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Responses of the Ant *Lasius niger* to Various Compounds Perceived as Sweet in Humans: a Structure–Activity Relationship Study

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Abstract

A behavioural study on the ant *Lasius niger* was performed by observing its feeding responses to 85 compounds presented in a two-choice situation (tested compound versus water control or sucrose solution). Among these compounds, only 21 were phagostimulating: six monosaccharides (D-glucose, 6-deoxy-D-glucose, L-galactose, L-fucose, D-fructose, L-sorbose), four derivatives of D-glucose (methyl α-D-glucoside, D-gluconolactone and 6-chloro- and 6-fluoro-deoxy-D-glucose), five disaccharides (sucrose, maltose, palatinose, turanose and isomaltose), one polyol glycoside (maltitol), three trisaccharides (melezitose, raffinose and maltotriose) and two polyols (sorbitol and L-iditol). None of the 16 non-carbohydrate non-polyol compounds tested, although perceived as sweet in humans, was found to be active in ants. The molar order of effectiveness of the major naturally occuring compounds (melezitose > sucrose = raffinose > D-glucose > D-fructose = maltose = sorbitol) is basically different from the molar order of their sweetness potency in humans (sucrose > D-fructose > melezitose > maltose > D-glucose = raffinose = sorbitol). On a molar basis melezitose is in *L. niger* about twice as effective as sucrose or raffinose, while D-glucose and D-fructose are three and four times less effective, respectively, than sucrose or raffinose. From a structure–activity relationship study it was inferred that the active monosaccharides and polyols should interact with the ant receptor through only one type of receptor, through the same binding pocket and the same binding residues, via a six-point interaction. The high effectiveness of melezitose in *L. niger* mirrors the feeding habits of these ants, which attend homopterans and are heavy feeders on their honeydew, which is very rich in this carbohydrate.

Introduction

Since the pioneering work of Schmidt (Schmidt, 1938), few studies on the feeding responses of ants towards carbohydrates (or related compounds) have been published (Ricks and Vinson, 1970; Vander Meer and Merdinger, 1990; Vander Meer *et al.*, 1995). The purpose of the present work has been to investigate, through an extensive study of 85 compounds (37 monosaccharides and derivatives, 16 oligosaccharides and derivatives, 16 polyols and 16 non-carbohydrate non-polyol compounds known to taste sweet in humans), the feeding responses of the ant *Lasius niger* (Hymenoptera: Formicidae) in order to analyse the possible structure–activity relationships that can be deduced from the data thus obtained.

Materials and methods

Materials

Lasius niger ants (black or common garden ants), living in their natural 'field' conditions, were used. This species is very common and widespread in Europe. The three colonies used in the present work were located in the area of Lyon (France). Tests were conducted during four successive years from mid-April to mid-June, in outdoor conditions and close to a nest entrance (<0.50 m). At the beginning of each annual session an artificial foraging area regularly visited by ants was created by using as initial bait a 200 g/l sucrose solution (placed in a multiwell plate with 48 wells of 25 µl).

Compounds in aqueous solution were placed on a polypropylene multiwell plate (GenNunc module no. 2-32298) provided with 48 wells of 25 µl. The dimensions of the wells (diameter 3 mm, depth 4 mm) were proportional to ant size (4–5 mm), which limited the number of insects (4–6) capable of feeding simultaneously at the same well. The short distance between the well sides (~6 mm) favoured methodical exploration of the wells by the ants. In order to reduce possible formation of pheromone trails and ensure a random comparison, wells were alternately filled with the test fluid and the water control.

All carbohydrates, derivatives and polyols were supplied by Sigma (except for α-D-glucose and L-threitol, which were obtained from Aldrich) and were tested at a concentration of 1 mol/l (pilot experiments with more dilute solutions gave noticeably lower recruitment, which increased the test duration and, as a result, fluid evaporation).

The reducing carbohydrates were dissolved in water ~24 h before the test in order to ensure completion of the

mutarotational equilibrium (except for α-D-glucose and β-D-glucose, which were tested in freshly prepared solutions to avoid α/β interconversion).

The 16 non-carbohydrate non-polyol compounds tested were obtained from commercial sources or synthesized according to published data. Each compound was tested: (i) at a concentration that was equivalent, on a sweetness basis, to that of a 50–100 g/l sucrose solution in humans; (ii) at its highest possible concentration. The compounds used (with their origin and their concentrations) were as follows: acesulfame-K (from Supelco; 5×10^{-3} and 1.6 mol/l); alitame [according to Brennan and Hendrick (Brennan and Hendrick, 1981); 0.15×10^{-3} and 0.39×10^{-3} mol/l]; aspartame (from Sigma; 1.7×10^{-3} and 34×10^{-3} mol/l); cyanosuosan [according to Tinti *et al.* (Tinti *et al.*, 1982); 0.64×10^{-3} and 2.1×10^{-3} mol/l]; sodium cyclamate (from Sigma; 15×10^{-3} and 1 mol/l); dulcin (from Interchim, France; 4.4×10^{-3} and 6.9×10^{-3} mol/l); magap (*N*-[(*S*)-2-methylhexanoyl]-α-L-glutamyl 5-aminopyridine 2-carbonitrile) [according to Nofre and Tinti (Nofre and Tinti, 1994); 0.014×10^{-3} and 0.11 mol/l]; neohesperidin dihydrochalcone (from Sigma; 0.16×10^{-3} mol/l); neotame [according to Nofre and Tinti (Nofre and Tinti, 1996, 2000); 26×10^{-6} and 27×10^{-3} mol/l]; perillartine (from Sigma; 1.8×10^{-3} and 3.6×10^{-3} mol/l); saccharin (from Aldrich; 2.2×10^{-3} and 18.5×10^{-3} mol/l); stevioside (from Fluka; 0.5×10^{-3} and 1.5×10^{-3} mol/l); sucralose (from Redpath, Canada; 0.4×10^{-3} and 0.63 mol/l); sucrononate (*N*-[*N*-cyclononylamino(4-cyanophenylimino)-methyl]-2-aminoethanoic acid) [according to Nofre *et al.* (Nofre *et al.*, 1990); 1.5×10^{-6} and 29.2×10^{-6} mol/l]; suosan [according to Petersen and Müller (Petersen and Müller, 1948); 0.63×10^{-3} and 2.1×10^{-3} mol/l]; superaspartame [*N*-(4-cyanophenylcarbamoyl)-L-aspartyl-L-phenylalanine methyl ester] [according to Nofre and Tinti (Nofre and Tinti, 1987); 23×10^{-3} mol/l].

Methods

Compounds were declared 'effective' when total consumption of the test solutions (24 × 25 µl, i.e. 600 µl/plate) was achieved within 1 h after arrival of the first forager; note that within this short 1 h test period spontaneous evaporation of the solution was always low (<10% of the initial volume). Results were validated by repeating the same procedure with the other two colonies. Note that the water control was not consumed by the ants, except during certain specific climatic conditions, such as high temperatures or prolonged drought; in this case the tests were discarded and repeated during more favourable weather conditions.

Compounds were declared 'ineffective' when the test solutions were not consumed after the 1 h test period and when no ant recruitment was observed. Results were validated by replicating the same procedure with the other two colonies.

The relative effectiveness of several of these compounds was evaluated in a 'pairwise' comparison with reference to sucrose. The evaluation was based on the observation that the rate of consumption of sucrose increases with its concentration; for example, we observed that the individual intake doubles when the sucrose concentration increases from 50 to 100 g/l, as already noted by Josens *et al.* (Josens *et al.*, 1998) in *Camponotus mus*. In practice, a sucrose solution of 100 g/l (0.292 mol/l) and the test solution were presented on the same plate (replacing the water control by the 100 g/l sucrose solution). When both the fluids elicited high level recruitment, tests were carefully monitored and interrupted as soon as the most preferred fluid was completely consumed; note that owing to the high number of ants recruited on the plate, lowering of the same fluid in the wells was uniform. Each compound was successively tested at concentrations in fractions or multiples of that of sucrose (i.e. at 0.0146, 0.292, 0.584, 0.876, 1.168 or 2.336 mol/l) until the consumption rate of the test fluid matched the consumption rate of the 100 g/l sucrose reference. Results were ascertained after three coherent tests had been observed in each colony.

Results

At the beginning of the annual session the recruitment level, the feeding time and the individual intake (i.e. the mean volume ingested by one ant during its visit to the plate) were assessed. Thus, with a 100 g/l sucrose solution (0.292 mol/l), ~100 (or more) ants were found on the plate, most staying motionless with their mouthparts in continuous contact with the fluid for an average time of feeding not less than 30–45 s, while many others were competing for access to the solutions; the individual volume intake, which was evaluated in the course of a separate experiment, was ~1.5 µl/ant at this sucrose concentration. With a 50 g/l sucrose solution (0.146 mol/l) the number of ants recruited was lower (20–50), with the feeding time unchanged and individual intake lowered to ~0.8 µl. With a 20 g/l sucrose solution (0.058 mol/l) consumption of the fluid was still observed, but with a brief feeding time and weak recruitment (10–20 ants). With more diluted sucrose solutions ant recruitment was very weak, but fluid consumption was still observed up to and including 5 g/l sucrose (0.0146 mol/l); lower concentrations of sucrose were always ineffective under our experimental conditions. Note that the lowest concentration value of sucrose still giving a feeding response in ants (~5 g/l or $\sim 14.6 \times 10^{-3}$ mol/l) is close to the value of the human recognition threshold for sucrose (~5.8 g/l or $\sim 17 \times 10^{-3}$ mol/l) (Amerine *et al.*, 1965), which reflects an approximately equal effectiveness between the physiological responses of ants and humans towards sucrose.

Among the 27 monosaccharides examined only six elicited a phagostimulating activity in *L. niger*: four aldohexoses (D-glucose, 6-deoxy-D-glucose, L-galactose and L-fucose) and two ketohexoses (D-fructose and L-sorbose) (Table 1). Note that for D-glucose only the α-anomer

Table 1 Responses of *L. niger* to monosaccharides and derivatives

Compound[a]	Response[b]
Aldohexoses	
D-Allose	–
D-Altrose	–
2-Deoxy-D-glucose	–
3-Deoxy-D-glucose	–
6-Deoxy-D-glucose	+
D-Fucose	–
L-Fucose	+
D-Galactose	–
L-Galactose	+
D-Glucose	+
α-Anomer	+
β-Anomer	–
L-Glucose	–
D-Gulose	–
D-Idose	–
D-Mannose	–
L-Rhamnose	–
D-Talose	–
Ketohexoses	
D-Fructose	+
D-Psicose	–
L-Sorbose	+
D-Tagatose	–
Aldopentoses	
D-Arabinose	–
2-Deoxy-D-ribose	–
D-Lyxose	–
D-Ribose	–
D-Xylose	–
L-Xylose	–
Ketopentose	
D-Xylulose	–
Monosaccharide derivatives	
6-Chloro-6-deoxy-D-glucose	+
1-Fluoro-1-deoxy-α-D-glucose	–
6-Fluoro-6-deoxy-D-glucose	+
D-Gluconolactone	+
3-*O*-Methyl-D-glucose	–
Methyl α-D-glucoside	+
Methyl β-D-glucoside[c]	–
Methyl α-D-mannoside	–
Sedoheptulose anhydride	–
5-Thio-D-glucose	–

[a]All compounds were tested at a concentration of 1 mol/l and, for the reducing carbohydrates, in mutarotational equilibrium (except for α- and β-D-glucose, which were tested as pure compounds).

[b]An effective response (+) means that the test solution elicits important recruitment of ants and total consumption of the fluid (24 × 25 μl, i.e. 600 μl/plate). A response is considered as ineffective (–) when the test solution does not elicit any characteristic ant recruitment and is not consumed.

[c]Note that methyl β-D-glucoside is not sweet in humans.

(α-D-glucopyranose), when tested pure in a freshly prepared solution, is active, while the pure β-anomer is inactive under the same conditions. Among the 10 monosaccharide derivatives examined only four were phagostimulants (methyl α-D-glucoside, D-gluconolactone and 6-chloro and 6-fluoro-6-deoxy-D-glucose).

Table 2 Responses of *L. niger* to oligosaccharides and derivatives (polyol glycosides)

Compound[a]	Structure	Response[b]
Disaccharides		
Cellobiose[b]	Glc*p*β1–4Glc	–
Isomaltose	Glc*p*α1–6Glc	+
Lactose	Gal*p*β1–4Glc	–
Lactulose	Gal*p*β1–4Fru	–
Maltose	Glc*p*α1–4Glc	+
Melibiose	Gal*p*α1–6Glc	–
Palatinose	Glc*p*α1–6Fru	+
Sucrose	Glc*p*α1–2βFru*f*	+
Trehalose	Glc*p*α1–1αGlc*p*	–
Turanose	Glc*p*α1–3Fru	+
Trisaccharides		
Maltotriose	Glc*p*α1–4Glc*p*α1–4Glc	+
Melezitose	Glc*p*α1–2βFru*f*3–1αGlc*p*	+
Raffinose	Gal*p*α1–6Glc*p*α1–2βFru*f*	+
Tetrasaccharide		
Stachyose	Gal*p*α1–6Gal*p*α1–6Glc*p*α1–2βFru*f*	–
Polyol glycosides		
Lactitol	Gal*p*β1–4Sorbitol	–
Maltitol	Glc*p*α1–4Sorbitol	+

[a]All compounds were tested at a concentration of 1 mol/l and, for the reducing carbohydrates, in mutarotational equilibrium.

[b]Note that cellobiose is not sweet in humans.

Of the 16 oligosaccharides and derivatives investigated eight were able to elicit a feeding response in the black ant: five disaccharides (sucrose, maltose, isomaltose, palatinose and turanose), a polyol glycoside (maltitol) and three trisaccharides (melezitose, raffinose and maltotriose) (Table 2).

Among the 16 polyols tested (namely: a diol, ethylene glycol; a triol, glycerol; three tetrols, erythritol, D-threitol and L-threitol; four pentols, adonitol, D-arabitol, L-arabitol and xylitol; four hexols, galactitol, L-iditol, mannitol and sorbitol; two cyclohexols, myo-inositol and quebrachitol; a polyol anhydride, 1,5-anhydro-D-mannitol) only two were phagostimulants, sorbitol (D-glucitol) and L-iditol.

It should be noted that among the 16 non-carbohydrate non-polyol compounds tested (namely acesulfame-K, alitame, aspartame, cyanosuosan, sodium cyclamate, dulcin, magap, neohesperidin dihydrochalcone, neotame, perillartine, saccharin, stevioside, sucralose, sucrononate, suosan and superaspartame) none was active in *L. niger*, although all are known to be perceived as sweet by humans.

The approximate effectiveness values in *L. niger* of 13 selected active compounds, on a molar basis and with regard to a 100 g/l sucrose solution, are reported in Table 3. We observed that under these conditions melezitose is approximately twice as effective as sucrose and raffinose is as

Table 3 Approximate effectiveness in *L. niger* of some carbohydrates and derivatives relative to sucrose; comparison with their sweetness potencies in humans relative to sucrose

Compound[a]	Isoeffective concentration[b] (mol/l)	Relative effectiveness in ants[c]	Relative sweetness potency in humans[d]
Melezitose	0.146	2	0.35
Sucrose	0.292	1	1
Raffinose	0.292	1	0.25
Maltitol	0.584	0.5	0.5
D-Glucose	0.876	0.33	0.25
6-Deoxy-Dglucose	0.876	0.33	0.20
L-Fucose	0.876	0.33	0.15
D-Fructose	1.168	0.25	0.50
Maltose	1.168	0.25	0.33
Palatinose	1.168	0.25	0.25
Sorbitol	1.168	0.25	0.25
L-Sorbose	1.168	0.25	0.25
Turanose	1.168	0.25	0.25–0.5
Methyl α-D-glucoside	2.336	0.125	0.25

[a]Reducing carbohydrates were tested in mutarotational equilibrium.

[b]For each compound tested increasing concentrations (0.146, 0.292, 0.584, 0.876, 1.168 and 2.336 mol/l) were successively compared to a 100 g/l (0.292 mol/l) sucrose solution presented simultaneously (Figure 1). The concentration of a compound is considered as 'isoeffective' when it elicits a consumption quantitatively similar to that of a 100 g/l sucrose solution.

[c]Note that the high effectiveness of melezitose and raffinose in *L. niger* has already been reported by Schmidt (1938).

[d]Sweetness potency in humans is given on a molar basis relative to a 20 g/l (0.0584 mol/l) sucrose solution.

effective as sucrose [the high effectiveness of melezitose and raffinose in starved *L. niger* has already been noted by Schmidt (1938)], while D-glucose and D-fructose (in their equilibrium isomeric mixtures) are about three and four times less active, respectively, than sucrose. If we consider only the major naturally occuring compounds, their molar order of effectiveness in *L. niger* is as follows: melezitose > sucrose = raffinose > D-glucose > D-fructose = maltose = sorbitol. Their molar order of effectiveness in ants is therefore essentially different from their molar order of sweetness in humans, which is: sucrose > D-fructose > melezitose > maltose > D-glucose = raffinose = sorbitol (Table 3).

Discussion

From the above results certain relationships between the structures of the compounds tested and their phagostimulating activity in *L. niger* can be deduced.

For the active aldohexoses (such as D-glucose and 6-deoxy-D-glucose) our data permit us to infer that these monosaccharides interact with the sugar receptor of *L. niger*

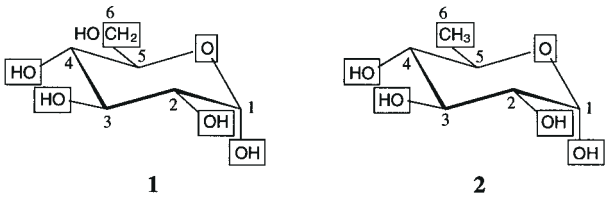

Figure 1 The putative interaction points (boxed groups and atoms) of (1) α-D-glucopyranose, (2) 6-deoxy-α-D-glucopyranose.

through six interaction points, namely through their 1-, 2-, 3- and 4-OH groups, their heterocyclic oxygen atom and their 6-CH_2 (or 6-CH_3) group (see Figure 1.1 and 1.2). For example, the 1α-OH hemiacetal group of D-glucopyranose must be required since only the α-anomer of D-glucopyranose (Figure 2.1) is effective and not the β-anomer (Figure 2.2); moreover, the oxygen atom of the 1α-OH group must be involved (possibly as a hydrogen bond acceptor) since methyl α-D-glucoside (Figure 2.3) and D-gluconolactone (Figure 2.4) are both active. As the anomer distribution at mutarotational equilibrium is ~36% α-D-glucopyranose and ~64% β-D-glucopyranose and as the phagostimulating activity of D-glucose at equilibrium is about one-third that of sucrose (Table 3), the calculated effectiveness value of α-D-glucopyranose should be near that of sucrose (~0.9 times that of sucrose on a molar basis); as a result, we deduce that sucrose should interact with the ant receptor in large part through its glucopyranosyl moiety. The three equatorial 2-, 3- and 4-OH alcohol groups of D-glucose must be involved since 2-deoxy-D-glucose (Figure 2.5), D-mannose (Figure 2.6), 3-deoxy-D-glucose (Figure 2.7), D-allose (Figure 2.8) and D-galactose (Figure 2.9) are ineffective. The 5-O heterocyclic oxygen is essential to the interaction, since 5-thio-D-glucose (Figure 2.10), a structural analogue of D-glucose, is inactive. Finally, only the 6-OH alcohol group of D-glucose can be deleted without affecting feeding stimulation: in fact, 6-deoxy-D-glucose (Figure 2.11) is as effective as D-glucose (Table 3). However, lack of the 6-CH_2OH group, as found in D-xylose (Figure 2.12), precludes a feeding response, which substantiates a contribution of the 6-CH_2 (or 6-CH_3) group to the interaction with the receptor, probably through a steric interaction.

For the active ketohexoses (D-fructose and L-sorbose) we assume that these compounds should also interact with the ant receptor through a six-point interaction, namely through their 1-, 2-, 3- and 4-OH groups, their heterocyclic oxygen atom and their 6-CH_2 group (see Figure 3.1 and 3.2), via the same sugar receptor type and the same receptor binding sites as those used by the active aldohexoses. For example, for β-D-fructopyranose (which is the major constituent of the D-fructose isomeric mixture in aqueous solution) the 1-CH_2OH alcohol group (Figure 4.1) must be required since D-arabinose (Figure 4.2) is ineffective. Its

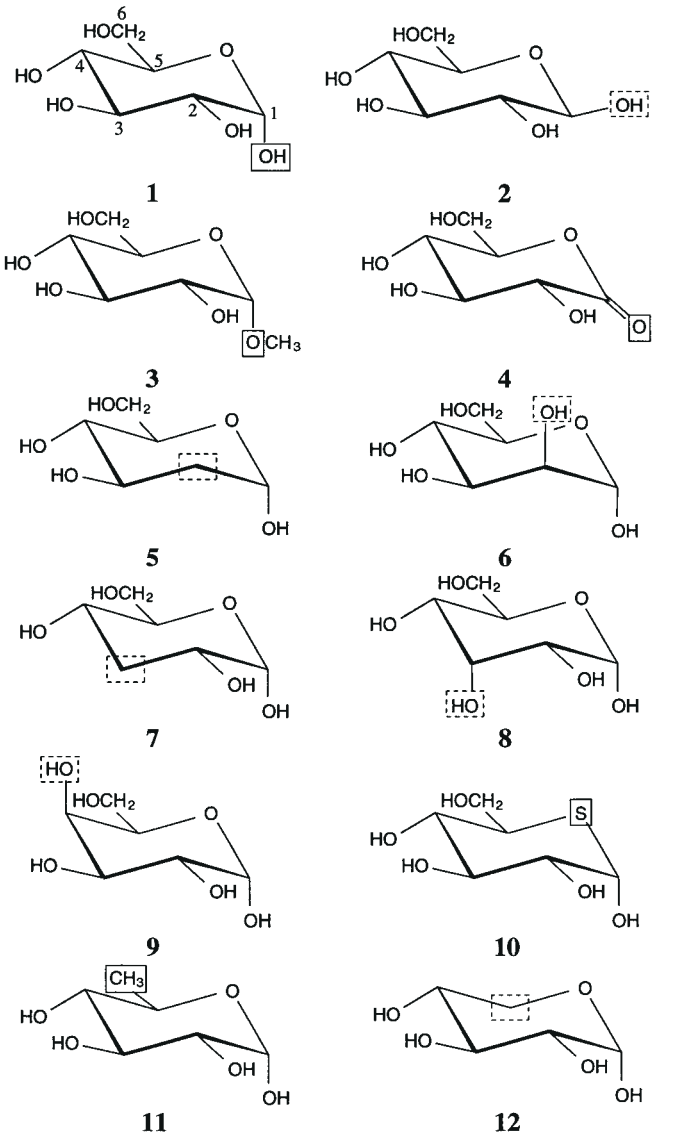

Figure 2 (1) α-D-Glucopyranose; (2) β-D-glucopyranose; (3) methyl α-D-glucopyranoside; (4) D-gluconolactone; (5) 2-deoxy-α-D-glucopyranose; (6) α-D-mannopyranose; (7) 3-deoxy-α-D-glucopyranose; (8) α-D-allopyranose; (9) α-D-galactopyranose; (10) 5-thio-α-D-glucopyranose; (11) 6-deoxy-α-D-glucopyranose; (12) α-D-xylopyranose.

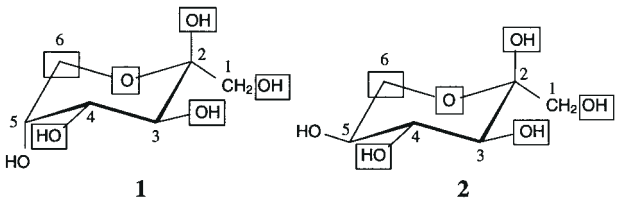

Figure 3 The putative interaction points (boxed groups and atoms) of (1) β-D-fructopyranose, (2) α-L-sorbopyranose.

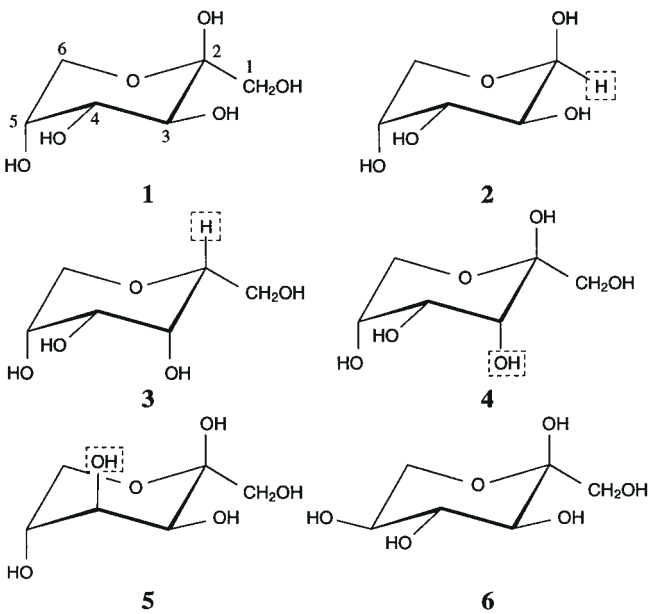

Figure 4 (1) β-D-Fructopyranose; (2) β-D-arabinopyranose; (3) 1,5-anhydro-D-mannitol; (4) β-D-psicopyranose; (5) β-D-tagatopyranose; (6) α-L-sorbopyranose.

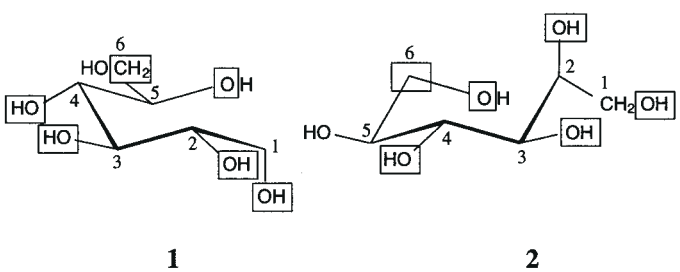

Figure 5 The putative interaction points (boxed groups and atoms) of (1) sorbitol (D-glucitol), (2) L-iditol.

2β-OH hemiacetal group is necessary since 1,5-anhydro-D-mannitol (Figure 4.3) is inactive. Its two equatorial 3- and 4-OH alcohol groups must also be involved since D-psicose (Figure 4.4) and D-tagatose (Figure 4.5) are not phagostimulants. As L-sorbose (Figure 4.6), which differs from D-fructose only in the configurational position of the 5-OH alcohol group (compare Figure 4.1 and 4.6), has the same effectiveness as D-fructose (Table 3), the 5-OH group of these molecules must not take part in the interaction. Finally, we assume, by analogy with D-glucopyranose, that their 6-O heterocyclic oxygen and their 6-CH_2 group should also be involved in the interaction. As the proportion of β-D-fructopyranose is ~73% in the isomer mixture at equilibrium and as the phagostimulating activity of D-fructose is, at equilibrium, about one-fourth that of sucrose (Table 3), the calculated effectiveness value of β-D-fructopyranose

should be approximately one-third (~0.33) that of sucrose on a molar basis, lower than that of α-D-glucopyranose (~0.9 times that of sucrose).

For the polyols the phagostimulating activities of sorbitol (Figure 5.1) and L-iditol (Figure 5.2) are probably the consequence of their ability to correctly position in space six groups able to play the role of the six interaction points postulated for the active aldohexoses and ketohexoses; compare, for example, the formulae of sorbitol (Figure 5.1) and α-D-glucopyranose (Figure 1.1) and those of L-iditol (Figure 5.2) and α-L-sorbopyranose (Figure 3.2). The inactivity of mannitol and galactitol, two epimers of sorbitol (D-glucitol), argue in favour of this view.

The foregoing structure–activity relationship data are consistent with the view that in *L. niger* all the effective monosaccharides (such as D-glucose and D-fructose) interact: (i) with only one type of carbohydrate gustatory receptor; (ii) through only one mode of interaction, i.e. via the same binding pocket and the same binding residues of the carbohydrate receptor. The mode of interaction of D-glucose and D-fructose in *L. niger* appears: (i) to be closely related to that reported in the acalyptrate fly *Drosophila melanogaster*, in which both these hexoses appear to interact with the receptor through the same mode of interaction (Wieczorek and Wolff, 1989); (ii) to be basically different from that recognised in the calyptrate flies (such as the blowfly *Phormia regina* or the fleshfly *Boettcherisca peregrina*), in which these two hexoses are known to interact with the receptor through two distinct modes of interaction (Evans, 1963; Omand and Dethier, 1969; Hanamori *et al.*, 1974; Shimada *et al.*, 1974; Shimada, 1987).

In conclusion, *L. niger*, which is polyphagous, like many ant species, is above all known for its preference for collecting nectar and attending aphids (El-Ziady and Kennedy, 1956; Kiss, 1981). Its gustatory preferences for certain specific carbohydrates, as noted in the present work, reflect its feeding choices, such as its marked predilection for the carbohydrates usually found in nectar (sucrose, D-fructose, D-glucose and raffinose) and in homopteran honeydew (mainly melezitose, but also its hydrolysis products, turanose, D-glucose and D-fructose) (Hussain *et al.*, 1974; Owen, 1978; Belliardo *et al.*, 1979; Lombard *et al.*, 1987; Hendrix *et al.*, 1992).

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