Reports

Taste-Structure Correlation with α-D-Mannose and β-D-Mannose

Abstract. The so-called ambiguity of taste perception of D-mannose has been traced to actual differences in taste between the two anomers of this substance. Preliminary data indicate strongly that the α-anomer is sweet (sucrose-like) and the β-anomer is bitter (quinine-like). The difference in taste is attributed to the slight difference in structures of the anomers.

It is well known that D-mannose is an ambiguous taste stimulator; that is, some subjects will taste it as sweet only, some as bitter only, and many as bitter-sweet or sweet-bitter in succession (*1*). We have hypothesized that the basic reason for the taste ambiguity is that D-mannose, as commonly obtained, usually consists of a mixture of two very slightly different molecules, α-D-mannose and β-D-mannose (Fig. 1), in different proportions depending upon the method of preparation, and that, because of the difference in structures, each of the anomers might be supposed to stimulate one or the other of the two types of taste. A further reason for ambiguity lies in the difficulty of reporting unfamiliar tastes, especially in cases in which two different substances are being tasted at the same time.

Ideally, the experiment should have consisted of testing the separate pure anomers for possible taste differences. However, because of the difficulty of separating the pure β-anomer from the equilibrium mixture (*2*), it was found possible at the present time to test only the α-anomer and the equilibrium mixture of anomers.

The α-anomer was obtained in almost pure form from the Fisher Scientific Company under the name of "d(+)Mannose" (*sic*). The specific rotation of this material was found to be $[\alpha]_D^{20} = +29.0°$ as compared with the value of +29.34° calculated from the data of Isbell and Pigman (*3*) for pure α-D-mannose. The equilibrium mixture of anomers was prepared by dissolving 20 g of α-D-mannose in 100 ml water and permitting to stand with occasional agitation for 5 hours. The water was then removed by vacuum evaporation at room temperature. The residue was redissolved in excess 95-percent ethyl alcohol, and the alcohol was allowed to evaporate at room temperature over a 12-hour period. The mannose crystallized in fine crystals and in excellent yield. After vacuum drying, the specific rotation of the product was found to be $[\alpha]_D^{20} = +14.2°$, in agreement with the value of +14.20° calculated from the data of Isbell and Pigman (*3*) for the equilibrium mixture, which consists of 69.3 percent α-D-mannose and 30.7 percent β-D-mannose. The mutarotation velocity constant (*4*) is 17.3×10^{-3} min^{-1} at 20°C (*3*).

To determine whether or not a difference in taste of the two substances could be perceived, a group of four subjects was given preliminary taste training with the materials. Under the informal conditions of familiarization, it was found that the α-anomer invariably elicited a response of "sweet," while the equilibrium mixture invariably stimulated a "sweet-then-bitter" response. A controlled test of taste-structure correlation was then carried out.

Each of the four subjects was given 10 taste tests of the α-anomer and the α-β equilibrium mixture, the order of testing being governed by four different random orders taken from Gellerman (*5*). Each subject was blindfolded at the time of the test and was not in the company of the other subjects while being tested. On each trial about 20 mg of the solid sample was placed on the middle of the subject's tongue (*6*). The subject then manipulated the sample between the tongue and the hard palate in such a way that the sample (which almost immediately dissolves in the saliva) reached all portions of the tongue. After 10 seconds the subject reported which kind of perceptual experience he had undergone: (i) a simple taste that did not change qualitatively with time, or (ii) a taste experience that did change with time. (The first experience, it will be noted, is that corresponding to the pure α-anomer, while the second experience is that corresponding to the equilibrium mixture.) After each trial the subject rinsed his mouth with distilled water and returned to the waiting room until the other three subjects had completed their next trials. The interval between tests for the same subject was about 2 minutes.

Of the total of 40 trials, 39 responses

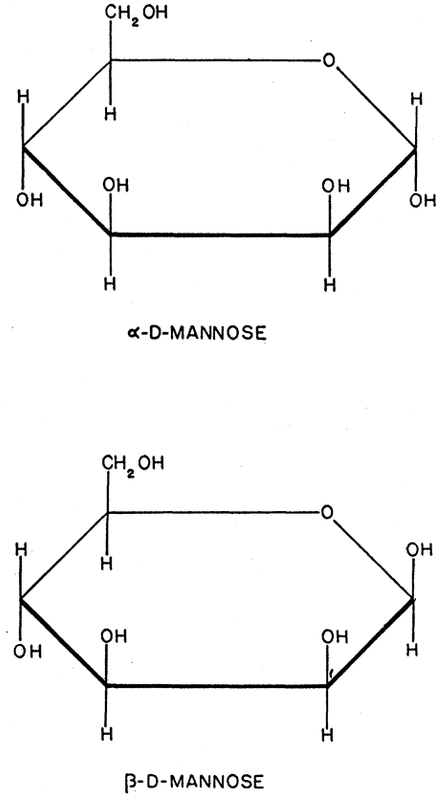

Fig. 1. Molecular structures of the α- and β-anomers of D-mannose.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

identified the sample by noting perceptual experience which was in agreement with that which was predicted solely on the basis of the composition of the sample in comparison with the results of the familiarization training. In the one case in which this was not so, the response was almost immediately changed by the subject. However, as this response change occurred after the 10-second deadline, the response was not scored as an agreement. The null hypothesis can be rejected with a P value considerably beyond the .01 value.

A further test was made with 12 subjects who had no previous taste training experience, had never tasted mannose before, and who had no knowledge of the experiment nor any intimation of the results expected. The same taste testing procedure was followed as outlined above, except that each subject was asked to tell in his own words the nature of the taste experience. Each subject was given only one test.

In the 12 tests, 10 responses were in agreement with predictions based on the composition of the administered sample ($P = .019$ one-tailed). One subject reported the equilibrium mixture as bitter only, while another stated that the α-anomer was initially sweet, but started to change "somewhat" within a few seconds and became "unpleasant" about 1 or 2 *minutes* later. (It should be noted that the taste threshold for the α-anomer appears to be significantly higher than that of the β-anomer; this may account in itself for the first disagreement. That is, the mild taste of the α-anomer was not perceived by this subject. In the second disagreement, the relatively long time that elapsed before the "unpleasant" report leads one to surmise that sufficient mutarotation may have occurred so that sufficient β-anomer may have been produced to stimulate an "unpleasant" response.)

It would appear that the extremely slight difference in molecular structure between the α- and β-anomers of D-mannose is sufficient to cause a rather dramatic difference in the taste of these substances. [It is interesting to note that in a similar but more complicated case of a disaccharide, 6-α-D-glucopyranosyl-D-glucose is sweet but its anomer, 6-β-D-glucopyranosyl-D-glucose, is bitter (*7*). Furthermore, Pangborn and Gee (*8*) have shown that the α-anomers of the monosaccharides D-glucose and D-galactose are sweeter than the β-anomers of these compounds.] It is apparent that a high degree of physicochemical stereospecificity must be exhibited by taste receptors.

This work is being continued by efforts to separate sufficient pure β-D-mannose so that a direct comparison of the tastes of these two anomers can be made (*9*).

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References and Notes

1. R. J. Williams, *Biochemical Individuality* (Wiley, New York, 1956), p. 128; *The Human Frontier* (Harcourt Brace, New York, 1946), pp. 71–72.
2. Harriet L. Frush, National Bureau of Standards, Washington, D.C., personal communication.
3. H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards* **18**, 141 (1937).
4. C. S. Hudson, *J. Am. Chem. Soc.* **26**, 1067 (1904).
5. L. W. Gellerman, "Chance orders of alternating stimuli in visual discrimination experiments," *J. Genet. Psychol.* **42**, 207 (1933).
6. The sample was administered in the solid state to obtain as intense a taste stimulation as possible. Through preliminary experiments it had been established that solution concentration does not appear to be a relevant variable in this case, the only effect of concentration being that of altering the intensity of taste. The effect of mutarotation in the α-anomer makes administration of the sample as the aqueous solution extremely inconvenient, as a new sample must be prepared rapidly before each individual test; sufficient β-anomer to affect the taste is developed within a few minutes after the preparation of an aqueous α-anomer solution.
7. L. N. Ferguson and A. R. Lawrence, *J. Chem. Educ.* **35**, 436 (1958).
8. R. M. Pangborn and S. C. Gee, *Nature* **191**, 810 (1961).
9. This work was supported in part by a grant from the Hollins College Faculty Travel and Research Fund, and in part under a grant from the National Science Foundation under the provisions of the Undergraduate Research Participation Program.
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Decrease of Acetylcholinesterase Activity along Peripheral Nerves

Abstract. Acetylcholinesterase activity was examined in segments of some peripheral nerves anatomically accessible throughout their length. It decreases linearly with distance from cell bodies, the activity of the terminal part of the nerve being about 30 to 40 percent lower than that of its most proximal part.

Although electrophysiological and morphological features of nerve fibers are constant throughout the length of unbranched parts of axons, evidence is accumulating that the biochemical organization of axons fails to exhibit similar uniformity. Gradients of composition and metabolic activity have been described in normal peripheral nerves (*1*, *2*). Indirect evidence of changes of biochemical properties along axons is provided by many peripheral neuropathies, in which the distal parts of nerves appear to be more vulnerable to circulating toxic agents than the proximal parts.

If a substance is produced in the perikaryon and metabolized in the cell processes, it has to be transferred from one site to the other. For these substances the axon, besides being the possible seat of metabolism, constitutes a traffic line. At each level of the axon the concentration of a substance originating from the perikaryon is determined by the rates of arrival, of utilization, and of transport to more distal portions of the axon. The distribution of the substance in the length of the axon would thus provide some information about its fate in the neuron.

With this in view we investigated the distribution of acetylcholinesterase in the peripheral nerves. The known physiological action of this enzyme occurs at the nerve endings, and there are manifold indications (*3*, *4*) that, like many other enzymes (*5*), it is synthesized in the perikarya.

In view of large differences in enzyme activity of individual neurons (*6*), the assembly of the same fibers, without admixture of bundles which join or leave the nerve at various levels, should be analyzed throughout. Moreover, all fibers should be approximately equally long and accessible throughout most of their length. In these respects most nerves to extremities are inconvenient for an analysis of biochemical gradients. In a preliminary note (*2*) we described a proximo-distal gradient of total cholinesterase activity in the phrenic nerve of dog in which these difficulties are relatively small.

In the present experiments acetylcholinesterase activity, which provides a clearer picture of neuronal distribution, was determined at various levels of the phrenic and the hypoglossal nerves and of the nerve to the levator scapulae, originating at C_5. These nerves run without anastomoses and either have fibers of fairly uniform length or may be rendered homogeneous in this respect by rejection of separable fascicles shorter than the