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Department of Chemistry, University of Agriculture, 31-120 Kraków, Mickiewicz Avenue 21, Poland

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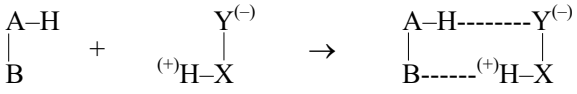
PM3 quantum chemical population analysis in the ground state was performed for sucrose and galactosucrose, their 8 chlorodeoxy derivatives and threonine as a moiety of sweet taste receptor. QSAR (Quantitative Structure-Activity Relationship) studies on RS_j relative sweetness of these sugars is carried out based on calculated quantum chemical parameters derived from independent particle model. It excellently explains very high sweetness of 1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose (RS_j = 2000), 1′,4,6,6′-tetrachloro-1′,4,6,6′-tetradeoxy-galactosucrose (RS_j =1000) and other chlorosugars. Presented QSAR analysis brings rather a limited contribution of the G_4(1′-CH_2) and G_1(6′-CH_2) dispersion fructofuranose subsites to Nofre-Tinti sweetener and reveals a great activity of E_4(Cl-1′) and E_1(Cl-6′) charge-transfer fructofuranose subsites in this sweetener. The latter subsites have a character of strong *n*-electron donors. According to Brand and Feigin, such a property may result in substantial increase of probability of a stimulus-gated ion channel transduction scheme for sweet taste.

S: E_1 and E_4 sweetener activity in chlorodeoxy sucrose derivatives, E_1 and E_4 sweetener activity in chlorodeoxy galactosucrose derivatives, QSAR computational model of Nofre-Tinti theory on sugar's high sweetness

Very high sweetness of sucrose/galactosucrose chlorodeoxy derivatives (Fig. 1a) has not been yet satisfactory explained in biorganic chemistry and sweet taste theories. The 1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose (RS_j = 2000) is about five times more sweet than saccharin and aspartame, whereas galactosucrose (RS_j < 0.2) is almost nonsweet [1]. Sweet taste of sugars is usually accompanied by pairs of functional groups, such as hydroxyl groups, aminogroups and ether oxygen. They were called "glycophores" by Shallenberger [2–4]. The sweet taste-eliciting group for the sugars was a glycol, (-CHOH-CHOH-) unit. According to Shallenberger, sweetener site pair of glycophore was marked by AH and B, where A and B are usually hydroxyl oxygen or amino nitrogen atoms. The sweet taste receptors have analogous $XH^{(+)}$, $Y^{(-)}$ dipoles. They interact with AH,B glycophore and form two hydrogen bonds:



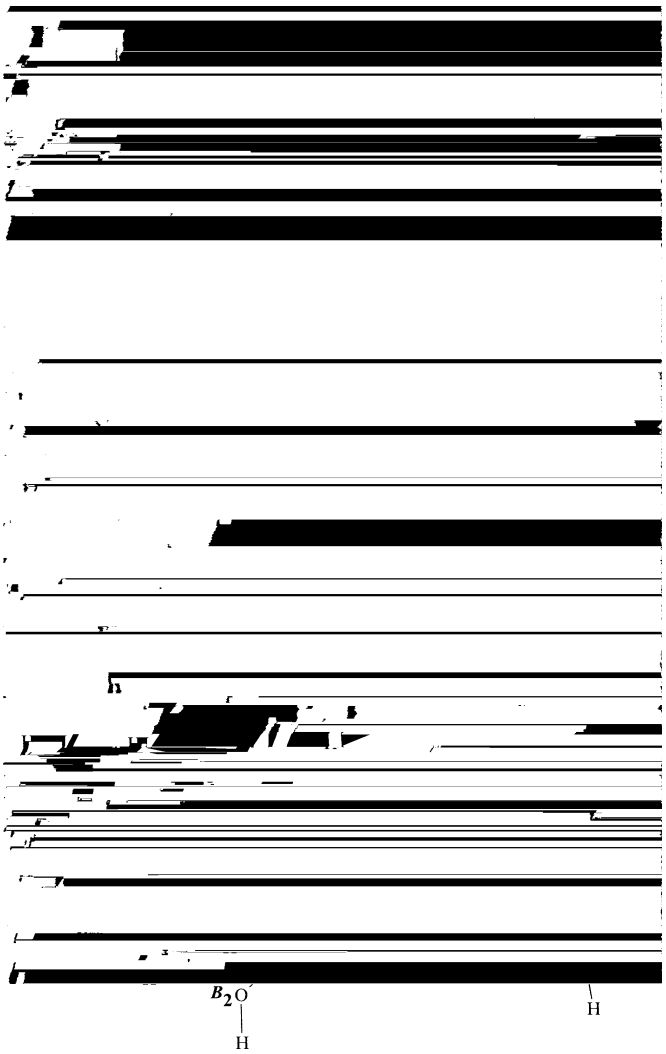
(1)

Glycophore Receptor Complex

The measurement of RS_j relative sweetness of j-th sugar is carried out in relation to sucrose (RS_0

All three types of receptors have their equivalents in sweet taste biochemistry. Several studies have shown that sweet taste stimuli enhanced the production of the cyclic AMP [10,11]. It suggests the prevailing hypothesis that cellular response is brought about by a receptor-mediated, G_s protein-coupled, AMP second messenger. Structure of sweet taste receptor is considered as similar to the structure of other G-protein receptors [8]. It shows polipeptide chain, distinguished by seven transmembrane domain segments, TM I – TM VII helices, forming a pocket, in which the sweet ligands are binded. Thus, sweetness of sucrose and other usual sugars belongs to β-adrenergic receptor scheme. On the other hand, very sweet substances have another transduction path mechanism. Artificial sweeteners, saccharin and the guanine sweetener SC-45647 induced the production of IP_3, when the epithelium from the vallate papilla of the rat was used as the tissue source [12]. This second messenger points rather for α_1-adrenergic receptor scheme.

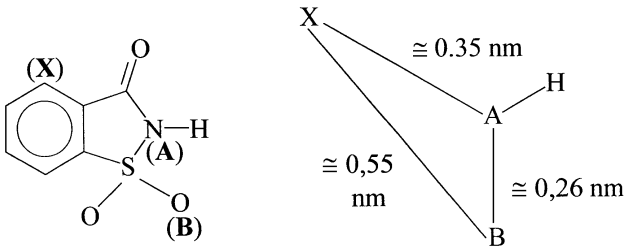
Under this biochemistry progress, Nofre and Tinti [13] have formulated Multipoint Attachment Theory (MPA), which may explain a binding of sweet ligands with the receptor in transmembrane pocket. According to this theory, sucrose (Fig. 1b) and galactosucrose (Fig. 1c) have 12 sweetener subsites. Glucopyranose AH_1, AH_2, XH_1 and XH_2 (hydrogen atoms in O-H groups) have positive atomic net charges and they may interact by electrostatic forces or they are acceptors of receptor *n*-electron pairs. Subsites B_1 and B_2 as oxygen atoms of 4-OH and 3-OH glucopyranose groups have



F **1.** a.) Numeration of carbon atoms (1–6,1′–6′) in *R* – substituted sucrose/galactosucrose derivatives. The *R*-substituent positions form the following derivatives:

	R_1	R_2	R_3	R_4	R_5	
1.)	H	Cl	OH	Cl	Cl	1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose
2.)	H	Cl	Cl	Cl	Cl	1′,4,6,6′-tetrachloro-1′,4,6,6′-tetradeoxy-galactosucrose
3.)	H	Cl	OH	OH	OH	1′,4-dichloro-1′,4-dideoxy-galactosucrose
4.)	OH	H	OH	Cl	Cl	1′,6′-dichloro-1′,6′-dideoxy-sucrose
5.)	Cl	H	Cl	Cl	Cl	1′,4,6,6′-tetrachloro-1′,4,6,6′-tetradeoxy-sucrose
6.)	OH	H	OH	OH	Cl	6′-chloro-6′-deoxy-sucrose
7.)	OH	H	OH	Cl	OH	1′-chloro-1′-deoxy-sucrose
8.)	Cl	H	OH	OH	OH	4-chloro-4-deoxy-sucrose
9.)	OH	H	OH	OH	OH	sucrose
10.)	H	OH	OH	OH	OH	galactosucrose

b.) Location of Nofre-Tinti sweetener subsites in sucrose molecule.
c.) Location of these subsites in galactosucrose.



F **2.** Location and dimensions of the Kier's triangle glycophore in saccharin.

some biochemical observations. The 1-methyl-4,6-dichloro-4,6,-dideoxy-D-galactopyranoside is sweet taste inhibitor (!) [16], which is effective in preventing the accumulation of cyclic AMP, due to stimulation by sugars. On the other hand, substituting 1-methyl group in this pyranoside by 2′-(1′,6′-dichloro-1′,6′-dideoxy-fructofuranoside) group, we obtain the mentioned very sweet 1′,4,6,6′-TClG (RS_j = 1200). Hence, one may suppose that high sweetness of the latter chlorosugar results from Cl-1′ and Cl-6′ fructofuranose chlorine atoms, described by Nofre and Tinti as E_4 and E_1 sweetener subsites. Thus, we are bound to throw off the pure electrostatic model, and try to form a new mechanism of sweetness.

It requires to build more wide theory than Kier's triangle, based on quantum population analysis, especially on energy and structure of molecular orbitals in frontier region. Nofre-Tinti model is qualitative and it cannot point the active sweetener subsites among 14 presented. Simple quantum chemical parameters of the ground state, like MO's energies and atomic net charges, include many hidden informations about the reception process and active sweetener subsites as well as sweet taste transduction. In this paper we present the method for reading over these informations.

METHOD OF THE CALCULATION

C **s**. Molecules of sucrose, galactosucrose and their 8 chlorodeoxy derivatives (Fig. 1a,b,c) are taken into consideration. The self-consistent geometries of these molecules are calculated applying at first the "*Add H & Model Build*" function under "*Build*" menu in HyperChem-5.0, then MM+ molecular mechanics self-consistent procedure [17] using Fletcher-Reeves convergence, and at last PM3 quantum procedure, applying Polak-Ribiere convergence in Hyperchem 5.0 standard [18]. PM3 population analysis was carried out for the self-consistent geometries of the all mentioned above molecules. It yields i-th atomic net charges, $Q_i(j)$, and k-th $\varepsilon_k(j)$ molecular energies (in eV units) for each j-th sugar. For each ψ_k molecular orbital with ε_k energy, the LCAO MO expansion by ϕ_λ atomic orbitals is considered,

$$\psi_k = \sum_\lambda c_{\lambda,k}\phi_\lambda \tag{3}$$

The $q_\mu(k)$ orbital electron density, belonging to k-th MO's on μ-th atom is calculated in the form:

$$q_\mu(k) = 2\sum_{\lambda\in\mu} c^2_{\lambda,k} \tag{4}$$

PM3 semiempirical quantum calculations are carried out in HyperChem-5.0 standard [18].

.) Eq. 4 RS_j(%/%) and RS_j(mole/mole) values. The RS_j^{exp}(%/%) value usually expresses a sweetness of 10% solution of j-th sugar measured in respect to the sweetness of 10% sucrose solution. Such a method is very suitable when molecular structure and molecular weight of given sugar is unknown. On the other hand, the concentrations of solutions in molecular theories are frequently expressed by mole/dcm^3 unit. We assume that RS_j(mole/mole) values are measured in the set of equimolar solutions. Recalculation of RS_j(%/%) values into RS_j(mole/mole) is carried out in this paper according to the expression:

$$RS_j(\text{mole/mole}) = \frac{d_0 M_j}{d_j M_0} RS_j(\%/\%) \tag{5}$$

M_j is molecular weight of j-th sugar, whereas M_0 molecular weight of standard sugar = sucrose. Additionally, we accept for simplicity that solutions of different sugars under the same small concentrations have approximately the same densities ($d_j = d_0$).

.) A relationship between RS_j(mole/mole) and free energy of sweetener–receptor complex formation. In accordance to Höltje and Kier (2), we may formulate the thermodynamic equation for sweetness

$$\log(RS_j) \cong b_1 \frac{E_{\text{int}}(j)}{RT} + b_0 \tag{6}$$

in which b_0 and b_1 are constants, if the following conditions, (i)–(iv), are fulfilled. For simplicity, all the chloro-sugars are called by "sugars" in further considerations.

(i) All sugars of the set react with the same taste receptor. Geometry of the receptor is common and unchanged for all sugars. Charge-transfer and dispersion interaction depend on the common HOMO and LUMO energies of threonine as a moiety of sweet taste receptor. They are $\varepsilon_{HO}^{Rec} = -9.724027$ eV and $\varepsilon_{LU}^{Rec} = 0.923911$ eV, respectively.

(ii) All sugars have a similar geometry. They own the same sucrose/galactosucrose structure. Chlorine substituent may be considered as a small perturbation of this structure. It does not change the number of valence electrons. The number of molecular orbitals occupied by valence electrons is the same for all sugars of a set.

(iii) Sweetener–receptor complexes have a similar geometry for each sugar of a set.

(iv) The overlap between sweetener and receptor orbitals in complex is small.

Simplified calculations of $E_{int}(j)$ sweetener-receptor interaction energy [19] in (6) are based on the sum (7) for the j-th arbitrary sugar

$$E_{int}(j) = E_{elst}^{(1)}(j) + E_{CT}^{(2)}(j) + E_{disp}^{(2)}(j) + E_{exch}^{(1)}(j) \tag{7}$$

$$\underbrace{\qquad\qquad}_{\text{attractive}} \quad \underbrace{\qquad}_{\text{repulsion}}$$

in which $E_{elst}^{(1)}(j)$ electrostatic term may be approximated by the sum of m-m monopol-monopol and m-d monopol-dipol terms [20]. They are linear functions in respect to any Q_a net charge of a-th atom in sweetener system. Since the overlap between glycophore and receptor orbitals is small, $E_{exch}^{(1)}(j)$ tends to vanish. Additionally, if the (i)–(iii) conditions are fulfilled, one can obtain simple formula for electrostatic, charge-transfer and dispersion energy of sweetener--receptor interaction

$$E_{elst}^{(1)}(j,a) = A_a \cdot Q_a(j), \quad E_{CT}^{(2)}(j,i) = \frac{C_i}{\varepsilon_{LU}^{Rec} - \varepsilon_i(j)}, \quad E_{disp}^{(2)}(j,i,x) = \frac{D_{ix}}{\varepsilon_x(j) - \varepsilon_i(j) + \varepsilon_{LU}^{Rec} - \varepsilon_{HO}^{Rec}} \tag{8}$$

in which A_a, C_i and D_{ix} are unknown constants and they are common for all the sugars of the set. CT interaction is attributed to electron transfer from i-th occupied orbital of j-th sugar to receptor's LUMO, In turn, dispersion interaction is attributed to electron transition from i-th occupied to x-th unoccupied MO's of sugar, coupled with receptor HO $\rightarrow$ LU transition. The A_a, C_i and D_{ix} unknown constants can be found by QSAR correlation technique [21]. At first, based on (8), the multiple regression equation is formed:

$$\log(RS_j^{\exp l}) \cong C_0 + \sum_{a \in Sug} C_a Q_a(j) + \sum_{i \in Sug}^{occ} \frac{C_i'}{\varepsilon_{LU}^{Rec} - \varepsilon_i(j)} + \sum_{i \in Sug,}^{occ} \sum_{x \in Sug}^{unocc} \frac{C_{ix}''}{\varepsilon_x(j) - \varepsilon_i(j) + \varepsilon_{LU}^{Rec} - \varepsilon_{HO}^{Rec}} \quad (9)$$

The C_a, C_i' and C_{ix}'' unknown coefficients (A_a, C_i and D_{ix} divided by $-RT$) and additionally unknown C_0 in QSAR equation (9) are common for all sugars from the set, $j = 1,\ldots N$. These coefficients are estimated by least-square procedure (9). In this equation $f_j(C_0, C_{a1}, C_{a2},\ldots C_{aq}, C_{i1}', C_{i2}', \ldots C_{ip}', C_{i1,x1}'', \ldots C_{im,xn}'')$ function is equal to $\log RS_j^{Calc}$.

$$\sum_{j=1}^{N}\left[f_j\left(C_0, C_{a1}, C_{a2}, \ldots C_{aq}, C_{i1}', C_{i2}', \ldots C_{ip}', C_{i1,x1}'', C_{i2,x2}'', \ldots C_{im,xn}''\right) - \log RS_j^{expl}\right]^2 = \min \quad (10)$$

Summation over j is turned over all N sugars. The $P = q + p + m \cdot n$ sum is a number of the coefficients. At the same time, we have $1 + P$ number of all free coefficients, together with the C_0 free coefficient for the estimation. The multiple correlation of $\log RS_j^{exp l}$ ***versus*** the particular Q_a net charges, $[\varepsilon_{LU}^{Rec} - \varepsilon_i(j)]^{-1}$ CT orbital parameters and $[\varepsilon_x(j) - \varepsilon_i(j) + \varepsilon_{LU}^{Rec} - \varepsilon_{HO}^{Rec}]^{-1}$ dispersion parameters is described by R coefficient of multiple correlation, calculated according to Czermiński *et al*. [22].

RESULTS AND DISCUSSION

Sucrose, galactosucrose and their chlorodeoxy derivatives have similar structures. The common structure of these compounds is presented in Fig. 1a. The substitution of the OH group, possessing 6 oxygen valence electrons and 1 hydrogen electron, by 7 chlorine valence electrons, does not change the number of all valence electrons. Under PM3 semiempirical basis approximation, all these 10 molecules are isoelectronic and have the same number of occupied orbitals. It allows to bind the sweetness of chlorodeoxy derivatives with the changes of occupied MO's energies followed by chlorosubstitution. The RS_j^{expl}(%/%) experimental values originate from papers [1,23–25].

Dependences of formation heats, bind energies, ε_{HOMO} and ε_5^{occ} energies on RS_j^{expl} relative sweetness of the sugars mentioned are shown in Table 1. The R correlation coefficients are 0.86527, 0.85705, 0.71997 and **0.97364**, respectively. The $\log RS_j^{expl}$ values indicate not high but visible correlations against three first quantities, but the correlation against energy of 5-th occupied MO's is unexpectedly high. Analysis of the corresponding values leads to conclusion that every substitution of successive chlorine atom lowers the heat of formation, bind energy (in relation to their absolute values) and raises MO's energy of a given sugar. It may point for more excitation of the ground state and an increase of chloro-sugar activity. Observed three first changes, however, cannot explain that stronger chloro-substituted 1′,4,6,6′-TClG (RS_j = 1000) is two times less sweet than 1′4,6′-TClG (RS_j = 2000), and analogously 1′,4,6,6′-TClS (RSj = 200) is also two time less sweet from 1′6′-DClS (RS_j = 500).

1. Calculated heats of formation, bind energies, energies of HOMO and 5-th occupied MO's, as well as RS_j experimental relative sweetnesses of chlorodeoxy derivatives of sucrose (S) and galactosucrose (G). PM3 semiempirical quantum calculations in the ground state. R are correlation coefficients for the correlation of the calculated quantities *versus* $\log RS_j$ (mole/mole).

No.	Derivative[a)]	Mol. Weight,	Heat of formation, kcal/mole	Bind energy, kcal/mole	ε_{HOMO} eV	ε_5^{occ} eV	RS_j (.) /	RS_j (expl.) %/%
1.	1′,4,6′-trichloro-G	397.635	–359.537	–3963.639	–10.259	–10.684	**2323.26**	2000
2.	1′,4,6,6′-tetrachloro-G	416.081	–326.555	–3847.944	–10.233	–10.713	**1215.52**	1000
3.	1′,4-dichloro-G	379.189	–403.594	–4090.325	–10.573	–10.865	**664.65**	600
4.	1′,6′-dichloro-S	379.189	–397.494	–4084.225	–10.567	–10.722	**553.87**	500
5.	1′,4,6,6′-tetrachloro-S	416.081	–325.059	–3846.448	–10.150	–10.715	**243.10**	200
6.	6′-chloro-S	360.743	–436.274	–4205.676	–10.345	–11.004	**21.08**	20
7.	1′-chloro-S	360.743	–439.312	–4208.714	–10.433	–11.048	**21.08**	20
8.	4-chloro-S	360.743	–440.771	–4210.173	–10.627	–11.231	**5.27**	5
9.	Sucrose	342.297	–478.706	–4330.779	–10.742	–11.316	**1.00**	1
10.	Galactosucrose	342.297	–481.233	–4333.306	–10.780	–11.476	**0.20**	0.2
	R		0.86527	0.85705	0.84998	**0.97364**		

[a)]"Deoxy" word is omitted for simplicity. For example, 1′,4,6′-trichloro-G is an abbreviation of 1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose.

In turn, we take into consideration $\varepsilon_i(j)$ energies of the occupied molecular orbitals in the frontier region. The $\log RS_j^{\text{expl}}$ indicates unexpectedly satisfactory correlations with $i = 4$, 5 and 6 MO's energy. (The sugar's HOMO owns $i = 1$ in this numeration). R correlation coefficients are equal to 0.93426, 0.97364 and 0.93758, respectively, (see Table 2). Thus, **5-** of the frontier region forms the parameters, which are best correlated *versus* $\log RS_j^{\text{expl}}$ ($\boldsymbol{R}$ **0.97**). It means that the *CT* terms in the multiple correlation equations should take the most part in the sweetness effect of the sugars considered.

2. R correlation coefficients for a correlation of $\log RS_j^{\text{expl}}$ (mole/mole) against ε_i energies of the individual sugar molecular occupied and unoccupied (#) orbitals in the frontier region.

i	R	i	R	i	R
$6^{\#}$.	0.748818	1.	0.719973	7.	0.890401
$5^{\#}$.	0.728712	2.	0.794896	8.	0.878288
$4^{\#}$.	0.631523	3.	0.915694	9.	0.878765
$3^{\#}$.	0.727830	**4.**	**0.934257**	10.	0.892143
$2^{\#}$.	**0.901944**	**5.**	**0.973638**	11.	0.913679
$1^{\#}$.	0.844136	**6.**	**0.937575**	12.	0.915447

To obtain a better view of the nature of sweetener-receptor interaction, QSAR correlation equation (9) with least square procedure (10) is successively carried out for arbitrary $P = 1$, $P = 2$, $P = 3$ and $P = 4$ parameters under $\boldsymbol{R} =$ condition for R multiple correlation coefficient. For $P = 1$, $P = 2$ and $P = 3$ the solutions are similar, yielding 100% of *CT* forces. Corresponding three QSAR linear correlation equations (A1–A3) are formed (see Appendix). In all the above three cases, the computer chooses pure *CT* sweetener-receptor interactions based on maximal R = 0.97300, 0.99145 and 0.99561, respectively. Electron donation into receptor's LUMO occurs from 5-th occupied MOs in eq. (A1), from 5-th and 6-th occupied MOs in (A2), and from 1, 5 and 6-th occupied MOs in (A3). One may notice that above 5-th MOs is the most important orbital in a frontier occupied orbital region. The results of RS_j calculation due to (A1–A3) one can find in Table 3. The RS_j values are too low for 1′,4,6′-TClG and 1′,4,6,6′-TClG , and, at the same time, too high for 1′,4,6,6′-TClS. In order to improve these results, the $P = 4$ case is considered with the following equation:

$$\log(RS_j) = -67.387 + 2.9473 \cdot Q_{XH_2}(j) + \frac{526.705}{\varepsilon_{LU}^{\text{Re}c} - \varepsilon_5(j)} + \tag{11}$$

$$+ \frac{1556.877}{\varepsilon_2^{\#}(j) - \varepsilon_2(j) + \varepsilon_{LU}^{\text{Re}c} - \varepsilon_{HO}^{\text{Re}c}} - \frac{1038.128}{\varepsilon_2^{\#}(j) - \varepsilon_7(j) + \varepsilon_{LU}^{\text{Re}c} - \varepsilon_{HO}^{\text{Re}c}}$$

The latter $P = 4$ parameter equation describes a more detailed nature of sugar-receptor interaction. Energy of this interaction indicates 1% electrostatic forces be-

3. Calculated values of the *RS* (mole/mole) relative sweetnesses of sucrose (S) and galactosucrose (G) chlorodeoxy derivatives. Calculations are carried out for: 1-, 2-, 3- and 4-parameters, according to the equations (A1), (A2), (A3) and (11) respectively. Correlation coefficients of the multiple regression (R) and the corresponding remainder variances (S^2).

Derivative	*RS* (Calculated)				***RS* (E)**	Sugar--receptor interaction energy in relation to the nonbonding galatosucrose--receptor energy {XH_2, 5, 2→2#, 7→2#}			
	{5}	{5,6}	{1,5,6}	{XH_2, 5, 2→2#,7→2#}		ELST	CT	DISP	**E**
	100%CT	100%CT	100%CT	1%ELST, 64%CT, 35%DISP		----------kcal/mole----------			
1′,4,6′-trichloro-(G)	1312.62	1903.07	1775.64	2656.06	**2323.26**	–0.003	–3.917	–1.622	**5.542**
1′,4,6,6′-tetrachloro-(G)	930.19	834.92	709.13	1230.95	**1215.52**	+0.760	–3.760	–2.091	**5.091**
1′,4-dichloro-(G)	165.78	526.98	750.99	566.59	**664.65**	–0.014	–2.973	–1.649	**4.636**
1′,6′-dichloro-(S)	841.10	377.40	669.80	537.92	**553.87**	–0.034	–3.714	–0.859	**4.607**
1′,4,6,6′-tetrachloro-(S)	915.47	531.33	374.49	239.14	**243.10**	+0.760	–3.752	–1.138	**4.130**
6′-chloro-(S)	35.56	40.02	29.18	20.05	**21.08**	–0.037	–2.270	–0.370	**2.677**
1′-chloro-(S)	21.96	21.27	17.80	23.15	**21.08**	–0.048	–2.051	–0.664	**2.763**
4-chloro-(S)	3.11	6.16	6.10	5.45	**5.27**	+0.003	–1.158	–0.757	**1.912**
sucrose	1.28	0.69	0.79	0.94	**1.00**	–0.044	–0.752	–0.089	**0.885**
galactosucrose	0.25	0.20	0.20	0.21	**0.20**	0.000	0.000	0.000	**0.000**
R	0.97300	0.99145	0.99561	0.99964					
S^2	0.61421	0.22490	0.13478	0.08152					

longing to hydrogen glucopyranose XH_2 sweetener subsite, 64% *CT* forces as electron donation from sugar 5-th occupied MOs to receptor LUMO, and finally 35% dispersion energy derived from sugar's $2 \rightarrow 2^{\#}$ and $7 \rightarrow 2^{\#}$ electron transitions in frontier MOs region accompanied by receptor's HOMO $\rightarrow$ LUMO transition. These per cent data belong to the first member of the set, 1′,4,6′-TClG. The RS_j sweetness calculation based on (11) leads to an excellent agreement with experimental sweetness, (Table 3). Detailed structures of the sweetener-receptor interaction for the individual sugars are shown in last four columns in Table 3. Exact values of $E^{(1)}_{elst}$, $E^{(2)}_{CT}$ and $E^{(2)}_{disp}$ cannot be however calculated from (11). On the other hand,

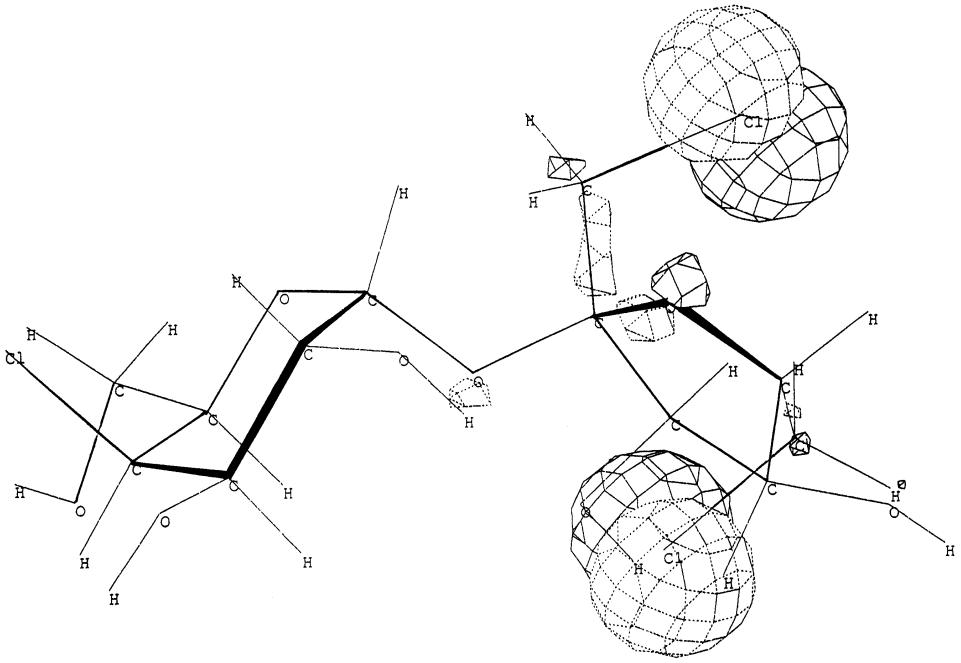
$$\mathrm{CT} = E^{(2)}_{CT}(j) - E^{(2)}_{CT}(\mathrm{G}), \quad \mathrm{DISP} = E^{(2)}_{disp}(j) - E^{(2)}_{disp}(\mathrm{G}), \quad \mathrm{ELST} = E^{(1)}_{elst}(j) - E^{(1)}_{elst}(\mathrm{G}) \qquad (12)$$

differences, as relative to nonsweet sugar G = galactosucrose, reveal improved values.

4. Energies (eV) of the $2^{\#}$-th unoccupied and 5-th molecular occupied orbitals in the frontier region as well as maximal $q_{\mu(k)}$ frontier orbital densities on the atoms. Contributions of fructo- furanose moiety to the frontier structure are underlined. Abbreviated names of sugars are used.

Name of sugar	MO's	Energy	Atom ($q_{\mu(k)}$ orbital density)
I.) 1′,4,6′-trichloro-galactosucrose	$2^{\#}$	0.87042	CH_2-6′ (2.23), Cl-6′ (1.61)
(*RS* = 2000)	5.	–10.68353	Cl-1′ (0.86), Cl-6′ (0.83)
II.) 1′,4,6,6′-tetrachloro-galactosucrose	$2^{\#}$	0.71451	CH_2-1′ (2.13), Cl-1′ (1.53)
(*RS* = 1000)	5.	–10.71337	Cl-1′(1.53)
III.) 1′,4-dichloro-galactosucrose	$2^{\#}$	0.80675	CH-4 (1.73), Cl-4 (1.68)
(*RS* = 600)	5.	–10.86521	Cl-1′ (1.39)
IV.) 1′,6′-dichloro-sucrose	$2^{\#}$	0.92375	CH_2-6′ (2.38), Cl-6′ (1.72)
(*RS* = 500)	5.	–10.72213	O-3 (0.59), Cl-1′ (0.53)
V.) 1′,4,6,6′-tetrachloro-sucrose	$2^{\#}$	0.78634	CH-4 (1.98), Cl-4 (1.53)
(*RS* = 200)	5.	–10.71476	Cl-6′ (1.24), Cl-1′ (0.48)
VI.) 6′-chloro-sucrose	$2^{\#}$	1.92966	C-2′ (1.48), C-3′ (1.14)
(*RS* = 20)	5.	–11.00412	O-3 (0.31), O-2 (0.22)
VII.) 1′-chloro-sucrose	$2^{\#}$	1.80450	C-1 (1.47), C-2 (0.94)
(*RS* = 20)	5.	–11.04827	O-4 (0.31), O-5′ (0.26)
VIII.) 4-chloro-sucrose	$2^{\#}$	1.65649	C-2′ (1.07), C-1 (0.94)
(*RS* = 5)	5.	–11.23087	O-6 (1.03)
IX.) Sucrose	$2^{\#}$	1.95579	C-5 (0.93), C-6 (0.87)
(*RS* = 1)	5.	–11.31583	O-2 (0.90)
X.) Galactosucrose	$2^{\#}$	1.95715	C-5 (1.16), C-6 (1.08)
(*RS* = 0.2)	5.	–11.47646	O-1 (0.36), O-1′ (0.31)

The primary sweetness effects belong to CT chlorosugar---receptor interactions, (64%). They are caused by *n*-electron transfer from **5-** s to receptor's LUMO. The orbital densities on atoms of this orbital yields the map of *n*-electron donor centers in molecule (Table 4). All five most sweet sugars (RS_j = 200–2000) have great electron orbital densities at fructofuranose Cl-1′ and Cl-6′ at-

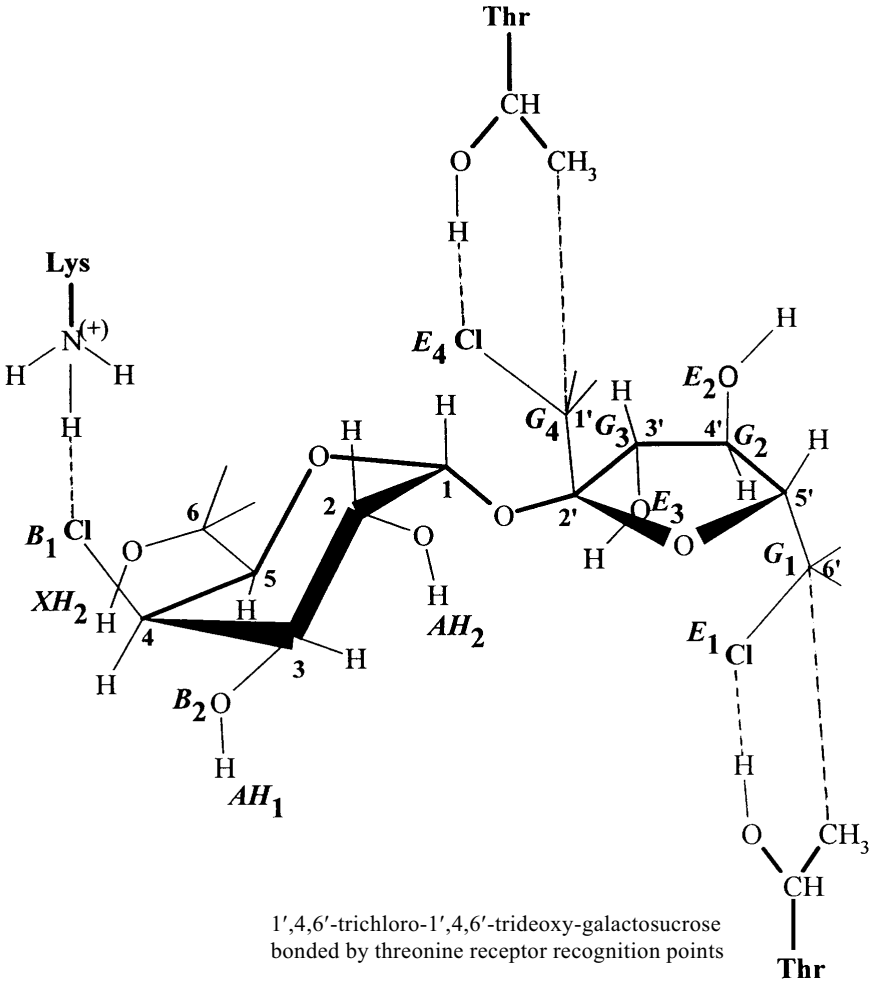


F 3. Space HyperChem-5.0 diagram of 5-th occupied molecular orbital in 1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose. Great contributions of Cl-1′(E_4) and Cl-6′(E_1) *n*-electron atomic orbitals to MO's structure are well observed.

oms, which strongly dominate in 5-th MO's structure, however, Cl-6′ is absent in some sugars. To convinced oneself, one may analyse a space molecular diagram of 5-th occupied MO's of 1′,4,6′-TClG in Fig. 3. These chlorines intensively raise this MO's energy. On the other hand, five smaller sweet sugars, RS_j = 0–21, do not possess either Cl-1′ or Cl-6′ chlorine *n*-electron donors on this orbital.

Secondary sweetness effects belong to sugar-receptor dispersion interactions (35%). Among two $2 \rightarrow 2^{\#}$ and $7 \rightarrow 2^{\#}$ sugar transitions coupled with HO(Rec) → LU(Rec) receptor transition, the first owns greater contribution ($C''_{22} = 1556$) in (11). Energies and orbital density structures are situated in Tab. 4. Energies of $2^{\#}$ unoccupied orbital for individual chlorosugars show the best correlation ($R = 0.90194$) *versus* $\log RS_j^{\exp l}$ among all unoccupied orbitals, (Table 2). For five most sweet chlorosugars, these energies are significantly lower, about 1 eV in relation to the remaining derivatives, causing an increase of DISP contribution, Tab. 4. The molecular orbital densities of $2^{\#}$ orbital distinguish fructofuranose 1′-CH_2 (G_4) and 6′-CH_2(G_1) as well as glucopyranose 4-CH atomic groups. Mentioned, weakly polarized fructofuranose groups are classified as G_4 and G_1 dispersion sweetener subsites in Nofre-Tinti theory, respectively.

The tertiary effects belong to electrostatic interactions. It is well observed that deoxychlorination at 6-th position of glucopyranose moiety yields a decrease of sweetness. So, less chlorinated 1′,4,6′-TClG is twice more sweet than greater chlorinated 1′,4,6,6′-TClG. Analogously, 1′,6′-DClS is twice more sweet than 1'4,6,6'-TClS. The glucopyranose H atom in 6-OH hydoxyl group (XH_2 sweetener subsite) is active in electrostatic sugar-receptor interaction, Eq. (11). Elimination of well charged XH_2 subsite (Q = 0.313), due to substituting of 6-OH group by weakly charged Cl-6 chlorine atom ($Q \approx -0.050$), leads to a decrease of ELST energy component for **0.76** in 1′,4,6,6′-TClG as well as in 1′,4,6,6′- TClS (Tab. 4). Calculated theoretical structure of the complex between 1′,4,6′-TClG and sweet taste receptor based on P = 4 parameter QSAR correlation equation is presented in Fig. 4.



F 4. Calculated theoretical structure of the complex between 1′,4,6′-trichloro-1′,4,6′-trideoxy-galactosucrose and sweet taste receptor based on the P = 4 parameter QSAR correlation equations.

Strong domination of the charge-transfer process, $5 \rightarrow \mathrm{LU(Rec)}$ in all $P = 1$–4 parameter correlation equations, can determine the biochemical transduction path. In such a case, transferred negative charge from Cl-1′(E_4) and Cl-6′(E_1) atoms to a receptor can form the potential stimuli, which may open Na^+ ionic channel closely associated with the receptor. According to Brand and Feigin [26], it may be the origin of the following biochemical transduction: then a positive charge flows into the cell. This influx brings about a depolarization, which, if sufficient, could trigger the opening or closing of voltage-dependent ion channels in baso-lateral portion of the cell. Channels are opened, Na^+ and Ca^{2+} flow into the cell, leading to further depolarization and release of neurotransmitter. Such transduction process may exist without cyclic AMP second messenger production. Above considerations support the favouritism of stimulus-gated ion channel transduction scheme for sweet taste of high deoxychlorinated galactosucrose/sucrose derivatives.

In the preliminary studies reported in [26], they have shown that some of high intensity sweeteners are capable of inducing ion channel-like activity in planar lipid bilayers. Thus, it seems, high sweetness of chlorodeoxy derivatives of sucrose and galactosucrose, belongs in great part to the cholinergic ionic receptor scheme (III).

Acknowledgment

I am indebted to Prof. Dr. P. Tomasik for presenting me Krustošikova and Uher monography [1]. This book, translated by him from Slovak language, supplies many valuable experimental data relating to the sweetness of chloro-sugars.

APPENDIX

$$\log(RS_j) = -55.192 + \frac{676.836}{\varepsilon_{LU}^{Rec} - \varepsilon_5(j)} \tag{A1}$$

$$\log(RS_j) = -64.893 + \frac{1573.563}{\varepsilon_{LU}^{Rec} - \varepsilon_5(j)} - \frac{789.205}{\varepsilon_{LU}^{Rec} - \varepsilon_6(j)} \tag{A2}$$

$$\log(RS_j) = -60.910 - \frac{123.012}{\varepsilon_{LU}^{Rec} - \varepsilon_1(j)} + \frac{1641.376}{\varepsilon_{LU}^{Rec} - \varepsilon_5(j)} - \frac{775.809}{\varepsilon_{LU}^{Rec} - \varepsilon_6(j)} \tag{A3}$$

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