Structural Functions of Taste in the Sugar Series VII: Taste Properties of Ketoses

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Abstract □ The preferable structural feature for sweetness is a lipophilic moiety, *e.g.*, a five- or six-membered ring with a polar substituent containing an A–H/B system outside the ring. In ketoses, this unit is probably the 1,2-glycol. If a third feature (the lipophilic, γ, site) is required for the attainment of optimum sweetness and if it is C-6 in ketoses, then this site can accommodate quite a large constituent, both above and below the plane of the ring. The removal of the hydroxyl group from the C-6 hydroxymethyl substituent to yield the 7-deoxy derivatives causes bitterness, thus implicating the primary hydroxymethyl group with bitterness. Therefore, the creation of lipophilic site(s) in the sugar ring causes the realignment of the sugar molecule on the taste receptor surface. The disturbance of the proposed A–H/B system, *e.g.*, the removal of the C-2 hydroxyl group, causes the ring α-glycol unit (most likely the ring C-3 and C-4 hydroxyl groups) to function as the A–H/B system.

Keyphrases □ Structure–activity relationships—sweetness of ketoses related to structural functions □ Ketoses—structural functions related to sweetness □ Sweetness—related to structural functions of ketoses □ Taste physiology—structural functions of ketoses related to sweetness

In considering the chemical characteristics of a biologically active compound, it is necessary to know at how many points in the molecule significant interaction can occur and also the nature of the molecules to which the compound may attach itself. Even with simple molecules, a study of the behavior of analogs is often necessary to determine which groupings are responsible for union with the receptor sites and which are responsible for the reactions concerned with function.

In studying the phenomenon of sweetness, evidence already published (1–3) suggests that a direct binding of saporific molecule with taste bud protein is the criterion of magnitude. Molecular patterns have been educed (1, 4–6) to account for the sweet taste of such a diverse group of chemical compounds as sugars, amino acids, synthetic sweetening compounds such as saccharin, cyclamates, dulcin, and 2-amino-4-nitrobenzenes, salts of beryllium, *etc.*

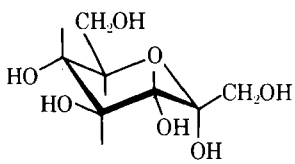
Although these patterns can explain the varying sweetness of sugars, it is nonetheless remarkable that such a vast difference in sweetness of sugars exists at all. Sucrose and fructose, the two sweetest simple sugars known, are not very sweet when compared with artificial sweeteners like saccharin and cyclamates, despite possessing several α-glycol groups, each satisfying Shallenberger's requirement of a geometrically suitable A–H/B system. Only one such system is present in the artificial sweeteners.

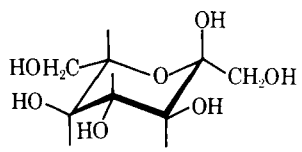
In previous publications (7, 8), it was suggested that although a high anionic character or the ability to form hydrogen bonds is an important prerequisite for sweetness, the preferable structural feature may be a five- or six-membered ring with a polar substituent including an A–H/B system outside the ring. Most sugars studied are aldoses and as such do not have this structure. Ketoses, on the other hand, would be ideal molecular models for such studies. Pentuloses, hexuloses, and various octuloses are found in various naturally occurring materials, *e.g.*, honey (9), passion fruits, avocado pears, and dried roots of primrose (*Primula officinalis* Jacq.) (10–12). Therefore, it is of particular fundamental interest that their sensory properties should be evaluated. Accordingly, this paper describes the structural functions of taste in a number of ketoses.

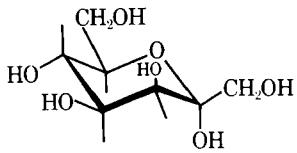
EXPERIMENTAL

Materials—The following sugars were used as received: D-*gluco*-heptulose[1], D-*manno*-heptulose[1], D-*talo*-heptulose[1], L-*gluco*-heptulose[1], L-*galacto*-heptulose[1], L-*allo*-heptulose[1], D-glycero-D-*gulo*-octulose[1], D-glycero-L-*gluco*-octulose[1], sedoheptulose hexaacetate[1], D-*altro*-3-heptulose[2] (coriose), D-*gluco*-heptulose[2], 7-deoxy-D-*altro*-heptulose[3], and 7-deoxy-L-*galacto*-heptulose[3]. All other parent sugars used were crystalline materials[4] (99% pure).

Tasting of Crystalline Substances—Panelists were selected and


α-D-*gluco*-heptulose


α-L-*gluco*-heptulose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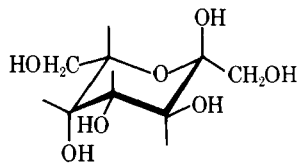
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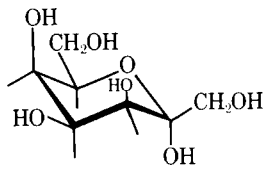
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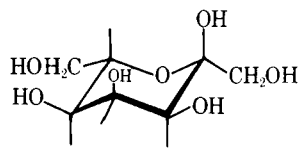
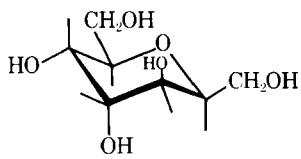
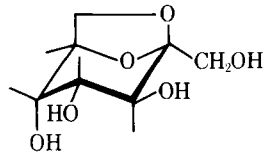

α-L-*allo*-heptulose


α-D-*altro*-heptulose (sedoheptulose)


sedoheptulosan

[1] Gift of Dr. Emmanuel Zissis, National Institutes of Health, Bethesda, Md.
[2] Gift of Professor T. Okuda, Okayama University, Japan.
[3] Gift of Dr. T. Ito, Meiji Seika Kaishi Ltd. Research Laboratories, Japan.
[4] Obtained from British Drug Houses, Chemicals, Poole, Dorset, England; Koch-Light Laboratories, Colnbrook, Berks, England; Pfanstiehl Laboratories, Ill.; Senn Chemicals, Switzerland; and T. I. Sas and Son Ltd., England.

trained from College personnel according to a previous publication (13). Each panelist was asked to place a few milligrams of each substance (finely powdered crystals) on the tongue and to comment whether it was trace sweet (tr. S), sweet (S), intensely sweet (SS), trace bitter (tr. B), bitter (B), or intensely bitter (BB). Tastelessness was designated zero (0). Panelists were presented with standards for comparison: *myo*-inositol, trace sweet, rating 0–0.35; methyl-α-D-glucopyranoside, sweet, rating 0.35–0.70; and sucrose, intensely sweet, rating 0.70 and above[5].

The decisions listed in Tables I and II are those obtained in at least 75% of the total judgments, with each panelist carrying out duplicate tasting sessions. Eight subjects formed the panel. Each panelist was asked to taste all substances once at each session, rinsing with distilled water between substances and pausing for a short interval (usually about 1 min) before passing on to the following substance.

Difference Testing—For comparing the intensity of sweetness, the two-sample difference testing method was used; each taster was asked to select the sweeter sample. At each sitting, the two possible orders of presentation, AB and BA, were presented, the tasting being carried out in duplicate. No retasting was allowed.

The number of correct selections of the sweeter sample within a pair in excess of chance expectation is defined in terms of standard deviations, σ, where:

$$\sigma = \frac{n - Np}{\sqrt{Npq}} \qquad \text{(Eq. 1)}$$

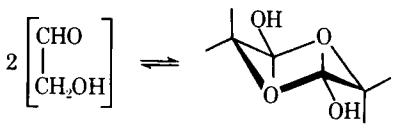
where N = total number of judgments, n = number of correct selections, p = probability of "correct" selection by chance, and q = probability of "incorrect" selection by chance. The level of significance was then obtained by referring to the table compiled by Yule and Kendall (14).

Rinsing with distilled water between substances and pausing 1 min before passing on to the following substances were required. Swallowing was permitted.

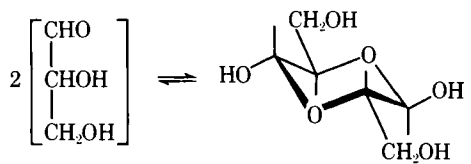
RESULTS AND DISCUSSION

The use of equilibrated solutions of reducing sugars in taste–structure studies has been criticized since, at equilibrium, solutions of such substances consist of a complex mixture of anomers. Therefore, it is not possible to relate taste with a particular structure (15). However, if such sugars are tasted in the crystalline state, the sweet taste appears to be perceived prior to any appreciable conformational change due to mutarotation. Electrophysiological studies (16) showed that the interval between initial stimulation of the receptors and the report of a reaction was 0.02–0.06 sec, and Kiesow (17) reported that oral response for sweet taste was about 0.5 sec. Thus, it could be reasonably assumed that the structure of the sugar (when tasted in the "solid" form) would be in its preferred conformation when stimulation occurred.

Dihydroxyacetone, like glyceraldehyde and glycolic aldehyde, exists as a crystalline dimeric compound (Schemes I–III) but gradually dissociates to the monomer in dilute aqueous solution (18). This fact was confirmed recently (19) using GC, NMR, and mass spectrometric analyses. Glyceraldehyde in its monomeric form is tasteless (20), but



Scheme I—Glycolic aldehyde



Scheme II—Glyceraldehyde

[5] Sucrose has a sweetness of SS (intensely sweet on this scale), but it is two or three orders of magnitude of sweetness lower than perillaldehyde *anti*-oxime and P-4000 and artificial sweeteners such as saccharin and cyclamates.

Table I—Taste Properties of Ketoses[a]

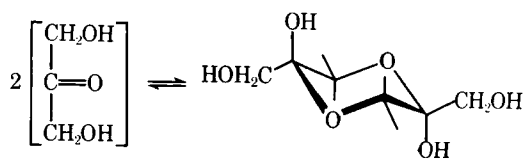
Sugar	Sweetness	Bitterness
Hexulose		
L-Sorbopyranose	S	0
D-Fructopyranose	SS	0
D-Tagatose	S	0
2-Deoxy-D-fructopyranose	S	0
Heptulose		
D-*gluco*-Heptulose	S	0
D-*manno*-Heptulose	S	0
D-*talo*-Heptulose	S	0
D-*altro*-Heptulose (sedoheptulose)	SS	0
Sedoheptulosan	SS	0
L-*gluco*-Heptulose	S	0
L-*galacto*-Heptulose	S	0
L-*allo*-Heptulose	S	0
1-Deoxy-D-*manno*-heptulose	S	0
D-*altro*-3-Heptulose	0-tr	0
7-Deoxy-D-*altro*-heptulose	S	B
7-Deoxy-L-*galacto*-heptulose	S	B
Octulose		
D-Glycero-L-*gluco*-octulose	S	0
D-Glycero-D-*gulo*-octulose	S	0
Disaccharide		
Maltulose (4-*O*-α-D-glucopyranosyl D-fructofuranose)	S	0
Lactulose (4-*O*-β-D-galactopyranosyl D-fructofuranose)	S	0
Palatinose (6-*O*-α-D-glucopyranosyl D-fructofuranose)	S	0
Turanose (3-*O*-α-D-glucopyranosyl D-fructopyranose)	SS	0

[a] Sucrose has a sweetness of SS (intensely sweet on this scale), but all of these values are two or three orders of magnitude of sweetness lower than perillaldehyde *anti*-oxime and P-4000 and artificial sweeteners such as saccharin and cyclamates.

the dimer is reported to be sweet (21). In the solid form, dihydroxyacetone was observed to be much sweeter than glyceraldehyde (which had only trace sweetness). This result, however, is not surprising since the dimeric form of dihydroxyacetone has an α-glycol system outside the ring (whereas glyceraldehyde does not), a structural feature we have maintained to be the preferable structure for sweetness and a feature found in β-D-fructopyranose and artificial sweeteners like saccharin and cyclamates.

The results in Table I give further proof of this hypothesis. All ketoses tested were consistently sweeter or of about the same sweetness as the corresponding aldoses and were never bitter. That they were consistently not bitter strongly suggests that binding to the taste receptor could be analogous to that in β-D-fructopyranose, *i.e.*, the 1,2-glycol unit functioning as the A–H/B system. Whether or not this 1,2-glycol unit does constitute the A–H/B system could, however, depend on the overall conformation concerned. Possibly, for molecules in the $_4C^1$ conformation, which are true analogs of an identical system in β-D-fructopyranose, the 1,2-glycol functions as the A–H/B, but this may not be the case in the $^4C_1$ conformation since, for example, 1-deoxy-D-*manno*-heptulose is only very slightly less sweet than D-*manno*-heptulose.

It was proposed previously (7, 22) that the hydrophilic–lipophilic ratio of a compound governs its binding behavior. A lipophilic moiety, *e.g.*, a five- or six-membered ring, with a polar substituent containing an A–H/B system outside the ring seems the preferable structural



Scheme III—Dihydroxyacetone

Table II—Comparison of Sweetness by Paired Comparison Test

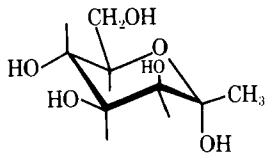
Sugar	Number Correct	Number Incorrect	Total	Percent Correct		Number Correct in Excess of Expectancy in σ Values	Significance Designation
				Obtained	Expected		
D-*manno*-Heptulose *versus* 1-deoxy-D-*manno*-heptulose—D-*manno*-heptulose sweeter	10	0	10	100	50	3.16	Very highly significant, $p < 0.001$
Palatinose *versus* isomaltose—palatinose sweeter	10	0	10	100	50	3.16	Very highly significant, $p < 0.001$
Maltulose *versus* maltose—maltulose sweeter	8	2	10	80	50	1.89	Significant, $p < 0.05$
Lactulose *versus* lactose—lactulose sweeter	9	1	10	90	10	2.53	Highly significant, $p < 0.01$

feature. Thus, for the attainment of optimum sweetness, a third binding site (as a lipophilic center) probably is required (23–25). Kier (4) proposed a dispersion or hydrophobic site, γ, located at about 3.5 Å from atom A of AH and about 5.5 Å from B, while Shallenberger[6] suggested that the "stereochemistry of this 'greasy' (γ) site, in relation to AH and B is defined by D-glucose where AH is the C-4 hydroxyl group, B the C-3 oxygen atom and γ is C-6." The center of the orbital A is quoted to be 2.9 Å from the center of B and 3.1 Å from γ, while the center of B is 5.13 Å from γ. These values are not very different from those of Kier (4).

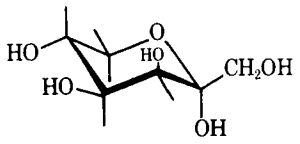
The intense sweetness of β-D-fructopyranose is significant because it possesses a ring methylene grouping, thus giving the sugar an element of lipoid character not present in all other sugars tested (except in tagatose, which exists in the stable $^4C_1$ conformation). The sweetness of heptuloses coupled with the pure sweet taste of octuloses clearly suggests that this greasy γ site, as proposed by Shallenberger and Kier, can accommodate quite a large substituent, both above and below the plane of the sugar ring. A greater insight into the molecular basis of the pharmacophore now appears to be available from the work of Holtje and Kier (26).

However, the removal of the hydroxyl group from the C-6 hydroxymethyl substituent to give the 7-deoxy derivatives (Table I) causes the development of bitterness, thus implicating the primary hydroxymethyl group with bitterness. This finding is in agreement with an earlier prediction (7), and it supports the contention that the presence of freshly available lipophilic sites causes the alignment of the sugar differently from the parent sugar, thus eliciting bitterness (8). Also, the slightly lower sweetness intensity of 1-deoxy-D-*manno*-heptulose compared to D-*manno*-heptulose (using paired comparison testing) (Table II) could be rationalized on this basis; *i.e.*, the removal of the C-1 hydroxyl group of *manno*-heptulose possibly causes the ring C-3 and C-4 (C-4 and C-5, respectively, in the normal carbohydrate nomenclature) hydroxyl groups to act as the A–H/B system, as in aldoses.

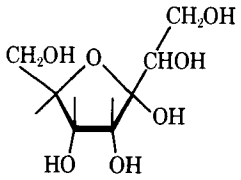
The sweetness intensity of 1-deoxy-D-*manno*-heptulose and β-D-arabinose (analogs of β-D-fructopyranose) demonstrate the importance of the C-1 hydroxyl group. This finding, coupled with the results of Birch and Lindley (27) on the importance of the C-2 hy-



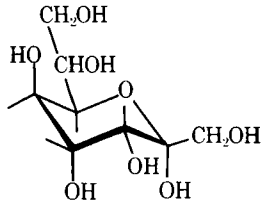
1-deoxy-α-D-*manno*-heptulose



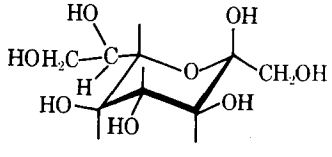
α-D-tagatose



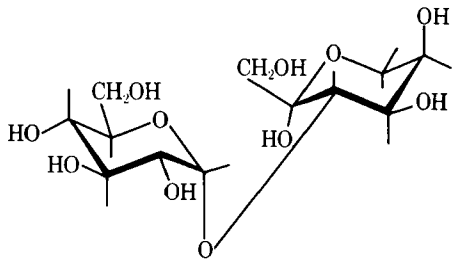
α-D-*altro*-3-heptulose



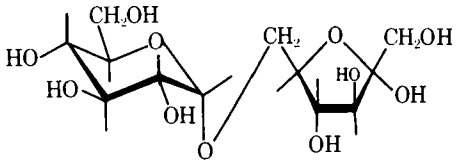
D-glycero-α-D-*gulo*-octulose



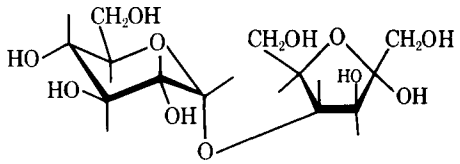
D-glycero-α-L-*gluco*-octulose



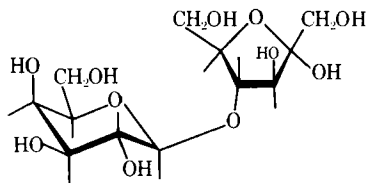
turanose (3-*O*-α-D-glucopyranosyl β-D-fructopyranose)



palatinose (6-*O*-α-D-glucopyranosyl α-D-fructofuranose)



maltulose (4-*O*-α-D-glucopyranosyl α-D-fructofuranose)



lactulose (4-*O*-β-D-galactopyranosyl α-D-fructofuranose)

[6] R. S. Shallenberger, New York State Agriculture Experimental Station, Cornell University, Geneva, N.Y., personal communication, 1975.

droxyl group in ketoses, thus offers an unequivocal support for the validity of the proposed A–H/B system for intense sweetness. The virtual tastelessness of D-*altro*-3-heptulose is not very surprising, being due to the free rotation of the primary hydroxyethyl grouping.

The sensory evaluation of β-D-fructopyranose and α-L-sorbopyranose implicated the configuration of the C-5 hydroxyl group as being of unique importance in sweetness. In fructose, the participation of this (axial) hydroxyl group with the ring oxygen in hydrogen bonding leaves the C-2 hydroxyl group free (this being also sterically disposed to hydrogen bond the ring oxygen) to exert the maximum effect on sweetness intensity (27). The intense sweetness of sedoheptulose could similarly be due to the availability of the C-3 axial hydroxyl group to hydrogen bond with the ring oxygen, a situation analogous to mannose (1).

A recent study on the binding behavior of reducing disaccharides (28) showed that the nonreducing glycosyl residue was involved in the binding to the taste receptor site. In disaccharides containing a terminal ketose, the presence of a 1,2-glycol unit in the ketose residue changes the binding behavior. Just as in the monosaccharide ketoses, this glycol unit preferentially becomes the A–H/B system. Thus, lactulose, turanose, palatinose, and maltulose are considerably sweeter than their corresponding aldose analogs (using the paired comparison test ($p < 0.05$) (Table II). Furthermore, the exceptional sweetness of turanose (3-*O*-α-D-glucopyranosyl D-fructopyranose) (judged to be nearly as sweet as sucrose) suggests that the C-3 hydroxyl group is not critical in eliciting sweetness. This finding, together with the lower sweetness intensity of maltulose and lactulose (both having 1 → 4 linkages), seems to suggest that the C-4 hydroxyl group (*i.e.*, ring C-3) is more critical than the C-3 hydroxyl group (*i.e.*, ring C-2 of the fructose ring) as regards sweetness.

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