, and found that there was no difference between the two. At the same time, we used different deuterated solvents to perform NMR tests on maltol-derived DDMP, and the results were consistent with those reported in the literature (in methanol-$d_4$, Kanzler, et al., 2016; in deuterium oxide, (Li et al., 2019a); in chloroform-*d*, Mills et al., 1970).

## 3.3. Stereochemistry

DDMP contains a chiral center in the C-3 position. It is known that

**Table 2**
Hydrolysis of DDMP-3-acetate.

| Entry | Catalyst | Time (h) | Conversion (%) |
|---|---|---|---|
| 1 | $CH_3ONa/CH_3OH$ | 0.2 | complex [a] |
| 2 | $K_2CO_3/CH_3OH$ | 1 | complex |
| 4 | DBU/ $CH_3OH$ | 2 | complex |
| 5 | $Na_2CO_3/H_2O$ | 0.5 | complex |
| 6 | $NaOH/H_2O$ | 0.2 | complex |
| 7 | Lipase from *Candida rugosa* | 1 | 100% [b] |
| 8 | Lipase from porcine pancreas | 10 | no reaction |
| 9 | Novozym 435 | 12 | 100% [b] |

*Note*: a. Conversion was not determined. b. Single enantiomer conversion.

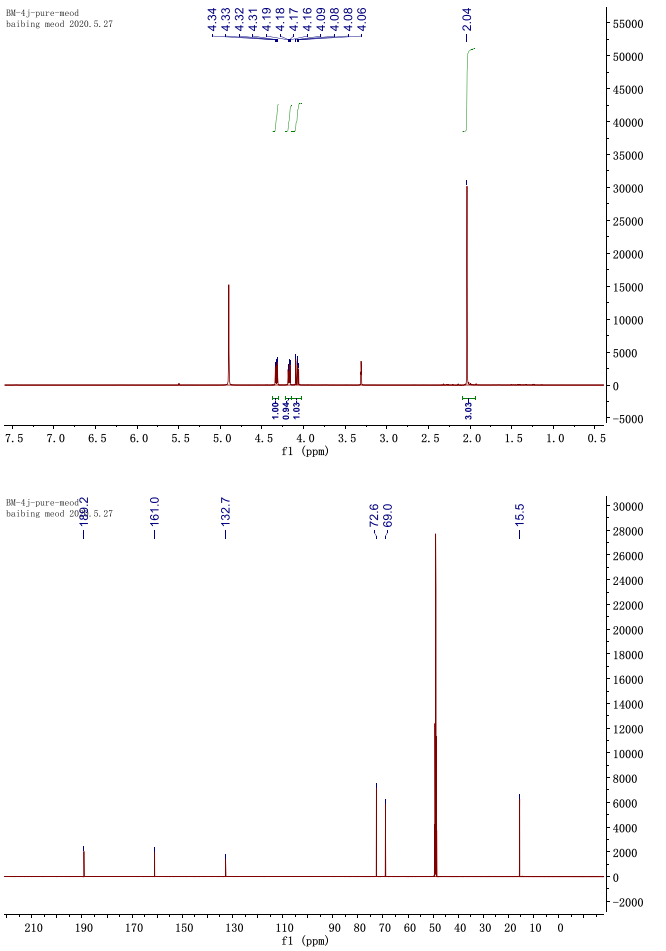



**Fig. 1.** $^{1}$H NMR and $^{13}$C NMR spectra (in methanol-$d_4$) of DDMP.

stereoisomers have different sensory properties, for examples D- and L-isomers of certain amino acids taste different. Perhaps the differences in reported DDMP sensory properties may be due to differences in the stereochemistry of the products. So, it was necessary to determine whether the absolute configuration of DDMP obtained by different methods is the same.

In the preparation of DDMP from D-glucose, the chirality of the C-5 position of D-glucose will be directly transferred to the C-3 position of DDMP. We also observed that the optical rotation of DDMP is (+) rotatory direction (DDMP from L-glucose is (–) rotatory direction, respectively). At the same time, after the condensation of DDMP with (+)-camphorsulfonyl chloride, the sulfonate product is a single compound rather than epimers, indicating that the DDMP from glucose is a single (+)-isomer rather than racemate. In addition, $^{1}$H NMR spectra showed that there was no H/D exchange in the C-3 position of DDMP in protic solvents ($D_2O$ and methanol-$d_4$), which indicated that enolization cannot occur in this position, and its chirality is very stable.

In the synthesis of DDMP from maltol, due to the good enantioselectivity of lipase for the hydrolysis of 3-ester group of racemic diacetate **4**, the final product DDMP also has optical activity. The (+) optical rotation in the experiment was identical with that of DDMP derived from glucose, so these two compounds were confirmed to be the same absolute configuration.

## 3.4. Taste evaluation

We conducted a taste test on the DDMP obtained by the two methods (Table 3). The results showed that the DDMP derived from glucose exhibited a bitter taste at a threshold of 1 mmol/kg of water, while the

**Table 3**
Taste quality and taste threshold.

| Entry | Compounds | Taste quality | Taste threshold (mmol/kg, in water) |
|---|---|---|---|
| 1 | DDMP-M [a] | tasteless | – |
| 2 | DDMP-G [b] | bitter | 1.2 |
| 3 | F-I | bitter | 0.08 |
| 4 | F-II | tasteless | – |
| 5 | pyrrolidinohexose reductone **6**[c] | bitter | 0.5 [d] |
| 6 | **7**[c] | bitter | 0.06[d] |
| 7 | **8**[c] | bitter | 0.8 |

*Note*: a. DDMP derived from maltol. b. DDMP derived from glucose. c. The structure is displayed in Fig. 2. d. The taste threshold was taken from Ottinger et al.

DDMP derived from maltol was tasteless even at 50 mmol/kg. This contradiction leads us to find the answer from the Maillard reaction which generates extremely complex products. The bitter compound pyrrolidinohexose reductone **6** (Fig. 2), formed by Maillard reactions from glucose and proline, showed low bitter taste threshold. Another reductone **8**, together with DDMP, both formed from glucose and piperidine, also showed bitter taste. There are good reasons for thinking that glucose-derived DDMP is mixed with other substances, which causes bitterness.

Accordingly, Sephadex LH-20 chromatography was used to further purify the glucose-derived DDMP, and the brown F-I fraction and white F-II fraction were obtained. The F-I fraction flowed out firstly, and its amount was rare, but exhibited an intense bitter taste at a low threshold of 0.07 mmol/kg of water, which was close to the threshold of bis (pyrrolidino)hexose reductone **7** (0.06 mmol/kg of water, Ottinger, & Hofmann, 2001). The F-II fraction afterwards was pure DDMP without color and bitterness. The results indicated that pure DDMP is a tasteless compound.

So far several types of bitter tastants formed during thermal food processing from Maillard reactions have been reported (Frank, Jezussek, & Hofmann, 2003; Wakamatsu, Stark, & Hofmann, 2016), such as pyrrolidinohexose reductone, quinizolate and (2*R*)-3-(allylthio)-2-((4*S*)-4-(allylthiomethyl)-6-formyl-3-oxo-3,4-dihydropyrrolo-[1,2-a]pyrazin-2 (1*H*)-yl) propanoic acid. Typically, most of the bitter tastants are nitrogen compounds. As for the F-I fraction, its composition was too complex to be fully clarified, although some piperidine substituents were detected by NMR spectra and GC–MS.

## 4. Conclusion

DDMP exists in many foods and is undoubtedly a very important compound. For the first time, DDMP was synthesized from maltol and obtained as a pure substance, and enzymatic synthesis played an important role in this process. Pure DDMP is tasteless with no bitter attribute. DDMP derived from glucose tastes bitter due to impurities produced in the Maillard reaction.

## CRediT authorship contribution statement

**Zhifei Chen:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Gaolei Xi:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing. **Yufeng Fu:** Formal analysis, Investigation, Resources. **Qingfu Wang:** Validation, Data curation. **Lili Cai:** Methodology, Visualization. **Zhiwei Zhao:** Formal analysis, Investigation. **Qiang Liu:** Formal analysis, Data curation. **Bing Bai:** Conceptualization, Supervision, Writing - review & editing. **Yuping Ma:** Supervision, Project administration, Conceptualization.

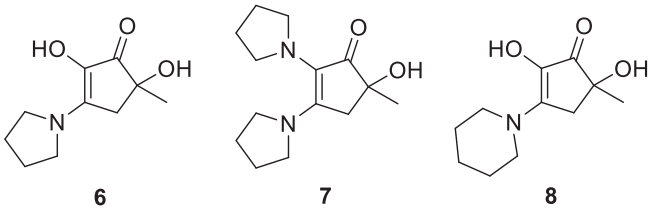



**Fig. 2.** Structures of pyrrolidinohexose reductone (**6**), pyrrolidinohexose reductone (**7**) and piperidinohexose reductone (**8**).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was partially supported by Technology Center, China Tobacco Henan Industrial Co., Ltd..

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