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Synthesis and taste properties of maltose and maltitol analogues

Imad Toufeili

Department of Food Technology and Nutrition, FAFS, American University of Beirut, Beirut, Lebanon

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Stanley Dziedzic

Department of Food Science, Food Studies Building, University of Reading, Whiteknights, PO Box 226, Reading, UK, RG6 2AP

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The hypothesis that intramolecular hydrogen bonding is responsible for the sweetness of maltitol is tested by the synthesis of maltitol analogues which differ in configuration at C_3'' and C_4' and sensory evaluation of the products. 3-*Allo*-maltitol and *galacto*maltitol were synthesised by treating suitably protected methanesulphonylated derivatives of benzyl β-maltoside with sodium benzoate followed by removal of the blocking groups and subsequent reduction with sodium borohydride. Sensory evaluation of maltose, maltitol and their analogues revealed that the non-reducing end is involved in the generation of the sweet response and that intramolecular hydrogen bonding governs the accession of this class of polyol sweeteners to the receptor site on the tongue.

INTRODUCTION

The pharmacophore which confers the quality of sweetness is generally viewed as two electronegative atoms A and B, A being chemically bonded to an acidic hydrogen atom, which can hydrogen bond with a commensurate AH,B system on the taste bud (Shallenberger & Acree, 1967). Furthermore, the presence of a lipophilic centre, X, with a well-defined geometrical relationship to the AH,B unit is acknowledged to be of importance in high-intensity sweeteners (Kier, 1972; Hough & Khan, 1978).

Evidence exists that in sugars, at least those of glucopyranoside-type structures, the third and fourth hydroxyl groups constitute the B and AH functions, respectively (Birch & Lee, 1974; Lindley & Birch, 1975). The hydrogen-bonding capacity of sugars has been invoked by many workers to explain differences in sweetness in this class of compound. Thus, the high sweetness of β-D-fructopyranose is believed to be due to intramolecular hydrogen bonding of the C_1 and C_2 hydroxyl groups (Shallenberger & Lindley, 1977). Likewise, the anomalously high sweetness of maltitol (Fig. 1), as compared to maltose, has been attributed to intramolecular hydrogen bonding interplay between the C_1 and/or C_3 hydroxyl groups of the aglycone and the C_4 hydroxyl in the glucose residue (Birch & Kearsley, 1977).

Accepting the hypothesis that the aforementioned intramolecular hydrogen bonding system is responsible for the high sweetness of maltitol then inversion of configuration either at C_3'' or C_4' would lower the affinity of the C_4' hydroxyl to the receptor site and lead to a reduction in sweetness. (Unprimed and primed carbons refer to the reducing and non-reducing units of maltose, respectively. Double primed carbons refer to the polyol chain of maltitol.) 3-*Allo*maltose (Fig. 3, **13**) and *galacto*maltose (Fig. 3, **14**) have been synthesised using β-maltose monohydrate (Takeo & Okano, 1977) and maltosan (Mori *et al.*, 1975), respectively. In the present work we report synthesis of these compounds using benzyl β-maltoside (Dutton & Slessor, 1964), as a starting material, their reduction to the corresponding alcohols and their taste characteristics.

MATERIALS AND METHODS

Reagents were obtained from British Drug Houses (Chemicals) Ltd (UK) and Sigma Chemical Company Ltd, Poole, Dorset (UK). Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded at room temperature with a P70-7 automatic polarimeter and a 5-cm cell. Thin-layer chromatography (t.l.c.) was

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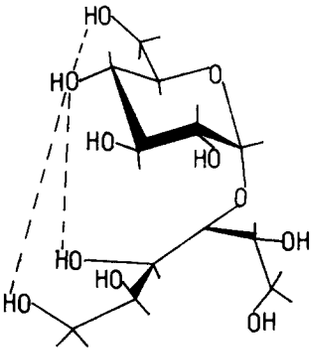
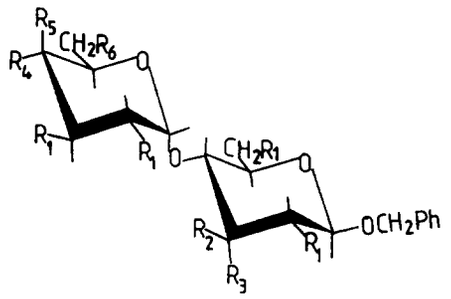


Fig. 1. Structure of maltitol showing multiple hydrogen bonding (– – – –, possible hydrogen bonds).

performed on precoated (0·25 mm) silica gel plates (Merck, Kieselgel $60F_{254}$) with detection by UV light or by charring with 10% ethanolic sulphuric acid. Column chromatography was carried out with Kieselgel 60 (Merck, 7734). ^{1}H-NMR spectra were measured on a Varian 220-MHz spectrometer with tetramethylsilane as internal standard. Sensory analysis was carried out as reported previously (Toufeili & Dziedzic, 1985); panellists were asked to rate sweetness according to the following descriptions: very sweet (SS), sweet (S), trace-sweetness (trS) and no sweetness (0).

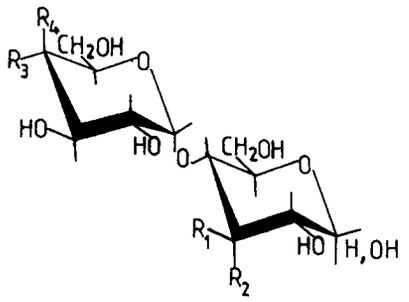
Chemical syntheses

3-*Allo*maltose (Fig. 3, **13**) was synthesised by treating benzyl 3-O-methanesulphonyl-hexa-O-benzoyl β-maltoside with sodium benzoate in hexamethylphosphoric triamide and subsequent removal of the protecting groups. Reduction of **13** with sodium borohydride gave 3-*allo*maltitol (Fig. 4, **16**). Treatment of benzyl 4,6-di-O-methanesulphonyl-penta-O-benzoyl β-maltoside (Fig. 2, **9**) with sodium benzoate and removal of protecting groups gave *galacto*maltose; reduction with sodium borohydride afforded the corresponding alcohol (Fig. 4, **17**).



1. $R_1 = R_2 = R_4 = R_6 = OH$; $R_3 = R_5 = H$.
2. $R_1 = R_4 = R_6 = OCOPh$; $R_2 = OH$; $R_3 = R_5 = H$.
3. $R_1 = R_4 = R_6 = OCOPh$; $R_2 = OMs$; $R_3 = R_5 = H$.
4. $R_1 = R_3 = R_4 = R_6 = OCOPh$; $R_2 = R_5 = H$.
5. $R_1 = R_3 = R_4 = R_6 = OH$; $R_2 = R_5 = H$.
6. $R_1 = R_2 = OH$; R_4, $R_6 = OCHPhO$; $R_3 = R_5 = H$.
7. $R_1 = R_2 = OCOPh$; R_4, $R_6 = OCHPhO$; $R_3 = R_5 = H$.
8. $R_1 = R_2 = OCOPh$; $R_4 = R_6 = OH$; $R_3 = R_5 = H$.
9. $R_1 = R_2 = OCOPh$; $R_4 = R_6 = OMs$; $R_3 = R_5 = H$.
10. $R_1 = R_2 = R_5 = R_6 = OCOPh$; $R_3 = R_4 = H$.
11. $R_1 = R_2 = R_5 = R_6 = OH$; $R_3 = R_4 = H$.

Fig. 2.



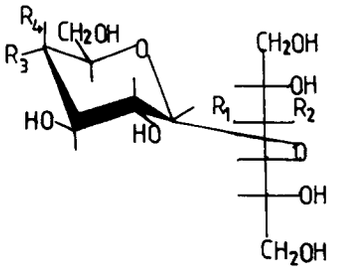
12. $R_1 = R_3 = OH$; $R_2 = R_4 = H$.
13. $R_1 = R_4 = H$; $R_2 = R_3 = OH$.
14. $R_2 = R_3 = H$; $R_1 = R_4 = OH$.

Fig. 3.

Benzyl 2,6-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**2**)
Benzyl β-maltoside (**1**) (bold numbers refer to Figs 2–4) (10 g) was dissolved in dry pyridine (200 ml) and treated with benzoyl chloride (30 ml) at 0°. After stirring for 48 h at room temperature, the reaction mixture was poured into ice-water (2 litres) and extracted with chloroform (300 ml). The chloroform layer was washed with water, dried ($MgSO_4$) and concentrated. Crystallisation from chloroform–light petroleum gave the title compound (20 g, 81%); m.p. 130–132°; $[\alpha]_D$ + 42° (*c*. 1·0, chloroform).

Benzyl 2,6-di-O-benzoyl-3-O-mesyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**3**)
The hexabenzoate (**2**) (10 g) was dissolved in pyridine (100 ml) and treated with methanesulphonyl chloride (MsCl; 3 ml) at 0°C. After stirring for 24 h at room temperature, the reaction mixture was treated with ice-water (5 ml) and concentrated to a syrup. Crystallisation from ethanol gave the 3-mesylate as needles (8 g, 74%), m.p. 147–148°; $[\alpha]_D$ + 51° (*c*. 1·0, chloroform).

Benzyl 2,3,6-tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-allopyranoside (**4**)
A solution of **3** (7 g) in dry hexamethylphosphoric triamide (40 ml) was heated with sodium benzoate (5 g) for 4 h at 90°. The reaction mixture was evaporated to dryness, dissolved in chloroform (150 ml) and washed with water (5 × 50 ml). Evaporation of chloroform and crystallisation from acetone–methanol gave the title



15. $R_1 = R_3 = OH$; $R_2 = R_4 = H$.
16. $R_2 = R_3 = OH$; $R_1 = R_4 = H$.
17. $R_1 = R_4 = OH$; $R_2 = R_3 = H$.

Fig. 4.

compound (5·4 g, 76%), m.p. 119–122°, $[\alpha]_D$ + 37° (*c.* 1·0, chloroform).

Benzyl 4-O-(α-D-glucopyranosyl)-β-D-allopyranoside (**5**)
Compound **4** (4 g) in anhydrous methanol (40 ml) was treated with M methanolic sodium methoxide (2 ml) and the reaction monitored by t.l.c. (ethyl acetate : ethanol : water, 10 : 2 : 1). After stirring for 24 h at room temperature, t.l.c. indicated the presence of a single product which co-chromatographed with benzyl β-maltoside (**1**). The reaction mixture was neutralised [Amberlite IR-120 (H^+)], filtered, evaporated to dryness and the residue extracted several times with ether to remove methyl benzoate. The title compound was obtained as an amorphous powder (0·8 g, 53%), $[\alpha]_D$ + 21° (*c.* 1·2, methanol).

4-O-α-D-Glucopyranosyl-D-allopyranose (**13**)
A solution of **5** (500 mg) in methanol (40 ml) was hydrogenated over 5% Pd/C (1 g) at room temperature and atmospheric pressure for 18 h. The catalyst was removed and the filtrate neutralised [Amberlite IR-45(OH^-)] and concentrated to yield the title compound as an amorphous solid (250 mg, 63%), $[\alpha]_D$ + 120° (*c.* 2·0, water); (Takeo & Okano, 1977), $[\alpha]_D$ + 118·9° (*c.* 3·0, water).

4-O-α-D-Glucopyranosyl-D-allitol (**16**)
A solution of **13** (200 mg) in water (10 ml) was treated dropwise with sodium borohydride (100 mg in 5 ml water) at 0°. After stirring for 4 h at 0°, the reaction mixture was treated with glacial acetic acid (2 ml), neutralised [Amberlite IR-120 (H^+)], filtered and concentrated. Crystallisation from 80% ethanol gave the title compound (100 mg, 50%), m.p. 106–107°, $[\alpha]_D$ + 88·6° (*c.* 1·4, water).

Benzyl 4-O-(4,6-O-benzylidene-α-D-glucopyranosyl)-β-D-glucopyranoside (**6**)
A solution of benzyl β-maltoside (20 g) and *p*-toluenesulphonic acid (500 mg) in dry dimethylformamide (150 ml) was treated with α,α-dimethoxytoluene (8 g). After stirring for 3 h at room temperature, t.l.c. (ethyl acetate : ethanol : water; 10 : 2 : 1) revealed the formation of a major product which co-chromatographed with authentic benzyl 4',6'-O-benzylidene β-maltoside (Klemer, 1959) in addition to several minor components. The mixture was neutralised [Amberlite IR-45(OH^-)], filtered and concentrated. Crystallisation from absolute ethanol at 0° gave the title compound (14 g, 54%), m.p. 112–116°, $[\alpha]_D$ + 17° (*c.* 1·0, pyridine); (Klemer, 1959) m.p. 112–116°, $[\alpha]_D$ + 15·8° (*c.* 1·2, pyridine).

Benzyl 2,3,6-tri-O-benzoyl-4-O-(2,3-di-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranosyl)-β-D-glucopyranoside (**7**)
Conventional benzoylation of **6** with benzoyl chloride in pyridine gave the title compound, m.p. 180° (chloroform–ethanol), $[\alpha]_D$ + 38·4° (*c.* 1·0, chloroform).

Benzyl 2,3,6-tri-O-benzoyl-4-O-(2,3-di-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**8**)
Aqueous 80% trifluoroacetic acid (10 ml) was added to a solution of **7** (15 g) in chloroform (150 ml). The solution was stirred for 20 min, diluted with chloroform (100 ml), washed with saturated sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated to a syrup (11 g, 80%). An analytical sample was obtained by chromatographing a small portion of the syrup with benzene : ethyl acetate (3 : 1). $[\alpha]_D$ + 64·3° (*c.* 1·0, chloroform).

Benzyl 2,3,6-tri-O-benzoyl-4-O-(2,3-di-O-benzoyl-4,6-di-O-mesyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**9**)
Compound **8** was mesylated as described above to give **9** (80%), m.p. 110° (chloroform–light petroleum), $[\alpha]_D$ + 57·6° (*c.* 1·0, chloroform).

Benzyl 2,3,6-tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl)-β-D-glucopyranoside (**10**)
Treatment of **9** with sodium benzoate, as described above, gave **10** (80%), m.p. 92–95° (isopropanol), $[\alpha]_D$ + 69° (*c.* 1·0, chloroform).

Benzyl 4-O-(α-D-galactopyranosyl)-β-D-glucopyranose (**11**)
Compound **10** was de-O-benzoylated to yield **11** as a syrup (90%), $[\alpha]_D$ + 32° (*c.* 1·0, methanol).

4-O-α-D-Galactopyranosyl-D-glucopyranoside (**14**)
Hydrogenation of **11**, as described above, gave **14** (55%), m.p. 228–229° (ethanol), $[\alpha]_D$ + 161° (*c.* 2·0, water); (Mori *et al.*, 1975), m.p. 227–229° (methanol), $[\alpha]_D$ + 159·6° (*c.* 1·02, water).

4-O-α-D-Galactopyranosyl-D-glucitol (**17**)
Compound **14** was reduced with sodium borohydride to yield **17** as an amorphous powder (60%); $[\alpha]_D$ + 76·2° (*c.* 1·0, water).

RESULTS AND DISCUSSION

The ^{1}H-n.m.r. data of maltose derivatives is shown in Table 1. The chemical shifts and coupling constants are consistent with the proposed structures. H_3 of **4** appeared as a triplet (τ value 3·92; $J_{2,3} = J_{3,4}$ = 2·5 Hz) and $H_{4'}$ of **10** as a double doublet (τ value 3·96; $J_{3',4'}$ = 2·5 Hz, $J_{4',5'}$ = 1·2 Hz) indicative of an axial, equatorial, axial arrangement of H_2, H_3, H_4 and $H_{3'}$, $H_{4'}$, $H_{5'}$. Attempts to confirm the structures of 3-*allo*maltitol (**16**) and *galacto*maltitol (**17**) by ^{13}C-n.m.r. spectroscopy were not successful as the spectra showed much overlapping of the ^{13}C-resonances and no unambiguous assignment of the signals could be made.

The taste properties of lactose, maltose, 3-*allo*maltose and *galacto*maltose and their corresponding alcohols are shown in Table 2. Inversion of configuration at C_4' of maltose led to a marked reduction in sweetness as evidenced by the similar taste properties of

Table 1. First-order chemical shifts (τ values) and coupling constants (Hz) in $CDCl_3$, unless otherwise stated (τ TMS = 10)

Compound	H-1 $J_{1,2}$	H-2 $J_{2,3}$	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6a}$	H-6a $J_{5,6b}$	H-6b $J_{6a,6b}$	H-1' $J_{1',2'}$	H-2' $J_{2',3'}$	H-3' $J_{3',4'}$	H-4' $J_{4',5'}$	H-5' $J_{5',6'a}$	H-6'a $J_{5',6'b}$	H-6'b $J_{6'a,6'b}$	Benzylidene; OBz; OMs
2[a]	5·39d	4·79t	5·32t	5·50t	5·82–5·90m	5·17dd	4·96dd	4·19d	4·62dd	3·88t	4·30t	5·42o	5·72dd	5·52dd	1·70–2·80 (35 H, m)
	7·5	9·5	9·5	9·5	2·5	3·5	−11·5	4·0	10·0	10·0	10·0	2·5	4·5	−12·5	
3	5·35d	4·82t	5·04t	5·47t	5·70–5·80m	5·17dd	5·01dd	4·00d	4·47dd	3·80t	4·24t	5·20–5·30m	5·64dd	5·15dd	1·80–2·80 (35 H, m); 7·00 (3 H, s)
	7·5	10·0	10·0	10·0	2·0	3·5	−12·5	3·5	10·0	10·0	10·0	3·0	4·5	−12·5	
4	5·17d	4·6dd	3·92t	5·35dd	5·70–5·90m	5·28dd	4·98dd	4·19d	4·52dd	3·83t	4·32t	5·40–5·50m	5·70dd	5·50dd	2·20–2·80 (40 H, m)
	7·5	2·5	2·5	10·0	2·5	5·0	−12·5	4·0	10·0	10·0	10·0	2·5	4·5	−12·0	
7	5·14d	4·58dd	4·29t	5·46t	5·84–6·00m	5·00dd	5·25dd	4·30d	4·70dd	4·02t	5·80dd	5·79dt	6·14t	6·28t	4·50 (1 H, s); 1·70–2·80 (40 H, m)
	7·5	9·0	9·0	9·0	2·0	3·5	−12·5	4·0	10·0	10·0	4·5	10·0	10·0	−10·0	
8[a]	5·19d	4·56dd	4·29t	5·57t	5·92–6·30m	5·04dd	5·28dd	4·37t	4·82dd	4·26dd	5·21dd		5·92–6·30m		1·70–2·80 (30 H, m)
	7·5	9·0	9·0	9·0	2·0	2·5	−11·0	4·0	10·5	9·0	10·0	—	—	—	—
9	5·18d	4·59dd	4·30t	5·52t	5·92–6·40m	5·08dd	5·32dd	4·29d	4·83dd	4·02t	4·92t	5·92–6·40m	5·68dd	5·73dd	1·70–2·80 (30 H, m); 7·00 (3 H, s)
	7·5	9·0	9·0	9·0	2·5	2·0	−12·5	4·0	10·0	9·5	9·5	2·5	2·0	−10·5	7·13 (3 H, s)
10	5·19d	4·57dd	4·28t	5·49t	5·20–6·00m	5·00dd	5·29dd	4·17d	4·30dd	4·06dd	3·96dd	5·20–6·00m	5·62dd	5·67dd	1·80–2·80 (40 H, m)
	7·5	9·0	9·0	9·0	2·0	3·5	−12·5	3·5	10·5	2·5	1·2	3·0	6·5	−12·5	

H-a, H-b of the anomeric benzyl group appeared at τ value: 5·06d, 5·29d; $J_{a,b} = -12{\cdot}5$ Hz.
s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, o = octet, m = multiplet.
[a] $CDCl_3 + D_2O$.

Table 2. Taste properties of lactose, maltose, maltose derivatives and polyol analogues

Carbohydrate	Taste
Lactose	trS
Lactitol	trS
Maltose (**12**)	S
Maltitol (**15**)	SS
3-*Allo*maltose (**13**)	S
3-*Allo*maltitol (**16**)	0
*Galacto*maltose (**14**)	trS
*Galacto*maltitol (**17**)	0

*galacto*maltose (**14**) and lactose. This could be attributed to intramolecular hydrogen bonding between the axial C_4' hydroxyl group and the ring oxygen (Lindley *et al.*, 1976) or to the conformation adopted by the CH_2OH group in *galacto* sugars (Mathlouthi *et al.*, 1986). The importance of the axial C_4 hydrogen atom, in sugars adopting the $^4C_1$ conformation in solution, in the generation of the sweet response is further highlighted in studies on structure–activity relationships of sucrose and solution properties of small carbohydrates. The lack of sweetness of *galacto*sucrose has been linked to the absence of the axial C_4 hydrogen atom believed to govern the accession of sucrose derivatives into the receptor site (Hough, 1989). Likewise, the low hydration capacity of the axial C_4 hydroxyl group of galactose hinders the diffusion of the molecule through the oral fluid and leads to a less efficient interaction with the receptor (Birch & Shamil, 1988).

The similar degrees of sweetness elicited by maltose and its 3-*allo* analogue (**13**) indicate that the tripartite glucophore AH, B, X (AH = 2-OH, B = 3-O, X = 6-CH_2), considered as one of the determinants of the sweetness of D-glucopyranose (Mathlouthi & Seuvre, 1988) contributes little to the sweetness of these molecules. The marginal effects of configurational changes at C_3 on taste properties coupled with the noted reduction in sweetness observed upon inversion of configuration at C_4' highlight the pivotal role of the C_4' hydroxyl group either as an AH function or as facilitating the accession of maltose derivatives into the vicinity of the receptor. These observations are in accord with previous findings which indicated that the AH,B unit is located at the non-reducing end of disaccharides and that only one half of the molecule is involved in the sensory response (Lee & Birch, 1975; O'Donnell, 1983).

Both alcohols (**16**, **17**) were completely devoid of sweetness, thus supporting the thesis of a precise stereochemical fit of sweet tastants with the receptor protein. The lack of sweetness of 3-*allo*sucrose has been related to its incongruity with the dimensions of the binding sites at the taste bud (Hough & Khan, 1991; Suami & Hough, 1991). In sugar alcohols, the geometrical relationships of hydroxyl groups have been shown to have profound effects on conformation in solution (Franks *et al.*, 1988, 1991). The low apparent molar volume of xylitol, as compared to ribitol, is believed to be responsible for its higher sweetness (Birch *et al.*, 1991). More recently, differences in the hydration behaviour of erythritol and L-threitol were shown to affect the dimensions of the sweet glycophore and were successfully correlated with their degree of sweetness (Howard & Grigera, 1992).

In conclusion, the tastelessness induced by configurational changes at C_3' and C_4' suggest that the conformation responsible for the sweetness of maltitol is governed by highly specific geometrical relationships between the aglycone and the sugar ring residues.

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