Physicochemical Stereospecificity in Taste Perception of α-D-Mannose and β-D-Mannose

THE anomers α- and β-D-mannose differ structurally only in that H and OH at the C_1 atom of the pyranose ring are interchanged. In spite of this apparently minor difference in structure, it has been suggested that the ambiguity of taste perception of D-mannose is due to actual differences in taste between the two anomers of this substance; the evidence presented in support of this view, however, was indirect because insufficient pure β-D-mannose was available for direct taste tests on that anomer[1]. Sufficient pure β-r -mannose was therefore prepared for a direct and statistically reliable comparison. It was found that the subjects reliably reported the α-anomer as sweet and the β-anomer as bitter. As before, the equilibrium mixture was found to be an ambiguous stimulator.

The β-anomer was prepared by dissolving 26 g of α-D-mannose in 15 ml. of water with heating. Glacial acetic acid (15 ml.) was added to the cool syrup and the resulting solution was filtered through finely divided charcoal under reduced pressure. The funnel was rinsed with 10 ml. of glacial acetic acid and the solution was seeded with about 1 mg of β-D-mannose[2]. A little crystal growth was noted after 24 h. Crystallization was allowed to proceed for 6.5 yr with occasional agitation, at which time sufficient (about 2 g) crystalline material was available for analysis and testing. The crystals were removed mechanically from the mother liquid, washed successively with glacial acetic acid and with absolute ethanol, and dried *in vacuo*. Infrared spectra were obtained using the KBr pellet technique. Peaks were found at 1170, 936, 899, 860, 772, 730, 620, and 448 cm^{-1}, corresponding to the characteristic peaks of β-D-mannose[2,3]. For the α-anomer peaks were found at 971, 960, 915, 885, 831, and 812 cm^{-1}, corresponding to the known characteristic peaks of this substance[2,3]. The equilibrium mixture used was that prepared by Steinhardt *et al.*[1], the spectrum of which confirmed that it contained both the α- and β-anomers in the equilibrium ratio.

Subjects for the taste discrimination tests were five males and five females, aged 19–24 yr. Subjects were instructed not to eat, smoke, nor to drink anything but water for 2 h before the taste tests. Each was given six to ten preliminary trials in which taste stimulators of known characteristics were presented for the purpose of subject screening and familiarization with experimental procedures. Dextrose and a dilute quinine solution were used to establish standards for sweet and bitter, respectively, and α-D-mannose containing a trace of β-D-mannose served as an ambiguous stimulus. The criteria for sensitivity and acceptability of the subject were the responses "sweet" to the dextrose and "bitter" to the quinine solution. After acceptable preliminary trials, each subject was given thirteen test trials which included four α-D-mannose samples, four β-D-mannose samples, four equilibrium mixture samples, and one dextrose sample as a control. The samples were arranged in different random orders for each subject and were administered in the solid state to avoid mutarotation. Weights were 14 mg of α-D-mannose, 6 mg of β-D-mannose, 20 mg of the equilibrium mixture, and 20 mg of dextrose. (The amounts of the α- and β-anomers correspond to the ratio in which they occur in the equilibrium mixture.) Tests were conducted using a double blind procedure. On each trial the experimenter placed the test material on the tongue of the subject who was then instructed to manipulate the substance between the tongue and the hard palate. During the test the subjects recorded their taste sensations as a function of time by depressing one of four appropriately labelled buttons on a response indicator and an operations recorder was used to record the responses "no taste," "sweet," "bitter," and "indistinguishable taste". Following each trial, after the "no taste" button had been pressed, the subject rinsed his mouth with water and was given a short rest period before the next trial.

The responses of two subjects were not included in the experimental results: One failed to follow the instructions not to smoke before the test and the other tasted the dextrose control as "bitter".

Table 1 Frequency of Taste Responses to Anomers of D-Mannose and to Dextrose

	Bitter	Sweet	Sweet-bitter	Indistinguishable	Total
β-D-Mannose	30 (93.75%)	0 (0.0%)	2 (6.25%)	0 (0.0%)	32
α-D-Mannose	0 (0.0%)	30 (93.75%)	0 (0.0%)	2 (6.25%)	32
Equilibrium mixture	23 (71.88%)	2 (6.25%)	5 (15.62%)	2 (6.25%)	32
Dextrose	0 (0.0%)	8 (100.0%)	0 (0.0%)	0 (0.0%)	8

Subjects' responses to the substances tested are summarized in Table 1. Each of the responses was coded +1 for sweet, −1 for bitter, and 0 for indistinguishable taste. The mean coded scores for the α- and β-anomers were +0.9375 and −0.9375, respectively. For purposes of statistical analysis it was assumed that the 64 observations made were randomly and independently chosen from 16 normal populations with equal variance. The analysis showed that the specific anomer administered significantly influenced the subjects' reports of their taste sensations ($f=787.5$, d.f. 1/7, $P<0.005$). Thus, subjects very reliably reported a sweet sensation in the presence of α-D-mannose and a bitter sensation in the presence of β-D-mannose.

It seems that the so-called "ambiguity" of taste associated with D-mannose is due solely to the slight difference in the molecular structure between α-D-mannose and β-D-mannose. These results confirm the conjecture that a high degree of physicochemical stereospecificity is exhibited by taste receptors.

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fi⁻ R Factors giving Chloramphenicol Resistance

ALTHOUGH many *fi*⁺ R factors confer resistance to chloramphenicol, *fi*⁻ R factors seldom do. Watanabe *et al.*[1] stated in 1964 that all the naturally occurring *fi*⁻ R factors which they had studied lacked chloramphenicol resistance. In our laboratory, no *fi*⁻ R factor giving chloramphenicol resistance has been observed among several hundred drug-resistant strains of enteric bacteria. In 1968 Watanabe, Furuse and Sakaizumi[2] reported an *fi*⁻ R factor (S-a) from *Shigella* which conferred chloramphenicol resistance at about the same level as *fi*⁺ R factors. Aoki, Egusa, Ogata and Watanabe[3] this year described R factors from the fish pathogen *Aeromonas liquefaciens*, which were all *fi*⁻ and some of which carried chloramphenicol resistance determinants.

Watanabe[4] found that factor S-a exhibited no superinfection immunity with any of several *fi*⁺ or *fi*⁻ R factors.