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The role of 2-hydroxypropanal in the biosynthesis of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one in strawberry (*Fragaria* × *ananassa*, cv. Elsanta) callus cultures

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Abstract

2-Hydroxypropanal was synthesized utilising a new approach via the Curtius rearrangement, which has the great advantage that the only co-product is ammonium chloride. 2-Hydroxypropanal was, without isolation, fed *in situ* to strawberry callus cultures in order to study the biosynthesis of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one. The high levels of the furanone obtained suggest that 2-hydroxy-propanal is a key precursor of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one in strawberry.

1. Introduction

2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one (DMHF) is one of the most important components of strawberry flavour (Honkanen and Hirvi, 1990; Larsen et al., 1992) but is also present in other fruits, such as pineapples (Rodin et al., 1965) and mango (Wilson et al., 1990). In strawberries, three derivatives of DMHF are also present: 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one-glucoside (DMHF glucoside), 2,5-dimethyl-4-methoxy-2*H*-furan-3-one (mesifuran) (Latrasse, 1991) and 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one-6'-*O*-malonyl-glucoside (Roscher et al., 1996). Despite the great commercial value and importance of DMHF and derivatives as flavourings and their broad use in numerous applications in the food industry, the biosynthesis of these furanones is unknown. The first studies on the bioformation of DMHF in *Zygosaccharomyces rouxii* (Hecquet et al., 1996) and strawberry callus cultures (Zabetakis and Holden, 1995) have recently been published. In the former, fructose-1,6-diphosphate was proposed as a key precursor of DMHF whereas in the latter, 6-deoxy-D-fructose was found to be a key precursor of DMHF glucoside and 2-hydroxypropanal (lactaldehyde) and dihydroxyacetonephosphate (DHAP) were proposed as precursors of DMHF glucoside (Zabetakis and Holden, 1995). It is suggested that the aldol condensation of these two compounds yields 6-deoxy-D-fructose and that this deoxysugar, in turn, may be the main precursor of DMHF glucoside (Zabetakis and Holden, 1995).

Given that lactaldehyde is possibly a key precursor of DMHF, lactaldehyde was synthesized using a new approach. In this way, the exact levels of lactaldehyde were known before the precursor feeding and no other compound, apart from ammonium chloride, was produced and co-fed to callus cultures.

2. Materials and methods

2.1. Synthesis

Melting points (m.p.) were taken in capillary tubes. Anhydrous solvents were used for the preparation of gem-diurethanes. Evaporation was carried out under reduced pressure at 35–40°C in a rotary evaporator, unless otherwise specified. When necessary, solutions in organic solvents were dried over sodium sulphate. Before analysis, compounds were dried over P_2O_5 at room temperature under high vacuum. R_f values refer to TLC on precoated plates Kieselgel 60 F_{254} (Merck) in the following solvent systems (proportions by volume):

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(A) chloroform—methanol (9:1), (B) ethyl acetate—light petroleum (60–80°C) (7:3). Plates were viewed under UV light.

2.2. N,N' *bis(benzyloxycarbonyl)-1,1-diamino-2-benzyloxypropane*

A suspension of *N*-benzyloxycarbonyl-*O*-benzyl-L-threonine dicyclohexylammonium salt (Bachem) (10.5 g, 20 mmol) in ether was shaken in a separatory funnel with sulphuric acid (0.1 M) until dissolved. The organic layer was washed with water (x3), dried and evaporated to ca 50 ml. A cooled (0°C) solution of diazomethane (Arndt, 1943) in ether was added until no more bubbles were formed. Some drops of glacial acetic acid were added (to destroy any excess of diazomethane) and the ethereal solution was washed with water, 5% aqueous potassium hydrogen carbonate, and water (to neutral pH), dried and evaporated. The residual oil, *N*-benzyloxycarbonyl-*O*-benzyl-L-threonine methyl ester, was homogeneous by TLC, R_f (A) = 0.88.

To a solution of the above oil in methanol (30 ml) hydrazine hydrate (4 ml) was added. After being stirred at room temperature overnight, the solvent was evaporated and the excess of hydrazine was removed in a desiccator over sulphuric acid. The product was precipitated by addition of ether-light petroleum. It was recrystallized from isopropyl alcohol, R_f (A) = 0.78.

A suspension of the hydrazide (3.6 g, 10 mmol) in tetrahydrofuran (5 ml) was cooled to −15°C. Hydrogen chloride in tetrahydrofuran (5 M; 5 ml) was added, followed by *t*-butyl nitrite (1.4 ml, 11 mmol). The mixture was stirred for about 30 min at −15°C until a clear solution was obtained. After evaporation of the solvent, the residue was extracted with ether, precooled to −10°C, then washed with cold water and dried. To the ethereal solution of the azide, benzyl alcohol (ca 10 ml) was added and the solvent evaporated at 5–10°C. The residual mixture of azide and alcohol was dissolved in benzene (15 ml) and heated under reflux for 2 h. The reaction mixture was left aside at room temperature. The product was crystallised from ethyl acetate-light petroleum and recrystallised from ethyl acetate; yield 2 g, m.p. 159–161°C; $[\alpha]_D^{25} = -9.4$°C (c 0.8 in dimethylformamide); R_f (B) 0.85; Found: C, 69.2; H, 6.3; N, 6.7. ($C_{25}H_{26}N_2O_5$ requires C, 69.6; H, 6.3; N, 6.2%).

2.3. Lactaldehyde

The gem diamine (2 g) was suspended in methanol and hydrochloric acid (5 ml, 4 N) was added. The mixture was subjected to hydrogenation at ambient pressure over Pd/C as catalyst for 4 h. The mixture was filtered, evaporated, water (10 ml) added and boiled for 15 min. Then it was evaporated, and the final aliquot (10 ml) added to culture medium. This medium was further sterilised by filtration (Whatman membrane filter; pore size: 0.2 μm).

A part of the mixture of lactaldehyde, after boiling, was added to a solution of 2,4-dinitrophenylhydrazine (0.1 M). The precipitate which formed was the 2,4-dinitrophenylhydrazone of the lactaldehyde. It was recrystallised from ethyl acetate-light petroleum and identified by comparison with an authentic sample by TLC-UV (Chrysochoou, 1973).

2.4. Callus cultures

Strawberry callus cultures were established and cultured as described elsewhere (Zabetakis and Holden, 1996). Calli were cultured in control medium and in a medium containing lactaldehyde for 4 weeks. The control medium consisted of Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal salt mixture, supplemented with agar (1% w/v), sucrose (2% w/v), benzylaminopurine (2.22 μM), and 2,4-dichlorophenoxy-acetic acid (2.26 μM). The pH of the medium was adjusted to 5.7 and the medium was sterilised. The precursor-supplemented culture medium contained a precursor in the form of lactaldehyde (0.5% w/v) synthesized *in situ*, as well as sucrose (2% w/v) and all other chemicals at the same level as in the control medium. All chemicals were purchased from Sigma, UK. At the end of the culture period, the calli were harvested, homogenised and analysed, using HPLC-UV, for DMHF and its derivatives as described elsewhere (Zabetakis and Holden, 1996).

3. Results and discussion

3.1. Preparation of lactaldehyde

Lactaldehyde has been synthesized, in its racemic form, from a suitable derivative of L-threonine. The conversion of the amino acid to the corresponding aldehyde has been achieved by applying the Curtius rearrangement (Bergmann and Zervas, 1936; Moutevelis-Minakakis and Photaki, 1985) to its azide (4), properly protected. As shown in Fig. 1, *N*-benzyloxycarbonyl-*O*-benzyl-L-threonine (1) is converted first to the gem diamine (5) and finally to the aldehyde (7) by heating in aqueous acidic solution.

3.2. Precursor feeding

The quantitative effect of lactaldehyde on the formation of DMHF and DMHF glucoside is shown in Table 1. In control experiments, neither DMHF nor DMHF glucoside were detected.

Free DMHF was the main product of the lactaldehyde-fed cultures as opposed to the detection of DMHF

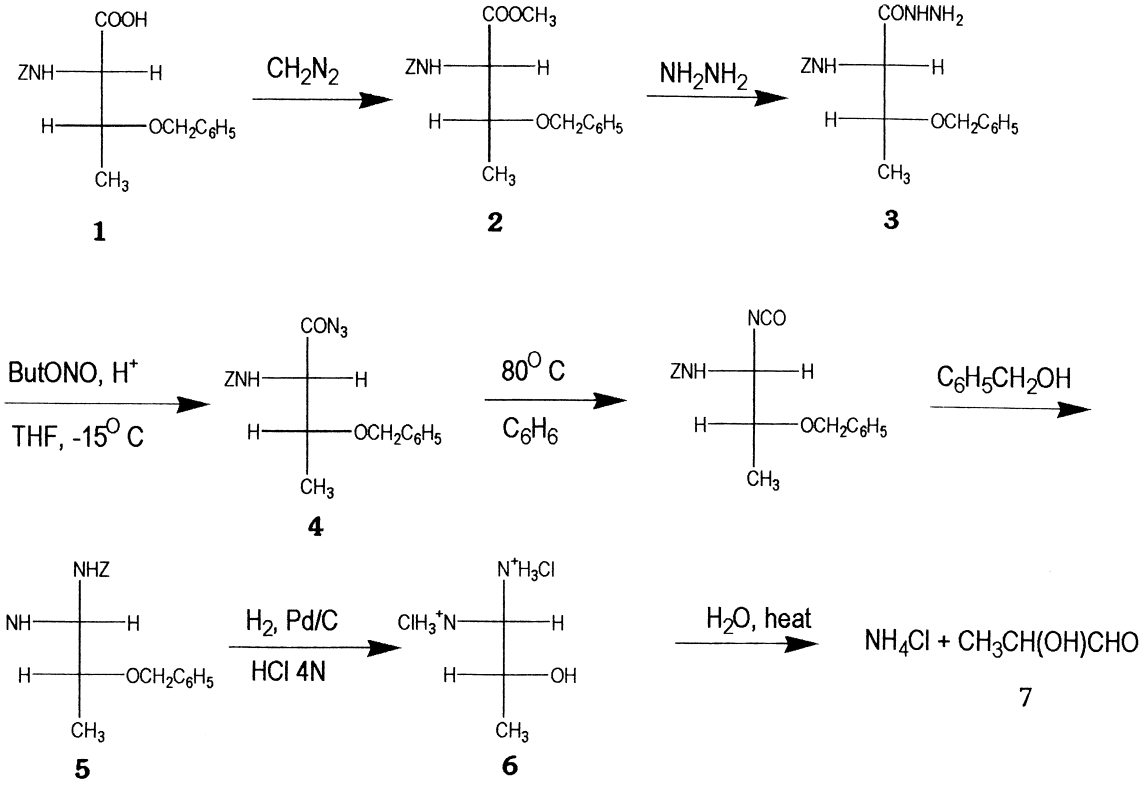

Fig. 1. Synthesis of lactaldehyde (7) from *N*-benzyloxycarbonyl-*O*-benzyl-L-threonine (1).

glucoside only when the callus were fed with 6-deoxy-D-fructose. This result shows that the biosynthesis of furaneol may proceed through a pathway different from that of DMHF glucoside. The putative pathway leading to the formation of free DMHF from lactaldehyde is given in Fig. 2.

3.3. The biosynthetic pathway of DMHF

The relatively high yield of furaneol in the lactaldehyde-fed cultures (0.015%) (Table 1) as opposed to the low yields of DMHF glucoside in 6-deoxysugar-fed cultures (0.00057%) (Zabetakis et al., 1996) highlights the significance of lactaldehyde as a key precursor of DMHF. It may also be proposed that the rate limiting step of the biosynthetic pathway to DMHF may be the formation of lactaldehyde from 1,2-propanediol (Zabetakis and Gramshaw, 1997). However, the occurrence of

Table 1
DMHF and its derivatives in control and precursor-supplemented cultures (μg per g of fresh weight of tissue)

	DMHF	DMHF glucoside	Mesifuran
Control	n.d.	n.d.	n.d.
Lactaldehyde	26.6 ± 1.44[a]	0.30 ± 0.03[a]	n.d.

n.d.: Not detected.
[a] Mean of three analyses (95% confidence level).

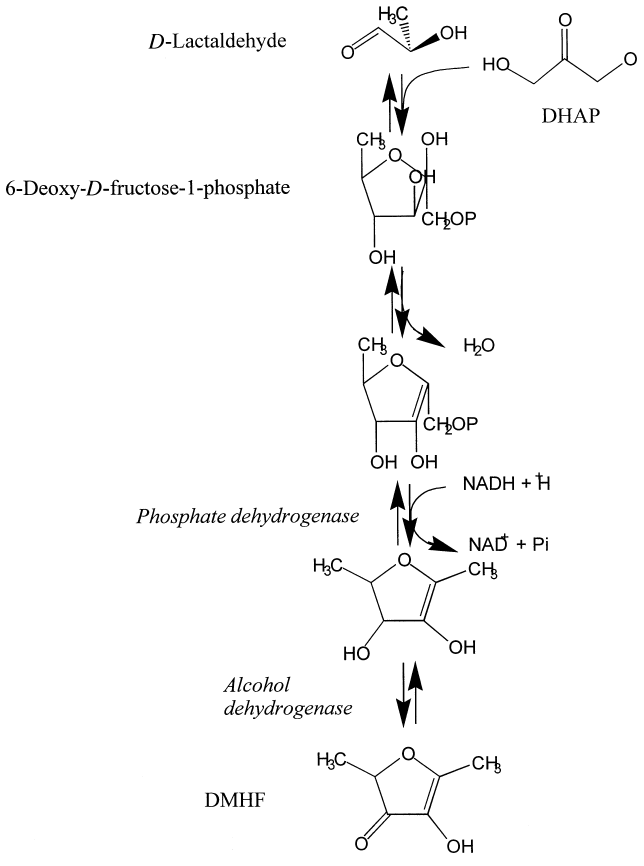

Fig. 2. A putative pathway for the biosynthesis of free DMHF.

1,2-propanediol in strawberry and consequent conversion to DMHF glucoside (Zabetakis and Gramshaw, 1997) suggests that the oxidation of one of the hydroxyl groups of the diol is a key stage where the pathways to DMHF and DMHF glucoside diverge and is thus worthy of further study.

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