Role of the anomeric centre in sugar sweetness

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Summary. Intensity-time studies of the sweetness of D-glucose solutions show that there are no major differences between α- and β-anomers. Nor do the α- and β-anomers exhibit any differences in apparent molar volumes. Contrary to previous reports, the anomeric centre of D-fructose may play no direct role in the sweetness response.
Key words. Sweetness; sugars; anomers; glucose; fructose; intensity; persistence; molar volume.

There has been some controversy over the role of the anomeric centre in the sweetness of sugar molecules. Shallenberger et al.[1] report that solid β-D-glucopyranose is sweeter than the α-form whereas Pangborn and Gee[2] report the opposite for D-glucose solutions. Evidence exists[3,4] that the glycophore is located at the opposite end of the glucopyranose molecule to the anomeric centre and bitter-sweet molecules may be 'polarised' on taste receptors[5], one 'end' eliciting sweetness and the other 'end' (the anomeric centre) eliciting bitterness. The 3,4 α-glycol group of glucopyranose structures evidently constitutes the primary AH, B system[6]. By contrast the primary alcohol group only plays a secondary role in augmenting the AH, B system by the Lemieux effect[7]. Further evidence to rule out the participation of the anomeric centre in sweetness is provided by the non-reducing sugars (e.g. sucrose, trehalose) which are sweet, the 1-deoxy sugars[3,4] (i.e. anhydropolyols), hydrogenated sugars, inositols[8] and pseudo-sugars[9] which are all sweet though they are devoid of a free anomeric centre. A problem arises with D-fructopyranose, the sweetest simple sugar, in which the AH, B system is thought to reside in the 1,2-α-glycol group[10,11]. Shallenberger and Acree[10] reached this conclusion because OH-2 has the most acidic proton but it is also known[11] that removal of either the primary alcohol group or HO-2 of D-fructopyranose (i.e. to form either D-arabinopyranose or 1-deoxy-D-mannopyranose) causes some loss of sweetness. It has also been suggested[11] that the axial OH at position 5 in D-fructopyranose is involved in sweetness because L-sorbopyranose is less sweet than D-fructopyranose. This was thought to be due to OH-5 forming an intramolecular H-bond with the ring oxygen atom, thus sparing OH-1 to act as AH in the glycophore. This idea, however, has recently been refuted by Martin et al.[12] who have synthesised 5-deoxy-D-fructopyranose and shown that it has a similar sweetness to D-fructopyranose. Moreover, 'pseudofructose' also has a similar sweetness[9] though it is devoid of a ring oxygen atom. Thus, in contrast to the D-glucopyranose structure (4_{C_1} conformation), the anomeric centre of D-fructopyranose (5^{C_2} conformation) does seem to play a role in the sweet response.

The anomaly may be resolved by viewing D-fructopyranose as conformationally analogous to both D-glucopyranose and D-mannopyranose (fig.). Thus 2-deoxy D-fructopyranose is identical to 1-deoxy D-mannopyranose (i.e. 1,5 anhydro-D-mannitol) and, like the aldopyranoses, the primary glucophore of D-fructopyranose is probably the 3,4-α-glycol system. All pyranoses may therefore be considered to be conformationally commensurate with the sweet taste receptor but their effective sweetening power depends on the geometry and intrinsic effectiveness of the glycophore as an AH, B system. Consequently mutarotation of reducing sugars should not modify their sweet tastes

Table 1. Intensities and persistences of 5% D-glucose for 1 h after dissolution compared with equilibrated (24 h) 5% D-glucose

Sample	Intensities (SMURF units) at following times after dissolution							Persistences (seconds) at following times after dissolution						
	5 min	10 min	20 min	30 min	40 min	50 min	60 min	5 min	10 min	20 min	30 min	40 min	50 min	60 min
α-D-Glucose	5.1	5.0	5.4	5.3	5.1	4.8	4.9	11.6	10.8	12.5	11.8	13.2	10.8	11.6
β-D-Glucose	7.0	7.0	6.8	6.5	6.5	6.6	–	12.4	12.0	12.0	10.2	10.1	10.8	–
Equilibrated α-D-glucose	5.6	5.7	5.8	6.1	6.3	5.5	5.1	11.9	11.1	12.4	14.8	12.7	11.2	11.8
Equilibrated β-D-glucose	6.7	6.4	6.9	6.5	6.5	6.5	–	12.3	11.0	11.4	11.3	11.0	11.9	–

Figures are means of 12 panellists tasting 2 ml solutions at 20 °C. Sugar solutions were made up as in table 3. Conditions of operation of SMURF were as in previous publications[5,13]. Intensity of 5% standard sucrose solution = 10 SMURF units. Chart speed 12.0 cm · min^{-1}. All of each anomer solutions were tasted on one day. Differences between mutarotating anomer and its equilibrated counterpart NS at all times. $[\alpha]_D^{20}$ (α-D-Glucose, c5, H_2O) + 112.0° (5 min) + 70.6° (60 min); $[\alpha]_D^{20}$ (β-D-Glucose, c5, H_2O) + 19.4° (5 min) + 41.0° (60 min); $[\alpha]_D^{20}$ (eqn.) = + 52.2° (c5, H_2O).

Table 2. Intensities and persistences of 15% D-glucose for 1 h after dissolution compared with equilibrated (24 h) 15% D-glucose

Sample	Intensities (SMURF units) at following times after dissolution							Persistences (seconds) at following times after dissolution						
	5 min	10 min	20 min	30 min	40 min	50 min	60 min	5 min	10 min	20 min	30 min	40 min	50 min	60 min
α-D-Glucose	8.8	8.7	8.6	8.1	8.4	8.3	8.1	16.0	14.6	14.1	13.3	13.5	13.1	12.0
β-D-Glucose	7.7	7.6	7.6	7.2	7.0	7.2	–	14.5	13.0	14.0	12.7	11.7	10.7	–
Equilibrated α-D-glucose	8.3	8.4	8.5	8.4	8.6	8.2	8.1	13.4	14.7	14.2	12.8	13.8	12.3	12.9
Equilibrated β-D-glucose	7.9	7.4	7.6	7.2	7.0	7.4	–	14.0	11.8	13.3	11.9	11.7	11.2	–

Figures are means of 9 panellists tasting 2 ml solutions at 20 °C. Sugar solutions were made up as in table 3. Conditions of operation of SMURF were as in previous publications[5,13]. Intensity of 15% standard sucrose solution = 10 SMURF units. Chart speed 12.0 cm · min^{-1}. All of each anomer solutions were tasted on one day. Differences between mutarotating anomer and its equilibrated counterpart NS at all times. $[\alpha]_D^{20}$ (α-D-Glucose, c15, H_2O) + 100.0° (5 min) + 64.0° (60 min); $[\alpha]_D^{20}$ (β-D-Glucose, c15, H_2O) + 20.7° (5 min) + 39.7° (60 min); $[\alpha]_D^{20}$ (eqn.) = + 53.0° (c15, H_2O).

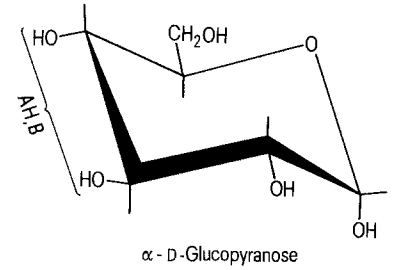
Table 3. Apparent molar volumes ($^{\phi}V$) of α and β-D-glucopyranose and β-D-fructopyranose and their mutarotated solutions in relation to concentration (A, D-Glucose; B, D-Fructose)

	A) D-Glucose Mutarotated D-glucose 66% β and 34%α		β-D-Glucopyranose		α-D-Glucopyranose		B) D-Fructose Mutarotated D-fructose 68% β-pyranose, 28% β-furanose, 4% α-pyranose		β-D-Fructopyranose	
Conc. w/w (%)	ϱ (g/cm^3)	$^{\phi}V$ (cm^3/mol)	ϱ (g/cm^3)	$^{\phi}V$ (cm^3/mol)	ϱ (g/cm^3)	$^{\phi}V$ (cm^3/mol)	ϱ (g/cm^3)	$^{\phi}V$ (cm^3/mol)	ϱ (g/cm^3)	$^{\phi}V$ (cm^3/mol)
30	1.125	112.8	1.125	112.8	1.124	112.9	1.128	111.4	1.128	111.4
20	1.080	112.2	1.079	112.5	1.079	112.6	1.082	110.8	1.082	110.8
10	1.038	111.8	1.037	112.1	1.038	111.8	1.039	110.1	1.038	110.6
5	1.018	111.3	1.017	111.7	1.017	111.7	1.018	108.2	1.018	110.3
3	1.010	110.8	1.010	111.4	1.010	110.8	1.010	107.3	1.010	110.2
1	1.002	110.3	1.002	110.3	1.002	110.3	1.002	104.9	1.002	108.5

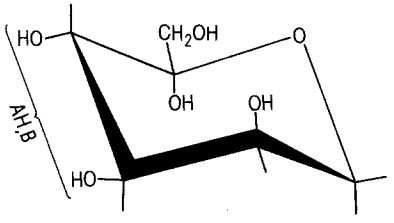
Sugars were purchased from the Sigma Chemical Co., Poole, Dorset. Densities (ϱ) were measured on a Parr Density Meter (DMA 60) and Density Measuring Cell (DMA 602) (Stanton Redcroft, London) equipped with an automatic sampler (SP2) and Anadex printer. Temperature control (20 ± 0.3 °C) was achieved with a Hetofrig bath (Heto Birkerod, Denmark), coupled to the density measuring cell. The density meter was calibrated with water and air. Water used for this and for sugar solutions was 'HiPerSolv' water for HPLC from BDH Chemicals Ltd., Poole, Dorset. Uncertainty in the density determinations was ± 3.0 × 10^{-6} g·cm^{-3}. Densities of sugar solutions were determined as quickly as practicable after dissolution.

unless the function of the glycophore itself is affected. The 33% loss of sweetness which occurs as D-fructopyranose mutarotates is accounted for by ring contraction to furanose form.

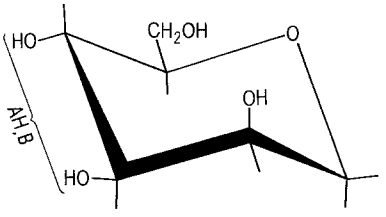
The above evidence does not rule out the *participation* of the anomeric centre in sweet taste chemoreception. However, unless anomerization is fast in contact with the receptor, it is clear that the configuration of the anomeric centre is irrelevant to the sweet response. Indeed the comparable sweetness values of parent sugars, 'pseudosugars' and '1-deoxy sugars' (i.e. 1,5-anhydrohexitols)[4,9] supports the view that the anomeric centre does not affect the degree of sweetness unless it interacts with the glycophore.



α-D-Glucopyranose



β-D-Fructopyranose



2-Deoxy-D-Fructopyranose
1-Deoxy-D-Mannopyranose

We now present data (tables 1 and 2) for both intensity and persistence of sweet taste of α- and β-D-glucopyranose measured at intervals during mutarotation by the SMURF technique[13]. There ar no major differences in either intensity or persistence of response during mutarotation, confirming our belief that the anomeric centre plays no part in the sweetness of these structures. The intensity and time facets of the taste response may reflect different stages of the taste chemoreception mechanism[14] and their analysis has been used to interpret possible differences between accession to and interaction with receptors. It seems likely that no substance can be tasted unless it is soluble and this has led[15] to a consideration of the role of water in the sweetness response. Presumably water molecules themselves have good access to the environs of receptors and therefore the compatibility of sapid solutes with water structure offers a clue to their accessibility to receptor sites. One way of measuring the disturbance of water structure is by determining apparent molar volume ($^{\phi}V$) and this is known[15] to give differences of over 1% between sugars differing only in the configuration of one carbon atom (e.g. D-glucose and D-galactose). When the anomers of D-glucopyranose are compared, however, there are no major differences in $^{\phi}V$ at any concentration (table 3) which indicates that α- and β-D-glucopyranose disturb water structure equally. A small noticeable difference in $^{\phi}V$ values between fresh and mutarotated β-D-fructopyranose solutions (at low concentrations), on the other hand, may be attributed to amounts of the smaller fructofuranose molecular skeleton.

We therefore propose that the anomeric centre plays no direct role in the sweetness of sugars as a binding site but rather it is the 3,4 α-glycol system of pyranoses which constitutes the AH, B glycophore. All taste effects are mediated by water and the solution properties of sugars offer the most immediate clue to their potential sapid effects. Although there are no real differences between the $^{\phi}V$ values of the D-glycopyranose anomers, the $^{\phi}V$ values of D-fructopyranose are consistently lower than D-glucopyranose at the same concentrations. This shows that D-fructopyranose is more compatible (less disturbance) with water structure than D-glucopyranose, reflecting the greater hydration of the former sugar. This effect may influence both access to and interaction with receptor sites.

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1 Shallenberger, R.S., Acree, T.E., and Guild, W.E., J. Fd Sci. *30* (1965) 560.
2 Pangborn, R.M., and Gee, S.C., Nature *191* (1961) 810.
3 Birch, G.G., Crit. Rev. Fd Sci. Nutr. *8* (1976) 57.
4 Birch, G.G., and Lee, C.K., in: Sweetness and Sweeteners, p. 95. Eds G.G. Birch, L.F. Green and C.B. Coulson. Applied Science, London 1971.
5 Birch, G.G., and Mylvaganam, A.R., Nature *260* (1976) 632.
6 Birch, G.G., in: Biochemistry of Taste and Olfaction, p. 163. Eds R.H. Cagan and M. Kare. Academic Press, London 1981.

7 Lemieux, R.U., and Brewer, J.T., in: Carbohydrates in Solution, p. 121. Adv. Chem. Series 117. Ed. H.S. Isbell, 1973.
8 Birch, G.G., and Lindley, M.G., J. Fd Sci. *38* (1973) 1179.
9 Suami, T., Ogawa, S., Takata, M., Yasuda, K., Suga, A., Takei, K., and Uematsu, Y., Chem. *6* (1985) 719.
10 Shallenberger, R.S., and Acree, T.E., Nature *216* (1967) 480.
11 Lindley, M.G., and Birch, G.G., J. Sci. Fd Agric. *26* (1975) 117.
12 Martin, O.R., Korpii-Tommola, S.L., and Szarek, W.A., Can. J. Chem. *60*, 1857.
13 Birch, G.G., and Munton, S.L., Chem. Senses *6* (1981) 45.
14 Birch, G.G., Latymer, Z., and Holloway, M., Chem. Senses *5* (1980) 63.
15 Birch, G.G., and Catsoulis, S., Chem. Senses *10* (1985) 325.

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A rapid HPLC method for determination of adenylate energy charge[1]

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Summary. A simple procedure is described for separation and analysis of adenine nucleotides in tissue extracts, utilizing anion exchange HPLC. Determination of AMP, ADP, and ATP takes 10 min per sample.
Key words. HPLC adenine nucleotide evaluation; adenylate energy charge; stress index.

Several biochemical indices for the assessment of sublethal stress in marine organisms have been described with the aim of evaluating the biological impact of pollutants[3]. Among these is the Adenylate Energy Charge (AEC), defined by Atkinson and Walton[4] as the ratio:

$$AEC = (ATP+0.5\ ADP)/(ATP+ADP+AMP)$$

where ATP, ADP, and AMP are the molar concentrations of adenosine-5'-triphosphate, -diphosphate, and -monophosphate. Advantages and limitations of AEC determinations in pollution monitoring have been reviewed[5]. It appears that the most important limitations are related to the methodology, in particular to the lack of analytical procedures allowing an accurate determination of the three nucleotides and suitable for routine analysis of a large number of samples. We describe a simple method which utilizes ion-exchange HPLC for rapid separation of adenine nucleotides, followed by spectrophotometric determination at 260 nm. This procedure was successfully employed in the evaluation of AEC in tissues of *Mytilus galloprovincialis* Lam. and *Palaemon elegans* L., organisms widely utilized in environmental studies.

Materials. Adenine nucleotides were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade and purchased from Riedel & De Haene (Hannover, West Germany). The liquid chromatograph was a Hewlett-Packard, model 1084, equipped with a high-speed spectrophotometric detector, model 1040, and facilities for data processing. The 100 × 4.6 mm analytical column was protected by a 30 × 4.6 guard column. Both were packed with 10 µm Aquapore anion exchanger (Brownlee Labs, Santa Clara, CA).

Sample preparation. The animal (*Mytilus galloprovincialis* Lam. or *Palaemon elegans* L.) was killed and immediately dissected: required organs or tissues were rapidly excised and pressed in liquid nitrogen. After evaporation of the nitrogen, the wet weight was determined and the material homogenized with 3 vols of ice-cold 7% (w/v) trichloroacetic acid (TCA) in a refrigerated homogenizer. After centrifugation at 35,000 × g for 10 min at 0 °C, the supernatant was removed. The pellet was resuspended in TCA, centrifuged again and the washing added to the first supernatant. The extract was neutralized with 5 M K_2CO_3 and filtered (Milliporefilter type Millex-HV, 0.45 µm). Some aliquots of the TCA extracts were also extracted five times with diethylether to remove the acid. This procedure which has been utilized by various authors[6], gave the same chromatographic results as the simplified procedure that was routinely adopted for the analysis.

Chromatographic procedure. Separation of AMP, ADP, and ATP was carried out using a gradient of phosphate buffer at pH 8.0. The mobile phase consisted of distilled water (A) and 200 mM phosphate buffer (B). Both solutions were filtered through 0.22 µm prior to use and the column was equilibrated with 3% B. After injection of the sample, the column was eluted with 3% B for 1.5 min, then B was increased up to 95% at 8 min and held up to 10 min. The gradient was then returned to 3% B and the initial conditions were restored in 5 min. Flow rate was 2 ml/min. Buffers and column temperatures were 35 °C. Detection was performed at 260 nm. Peak identities were confirmed by coelution with standards. Peak purity was verified by recording the UV-spectra at the apex and at the inflection points on the up- and down-slopes of each peak: after normalization, the three spectra should be superimposable. Quantitative measurements were carried out by injection of standard solutions at known concentrations. A molar absorption coefficient of 15,400 cm^{-1} M^{-1} was assumed for adenine nucleotides.

Results and discussion. Figure A shows the separation of a standard mixture of AMP, ADP, and ATP: the nucleotides are completely separated in 10 min, and thus there is an improvement with respect to reported HPLC separations[7], often obtained by reverse-phase or ion-paired chromatography. Furthermore, ion-exchange separation does not requires high-purity organic solvents or chemical modifiers. A chromatogram of an extract of the digestive gland of *Mytilus galloprovincialis* Lam. is reported in figure B: the nucleotides are completely separated from other components and the gradient used does not cause an appreciable baseline drift. It should be pointed out that the peaks corresponding to AMP, ADP, and ATP, extracted from several organs or tissues of the mussel *Mytilus galloprovincialis* Lam. and the shrimp *Palaemon elegans* L. were absolutely pure when checked spectrophotometrically as described under Meàthods. To ensure a long lifetime for the analytical column, the guard column was replaced every 150 injections, when analyzing 10-µl samples.

Reproducibility was tested by injecting the same extract ten times; the variation coefficients of retention times and nucleotide concentrations are reported in the table. Stability of adenine

Reproducibility of HPLC determination of adenine nucleotides. Mean values, standard errors, and coefficients of variation for retention times and concentrations obtained from 10 determinations carried out on the same extract of muscular tissue of *Palaemon elegans* L.

	AMP		ADP		ATP	
	R.T. min	Conc. µM	R.T. min	Conc. µM	R.T. min	Conc. µM
Mean value	3.75	374.10	5.82	359.9	7.52	1852.1
Standard error	0.03	20.87	0.007	3.71	0.007	13.20
Coeff. variation	0.008	0.056	0.001	0.010	0.001	0.007