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Structure-Activity Relationship in the Taste Effect of Ribonucleotide Derivatives[1]

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Flavor enhancing activities synergistic with monosodium L-glutamate and stimulus thresholds of ribonucleotide derivatives have been correlated with their chemical structures following Hansch–Fujita's method. Regression analyses were made separately for two groups, 2-substituted inosine 5′-phosphates and S-substituted 2-mercaptoinosine 5′-phosphates. Superdelocalizability, net π-charge, Hammett σ_m, and hydrophobic parameter π were used in the analyses. Nuclear magnetic resonance chemical shift of the $-SCH_2X$ group and a dummy variable D, which represents a proton accepting oxygen atom at the γ- or δ-position from the sulfur atom in substituents attached to the 2-position of purine skeleton, were employed successfully in a series of 2-S-substituted derivatives. In a series of 2-substituted derivatives, it has been recognized that flavor enhancing activities are influenced by Hammett σ_m and electrophilic superdelocalizability at the α-position of substituents ($S_2\alpha$), whereas thresholds are correlated with $S_2\alpha$ and π. In a series of 2-S-substituted derivatives, it has been shown that π, D, and electrophilic superdelocalizability of the methylene protons in the $-CH_2S-$ group of substituents play important roles in the structure–activity relationships.

Hansch and Fujita have shown that biological response to congeneric drugs may be quantitatively correlated with physicochemical properties of drugs by means of substituent constants.[3] They found that, by using the Hammett σ constant for the electronic effect and a constant π for the hydrophobic binding power of a substituent, the biological activity of a series of substituted compounds can be expressed by Eq. 1.

$$\log 1/C = -k\pi^2 + k'\pi + \rho\sigma + k'' \qquad \text{Eq. 1}$$

In Eq. 1, C is the molar concentration of a compound causing an equivalent biological response and π is defined as $\pi = \log P_X - \log P_H$ where P_X is octanol–water partition coefficient of a derivative X, and P_H is that of the standard compound. ρ is a constant characteristic of the reaction and reaction conditions. The constants k, k', k'', and ρ are obtained by the regression analysis. Recently, electronic polarizability (P_E) of a substituent,[4] molecular weight,[4a] resonance substituent constant,[4b] parachor (Pr),[4c] molar attraction constnat,[4c,5] superdelocalizability,[6] frontier electron density,[7] total charge density,[7] hydrolysis constant,[8] dissociation constant,[9] dipole moment,[10] nuclear magnetic resonance (NMR) chemical shift,[9]

1) Paper read at the 90th Annual Meeting of the Pharmaceutical Society of Japan, Hokkaido, July 1970.
2) Location: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*
3) C. Hansch, R.M. Muir, T. Fujita, P.P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963); C. Hansch and T. Fujita, *ibid.*, **86**, 1616 (1964); J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965).
4) *a*) A. Cammarate, *J. Med. Chem.*, **9**, 203 (1966); *b*) C. Hansch, E. Kutter, and A. Leo, *ibid.*, **12**, 746 (1969); *c*) A. Leo, C. Hansch, and C. Church, *ibid.*, **12**, 766 (1969); *d*) K. Bowden and R.C. Young, *ibid.*, **13**, 225 (1970).
5) J.A. Ostrenga, *J. Med. Chem.*, **12**, 349 (1969).
6) A. Cammarata, *J. Med. Chem.*, **11**, 1111 (1968).
7) Y.C. Martin, *J. Med. Chem.*, **13**, 145 (1970).
8) W.B. Neely, W.E. Allison, W.E. Crummett, K. Kauer, and W. Reifschneider, *J. Agr. Food Chem.*, **18**, 45 (1970).
9) N. Kakeya, N. Yata, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 2558 (1969).
10) M. Goulian and W.S. Beck, *Chem. Eng. News*, **46**, 38 (1968).

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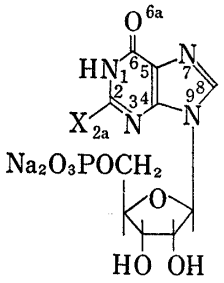
Taft's steric parameter (Es),[4d,11] steric substituent constant,[4d] *etc.*, have also been employed as physicochemical parameters. In the present study, Hansch–Fujita's method was applied in analyzing a possible structure-activity relationship between a taste effect and physicochemical parameters of ribonucleotide derivatives. Taste response might be elicited by a series of physiological reactions following a complex formation between the taste bud receptor and tasting compound, and intensity of the taste would be related to the magnitude of these reactions. It could be assumed that the complex-forming power of a tasting compound is not limited to one part of the molecule, but several functional groups in the molecule participate in intermolecular interactions. In such a case, the structure-activity relationship may be formulated as in Eq. 2 or 3. In Eq. 2 and 3, C_X is the equi-effective molar concen-

$$\log 1/C_X = -k\pi^2 + k'\pi + \sum_i a_i \log K_X^i/K_H^i + k'' \qquad \text{Eq. 2}$$

$$\log C_S/C_X = -k\pi^2 + k'\pi + \sum_i a_i \log K_X^i/K_H^i + c \qquad \text{Eq. 3}$$

tration of a derivative X and C_S is that of a compound S used as the standard in determining the biological response. K_X^i/K_H^i is the ratio of the i-th physicochemical constant (including the Hammett σ) at various positions of derivative X to that of the standard compound H which is not necessarily the same as the standard compound S for biological response. The constants a_i and c, as well as k, k', and k'', are obtained by the regression analysis. When the standard compound S is the same as the standard compound H, the constant c is theoretically equal to zero.

In recent years, a number of ribonucleotide derivatives were synthesized by Honjo, *et al.*[12] and by Yamazaki, *et al.*,[13] and their flavor-enhancing activities and stimulus thresholds were measured by Toda, *et al.*[14] and by Yamaguchi, *et al.*[15] Honjo[12a] has suggested that the taste effect would be affected by a substituent at the position 2, when he and his co-workers synthesized 2-chloroinosine 5′-phosphate and found its stronger taste effect than that of the parent compound.



X
H, Cl, C_6H_5
CH_3, CH_2R
OR, SR
NH_2, NHR, NR_2

Fig. 1. Chemical Structure of Ribonucleotide Derivatives

We attempted to analyze a structure-activity relationship between the taste effect and physicochemical parameters by using Eq. 2 and 3.

Method

The net π-charge and superdelocalizability were calculated by the ω-technique[16] (ω=0.55[17]) and were employed, in addition to Hammett σ_m of the substituent at the position 2, as physicochemical constants at various parts of tasting compound. For the determination of electronic structure of compounds treated in this study, ultraviolet (UV) spectra of guanosine 5′-phosphate (GMP) and 2-allylthioinosine 5′-phosphate were measured at various pH's and were compared with those of purine and pyrimidine bases determined

11) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).
12) *a*) M. Honjo, K. Imai, Y. Furukawa, Y. Kanai, R. Marumoto, H. Honda, H. Aoki, and T. Hirata, *Takeda Kenkyusho Nempo*, **25**, 74 (1966); *b*) R. Imai, R. Marumoto, K. Kobayashi, Y. Yoshioka, J. Toda, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **19**, 576 (1971).
13) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968).
14) J. Toda, H. Aoki, and K. Taniguchi, unpublished data.
15) S. Yamaguchi, T. Yoshikawa, S. Ikeda, and T. Ninomiya, *Agr. Biol. Chem.* (Tokyo), **32**, 797 (1968).
16) A. Streitwieser, *J. Am. Chem. Soc.*, **82**, 4123 (1960).
17) M. Kubo and Y. Kurita, *Bull. Chem. Soc. Japan*, **24**, 13 (1951); M. Ochiai, E. Mizuta, Y. Asahi, and K. Morita, *Tetrahedron*, **24**, 5861 (1968).

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by Clark and Tinoco,[18] the results of which showed that in neutral solutions purine moieties of these nucleotides exist almost completely in the keto-form. It will be reasonable to assume that other ribonucleotide derivatives used in the present work also exist in the keto-form in the pH range of 7—8 at which taste effects were determined. The superdelocalizability and net π-charge of purine moieties were, therefore, calculated in the keto-form. The part of the ribose 5′-pohsphate group bonded to the 9-position of purine bases was neglected from the calculation, since little effect of 2-substituent will be exercised on this group. Electron densities of methyl and methylene groups were calculated with a conjugation model.[19] The parameters[20] employed for the calculation are shown in Table I.

Table I. Parmameter Values Used for Calculation by the ω-Technique

X-Y-Z	a_X[a]	a_Y[a]	a_Z[a]	l_{XY}[b]	l_{ZY}[b]
C–O	0	2.0		0.8	
C=O	0	1.4		1.2	
C–N (ring)	0	1.5		0.8 (0.9)	
C=N	0	0.7		1.0	
C–S	0	1.0		0.7	
C–Cl	0	2.0		0.7	
C–C≡H_3	0	−0.1	−0.5	0.8	2.5
C–C=H_2	0	−0.1	−0.5	0.7	2.5
O–C≡H_3 (=H_2)	2.0	−0.1	−0.5	0.6	2.5
N–C≡H_3 (=H_2)	1.5	−0.1	−0.5	0.6	2.5
S–C≡H_3	1.0	−0.1	−0.5	0.6	2.5
S–C=H_2	1.0	−0.1	−0.5	0.5	2.5

a) coulomb integral of the substituent X, Y and Z: $a_X=a+a_X\beta$, $a_Y=a+a_Y\beta$, $a_Z=a+a_Z\beta$
b) resonance integral of the substituent X and Y, and Y and Z: $\beta_{X-Y}=l_{XY}\beta$, $\beta_{Y-Z}=l_{YZ}\beta$

Table II. Parameter Values Used in the Regression Analysis of 2-Substituted Inosine 5′-Phosphate

Substituent X	π[a]	σ_m[b]	S_{1a}	$S_1\times10^2$	$S_{6a}\times10^2$	$S_7\times10^2$	$Q_1\times10^2$	$Q_{6a}\times10^2$	$Q_7\times10^2$
$SCH_2CH_2CH_3$	2.91	0.30	0.519	0.77	0.53	0.35	−0.16	0.013	0.008
SCH_2CH_3	2.41	0.30	0.519	0.77	0.53	0.35	−0.16	0.013	0.008
SCH_3	1.91	0.30	0.527	0.82	0.56	0.37	−0.17	0.014	0.008
$OCH(CH_3)_2$	2.21	0.31	−0.268	−0.55	−1.03	−0.65	0.47	−0.063	−0.061
OCH_2CH_3	1.91	0.31	−0.257	−0.49	−1.00	−0.62	0.45	−0.063	−0.060
OCH_3	1.41	0.28	−0.261	−0.51	−1.00	−0.63	0.46	−0.063	−0.060
NH_2	0	0	0	0	0	0	0	0	0
$NH(CH_3)$	0.75	−0.14	0.015	0.10	0.05	0.04	−0.02	0.000	0.000
$N(CH_3)_2$	1.39	−0.05	0.030	0.19	0.11	0.07	−0.03	0.001	0.000
CH_3	1.85	0.09	−0.317[c]	−0.74	−3.75	−2.38	1.46	−0.291	−0.315
CH_2CH_3	2.35	0.09	−0.309[c]	−0.85	−3.90	−2.47	1.61	−0.300	−0.322
Cl	2.33	0.53	−0.260	−0.73	−1.80	−1.13	0.83	−0.120	−0.118
C_6H_5	3.42	0.22	−0.449	0.02	−3.94	−2.49	1.13	−0.367	−0.409
H(IMP)	1.29	0.16	−0.937[d]	−1.22	−4.73	−2.81	2.09	−0.330	−0.346

a) π values were recalculated from those in Ref. 21 by shifting the standard to NH_2-group (π NH_2=O).
b) σ_m values were recalculated from those in Ref. 21 and 22 by shifting the standard to NH_2-group (σ_m NH_2=O).
c) For the value of superdelocalizability at the 2a-position, that of methyl or methylene group was used.
d) The value of superdelocalizability for the hydrogen atom at the 2a-position was taken as 0.30 in calculating S_{2a}.

18) L.B. Clark and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **87**, 11 (1965).
19) Y. Ihara, *Bull. Chem. Soc. Japan*, **28**, 369 (1960).
20) T. Yonezawa, C. Nagata, H. Kato, A. Imamura, and K. Morokuma, "Ryoshi-Kagaku Nyumon," Vol. 1, Kagaku-Dojin, Kyoto, 1968.
21) *a*) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964); *b*) E.J. Lien, C. Hansch, and S.M. Anderson, *J. Med. Chem.*, **11**, 430 (1968); *c*) C. Hansch and E.W. Deutsch, *Biochim. Biophys. Acta*, **126**, 117 (1966).
22) J. Hine, "Physical Organic Chemistry," McGraw-Hill, 1962, p. 87.

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In the analysis of 2-substituted inosine 5′-phosphates listed in Table II, inosine 5′-phosphate (IMP), the parent compound, was used as the standard of the flavor-enhancing activity. For the standard of physicochemical parameters, however, GMP was adopted, since the π-electron density at the 2a-position[23] of IMP could not be obtained. π and σ_m in Table II respectively correspond to the hydrophobic parameter and Hammett constant of the substituent X attached to the 2-position relative to the 2-amino group in GMP. S_{2a}, S_1, S_{6a}, S_7, Q_1, Q_{6a}, and Q_7 in Table II are the constants corresponding to the log K_X^i/K_H^i in Eq. 3. The subscripts 2a, 1, 6a, and 7 designate the position of the compounds (Fig. 1). Si's and Qi's are parameters related to superdelocalizabilities and net charges, respectively. Since the term log K_X^i/K_H^i is free energy-related as is clear and superdelocalizability is also a free energy-related constant, the parameters Si in Table II are defined as Eq. 4, using the electrophilic superdelocalizability (S_r^i) at the i-th atom of the compounds. The parameters Q_i may be defined as Q_i' in Eq. 5 using net π-charge (Q^i) at the i-th atom of the compounds. However, as the change of net π-charge on the purine skeleton due to functional groups is relatively small, Eq. 5b will be used instead of Eq. 5. Equation 5a indicates the validity of this substitution.

$$S_i = S^i_{r_X} - S^i_{r_{NH_2}} \qquad \text{Eq. 4}$$

$$Q_i' = \log Q^i_X/Q^i_{NH_2} \qquad \text{Eq. 5}$$

$$\log Q^i_X/Q^i_{NH_2} = \log \{1+(Q^i_X-Q^i_{NH_2})/Q^i_{NH_2}\} \fallingdotseq (Q^i_X-Q^i_{NH_2})/2.3Q^i_{NH_2} \qquad \text{Eq. 5a}$$

$$Q_i = Q^i_X - Q^i_{NH_2} \qquad \text{Eq. 5b}$$

In Eqs. 4, 5, 5a, and 5b, the subscripts of NH_2 and X designate GMP and 2-substituted IMP derivative X, respectively.

Table III. Parameter Values Used in the Regression Analysis of S-Substituted 2-Mercaptoinosine 5′-Phosphates

Substituent X	π[a]	D[b]	S×10²	Q×10²	ν
SCH_2	1.31	1	5.27	0.92	1.99
$SCH_2CH_2COOC_2H_5$	0.09	1	−1.74	0.57	0.82
$SCH_2CH_2OC_2H_5$	0.02	1	−1.68	0.56	0.82
$SCH_2CH{=}C(CH_3)_2$	1.50	0	3.60	1.09	1.28
$SCH_2CH{=}CHCH_3$	1.20	0	2.83	1.17	1.25
$SCH_2CH{=}CH_2$	0.70	0	2.06	1.25	1.33
$SCH(CH_3){=}CH_2$	1.00	0	2.02	1.24	1.35
$SCH_2CH_2CH_3$	1.00	0	−1.70	0.56	0.60
$SCH_2C_6H_5$	2.13	0	1.01	1.16	1.92
$SCH_2CH{=}CHC_6H_5$	2.83	0	3.98	1.36	1.48
$SCH_2CH(CH_3)_2$	1.30	0	−2.67	0.63	0.55
$SCH_2CH_2CH(CH_3)_2$	1.80	0	−1.70	0.56	0.60
$SCH_2CH<^{C_2H_5}_{C_4H_9}$	3.30	0	−2.67	0.63	0.65
SCH_2CH_3	0.50	0	−1.27	0.53	0.60
SCH_3	0	0	0	0	0

a) π values were recalculated from those in Ref. 21 taking the S-CH_3 as the standard reference group.
b) dummy variable

For the analysis of S-substituted-2-mercaptoinosine 5′-phosphates listed in Table III, 2-methylthio IMP was used as the standard of physicochemical parameters. Thus, in Table III the parameters S, Q, and ν are calculated by Eq. 6, 7, and 8, respectively, where Sr_{SCH_3}, Q_{SCH_3}, and ν_{SCH_3} are superdelocalizability, net π-charge and NMR chemical shift of hydrogen atoms in the -SCH_3 group of the standard compound and Sr_{SCH_2X}, Q_{SCH_2X}, and ν_{SCH_2X} are those of hydrogen atoms in the -SCH_2X group of 2-S-substituted IMP derivative X.

$$S = S_{r_{SCH_2X}} - S_{r_{SCH_3}} \qquad \text{Eq. 6}$$

$$Q = Q_{SCH_2X} - Q_{SCH_3} \qquad \text{Eq. 7}$$

$$\nu = \nu_{SCH_2X} - \nu_{SCH_3} \qquad \text{Eq. 8}$$

23) The α-position of a substituent group attached to the 2-position of purine base was termed 2a-position for the sake of convenience. The 6a-position is defined in a similar manner.

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The chemical shifts of methyl and methylene protons were measured using tetramethylsilane as an internal standard.

Result

Disodium salts of ribonucleotide derivatives were assessed with regard to their flavor enhancing activity synergistic with monosodium L-glutamate (MSG). Ratios of activities of the compounds to that of disodium salt of IMP, $1/C_X/1/C_S$, *i.e.*, C_S/C_X, are given in Table IV and VII, together with standard deviations and number of measurements. For the regression analysis of structure-activity relationship for 14 kinds of 2-substituted derivatives in Table IV, a least squares fit of the values of log C_S/C_X was made by a computer to Eq. 3, using several promising parameters. Instead of using the average of individual measurements on each compound, all individual values were taken into calculation in the analysis. A computer program was written so as to omit, successively, insignificant parameters which were found to make little contribution to the regression and then to recalculate. By applying this program to the analysis, Eq. 15a and 16a were obtained after several cycles of omission and recalculation. For comparison, Eq. 9—14b, 15b, and 16b were derived by the usual method.

$$\log C_S/C_X = 0.133\pi + 0.3117 \quad \text{Eq. 9}$$
$$n=48,\ r=0.472,\ s=0.206$$
$$\log C_S/C_X = 0.831\sigma_m + 0.4056 \quad \text{Eq. 10}$$
$$n=48,\ r=0.646,\ s=0.179$$
$$\log C_S/C_X = 0.030\pi + 0.745\sigma_m + 0.3662 \quad \text{Eq. 11}$$
$$n=48,\ r=0.651,\ s=0.180$$
$$\log C_S/C_X = 0.030\pi^2 - 0.001\pi + 0795\sigma_m + 0.4348 \quad \text{Eq. 12}$$
$$n=48,\ r=0.661,\ s=0.180$$
$$\log C_S/C_X = 0.472S_{2a} + 0.5690 \quad \text{Eq. 13a}$$
$$n=48,\ r=0.764,\ s=0.151$$
$$\log C_S/C_X = 0.591S_{2a} + 0.5535 \quad \text{Eq. 13b}$$
$$n=47,\ r=0.735,\ s=0.157$$
$$\log C_S/C_X = 0.122\pi + 0.458S_{2a} + 0.3407 \quad \text{Eq. 14a}$$
$$n=48,\ r=0.879,\ s=0.113$$
$$\log C_S/C_X = 0.129\pi + 0.519S_{2a} + 0.3203 \quad \text{Eq. 14b}$$
$$n=47,\ r=0.872,\ s=0.115$$
$$\log C_S/C_X = 0.716\sigma_m + 0.427S_{2a} + 0.4356 \quad \text{Eq. 15a}$$
$$n=48,\ r=0.943,\ s=0.079$$
$$\log C_S/C_X = 0.695\sigma_m + 0.568S_{2a} + 0.4214 \quad \text{Eq. 15b}$$
$$n=47,\ r=0.910,\ s=0.097$$
$$\log C_S/C_X = 0.049\pi + 0.565\sigma_m + 0.425S_{2a} + 0.3748 \quad \text{Eq. 16a}$$
$$n=48,\ r=0.951,\ s=0.074$$
$$\log C_S/C_X = 0.068\pi + 0.489\sigma_m + 0.540S_{2a} + 0.3399 \quad \text{Eq. 16b}$$
$$n=47,\ r=0.929,\ s=0.088$$

In these equations, n is the number of points used in finding the constants. The multiple correlation coefficient is represented by r, and s is the standard deviation. The value of C_S/C_X is the ratio of the flavor enhancing activity, synergistic with MSG, of the derivative X to that of IMP. The correlations of the activity with Eqs. 9 and 10 were poor. Combination of π and σ_m (Eq. 11) did not result in a reduction in variance. Addition of π^2 term

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(Eq. 12) did not improve the situation either (compare values of s). Moreover, the positive sign of coefficient for π^2 in Eq. 12 is in conflict with the logical assumption of Hansch-Fujita's method. The activity of IMP calculated from Eq. 9—12 considerably derivated from unity. The correlation of the activity with S_{2a}, which is the most reasonable parameter obtained from the calculation of electron density, was fairly good (Eq. 13a), the activity of IMP being close to unity. Combining S_{2a} and π, we obtained Eq. 14a which accounted for 77.3% of the variance in the data *vs.* 58.4% being accounted for by Eq. 13a. Eq. 15a obtained by the automatic selection of parameters, accounted for 88.8% of the variance in the data.

These results, indicated that the electronic parameter, σ_m, is more useful than hydrophobic parameter, π, for the structure-activity correlation with the flavor enhancing activity synergistic with MSG. Eq. 16a rationalized 90.5% of the variance in the data. Although the difference of r^2 between Eq. 15a and 16a was small, it was statistically significant ($F_{1,44}$= 7.72, $F_{1,44,0.05}$=4.06). Eq. 13b, 14b, 15b, and 16b were derived under a condition in which the activity of IMP was unity. Each of them was closely similar with Eq. 13a, 14a, 15a, and 16a, respectively. F tests indicated that difference in correlations between Eq. 13a and 13b, and between 14a and 14b, was statistically insignificant at the 0.90 level. Eq. 15a and 16a were significant at better than 0.95 level when compared with Eq. 15b ($F_{1,45}$=24.2)

Table IV. Observed and Calculated Flavor Enhancing Activities of 2-Substituted Inosine 5'-Phosphates

Substituent X	Number of measurements	Cs/Cx		
		Obsd.[a]	Calcd.[b]	Calcd.[c]
SC_3H_7	3	8.49 (0.50)	7.45	8.09
SC_2H_5	5	6.64 (1.20)	7.45	7.64
SCH_3	5	7.64 (0.60)	7.51	7.28
$OCH(CH_3)_2$	3	3.86 (0.40)	3.49	3.50
OC_2H_5	3	4.80 (0.71)	3.53	3.42
OCH_3	3	3.06 (0.24)	3.35	3.10
NH_2	3	2.78 (0.10)	2.73	2.37
$NHCH_3$	5	2.24[d] (0.12)	2.20	2.18
$N(CH_3)_2$	3	2.17 (0.11)	2.59	2.68
CH_3	5	2.20[d] (0.13)	2.32	2.41
C_2H_5	3	2.17 (0.03)	2.33	2.29
Cl	3	3.81 (0.29)	5.06	4.77
C_6H_5	3	3.58 (0.13)	2.52	3.00
H (IMP)	1	1		

a) The values in parentheses are the standard deviation.
b) calculated by using Eq. 15a
c) calculated by using Eq. 16a
d) from Ref. 15; all other values wer taken from Ref. 14

Table V. Predicted Flavor Enhancing Activities and Stimulus Thresholds of Some Ribonucleotide Derivatives

Substituent X	π[a]	σ_m[b]	S_{2a}	Cs/Cx		log 1/C[d]
				Calcd.[c]	Corrected	
Br	2.46	0.56	−0.047	6.19	4.66[e]	3.914
I	2.56	0.51	0.309	8.31	6.26[e]	4.102
p-$SCH_3C_6H_4$-	4.04	0.22	−0.296	3.73	5.30[f]	4.008
p-$N(CH_3)_2C_6H_4$-	3.52	0.22	−0.320	3.43	4.87[f]	3.926
$C_6H_5CH_2$-	3.92	0.26	−0.292	3.89		3.994

a) π values were recalculated from those in Ref. 21 by shifting the standard to NH_2-group (π NH_2=0).
b) σ_m values were recalculated from those in Ref. 21 and 22 by shifting the standard to NH_2-group NH_2=0).
c) calculated by using Eq. 16a
d) calculated by using Eq. 21
e) The calculated value (*c*) was multiplied by a factor, 0.75, the ratio of $(Cs/Cx)_{obsd.}/(Cs/Cx)_{calcd.}$ for 2-chloro-IMP in Table IV.
f) The value (*c*) was multiplied by a factor, 1.42, the ratio of $(Cs/Cx)_{obsd.}/(Cs/Cx)_{calcd.}$ for 2-phenyl-IMP in Table IV.

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and 16b ($F_{1,44}$=19.9), respectively. The calculated values of activities of the 2-substituted derivatives using Eq. 15a and 16a are given in Table IV with their observed values. The values of activities of some ribonucleotide derivatives, which had not yet been synthesized, are predicted as shown in Table V with their physicochemical parameters.

Relationship between observed stimulus thresholds of 15 kinds of 2-substituted derivatives (Table VI) and their physicochemical parameters (Tables II and III) was analyzed by Eq. 2. Using the method of least squares, Eqs. 17—25 were obtained.

$$\log 1/C = 0.221\pi + 3.383 \quad \text{Eq. 17}$$
$$n=15,\ r=0.618,\ s=0.241$$
$$\log 1/C = 0.680\sigma_m + 3.702 \quad \text{Eq. 18}$$
$$n=15,\ r=0.388,\ s=0.283$$
$$\log 1/C = 0.203\pi + 0.169\sigma_m + 3.383 \quad \text{Eq. 19}$$
$$n=15,\ r=0.623,\ s=0.250$$
$$\log 1/C = 0.012\pi^2 + 0.140\pi + 0.211\sigma_m + 3.444 \quad \text{Eq. 20}$$
$$n=15,\ r=0.624,\ s=0.260$$
$$\log 1/C = 0.575S_{2a} + 3.900 \quad \text{Eq. 21}$$
$$n=15,\ r=0.840,\ s=0.166$$
$$\log 1/C = 0.137\pi + 0.489S_{2a} + 3.600 \quad \text{Eq. 22}$$
$$n=15,\ r=0.915,\ s=0.129$$
$$\log 1/C = 0.478\sigma_m + 0.548S_{2a} + 3.791 \quad \text{Eq. 23}$$
$$n=15,\ r=0.882,\ s=0.150$$
$$\log 1/C = 0.115\pi + 0.209\sigma_m + 0.491S_{2a} + 3.601 \quad \text{Eq. 24}$$
$$n=15,\ r=0.921,\ s=0.130$$
$$\log 1/C = -0.0056\pi^2 + 0.144\pi + 0.190\sigma_m + 0.492S_{2a} + 3573 \quad \text{Eq. 25}$$
$$n=15,\ r=0.921,\ s=0.136$$

Among three monoparameter equations (Eq. 17, 18, 21), the most significant correlation was obtained for an electronic parameter S_{2a} (Eq. 21), and a significant correlation was observed for the hydrophobic parameter π (Eq. 17). The correlation coefficient of Eq. 18 was not significantly different from zero ($F_{1,13}$=2.13). Variance of the correlation was not reduced by the combination of π and σ_m, as in Eq. 19 and 20, in comparison with Eq. 17. In Eq. 22 and 23, we examined linear combinations of S_{2a} with π, and of S_{2a} with σ_m. Eq. 22 resulted in a statistically significant improvement in correlation ($F_{1,12}$=9.73), but Eq. 23

Table VI. Observed and Calculated Stimulus Thresholds of 2-Substituted Inosine 5′-Phosphate

Substituent X	log 1/C Obsd.[a]	log 1/C Calcd.[b]	|log 1/C|	Substituent X	log 1/C Obsd.[a]	log 1/C Calcd.[b]	|log 1/C|
$SCH_2CH=CH_2$[c]	4.365	4.212	0.153	$NHCH_3$	3.725	3.710	0.015
$SCH_2C_6H_5$[c]	4.303	4.408	0.105	$N(CH_3)_2$	3.711	3.806	0.095
SCH_2CH_3	4.140	4.185	0.045	CH_3	3.658	3.699	0.041
SCH_3	4.221	4.120	0.101	CH_2CH_3	3.560	3.772	0.212
$OCH_2CH=CH_2$	3.947	3.764	0.183	Cl	3.755	3.793	0.038
$OCH(CH_3)_2$	3.750	3.772	0.022	C_6H_5	3.945	3.850	0.095
OCH_2CH_3	3.849	3.737	0.112	H (IMP)	3.399	3.319	0.080
OCH_3	3.485	3.666	0.181				

a) from Ref. 14
b) calculated by using Eq. 21
c) For these compounds, we obtained S_{2a}=0.152 from the calculation of electron density.

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was not significant at the 0.90 level when compared with Eq. 21 ($F_{1,12}$=3.95). In Eq. 24 and 25 of the highest order approximation, the standard deviations were not improved as compared to Eq. 22. Observed and calculated values, using Eq. 22, of thresholds of the 2-substituted derivatives are given in Table VI.

Difference in flavor enhancing activities of a series of 2-S-substituted derivatives would not be explained adequately with parameters concerning the electronic state on the purine skeleton, since the parameters were little influenced by structural changes remote from the skeleton by $-SCH_2-$. Therefore, analysis of structure-activity relationship for 15 kinds of 2-S-substituted derivatives in Table VII was made according to Eq. 3 with the parameters in Table III which were obtained from physicochemical properties of the substituents in the compounds. Using the method of least squares, Eq. 26—31b were obtained from the data in Table VII.

$$\log C_S/C_X = -0.127\pi + 1.0444 \quad \text{Eq. 26}$$
$$n=46,\ r=0.558,\ s=0.175$$

$$\log C_S/C_X = -0.107\pi^2 + 0.182\pi + 0.9232 \quad \text{Eq. 27}$$
$$n=46,\ r=0.749,\ s=0.142$$

$$\log C_S/C_X = -0.067\pi^2 + 0.035\pi + 4.24S + 0.9806 \quad \text{Eq. 28}$$
$$n=46,\ r=0.873,\ s=0.105$$

$$\log C_S/C_X = -0.052\pi^2 - 0.032\pi + 2.43S - 13.3Q + 0.221v + 0.9187 \quad \text{Eq. 29a}$$
$$n=46,\ r=0.917,\ s=0.088$$

$$\log C_S/C_X = -0.060\pi^2 + 2.45S - 14.7Q + 0.217v + 0.9162 \quad \text{Eq. 29b}$$
$$n=46,\ r=0.916,\ s=0.088$$

$$\log C_S/C_X = -0.125\pi^2 + 0.273\pi + 0.259D + 0.8101 \quad \text{Eq. 30}$$
$$n=46,\ r=0.880,\ s=0.103$$

$$\log C_S/C_X = -0.090\pi^2 + 0.137\pi + 3.48S + 0.216D + 0.8762 \quad \text{Eq. 31a}$$
$$n=46,\ r=0.950,\ s=0.068$$

$$\log C_S/C_X = -0.051\pi^2 + 4.29S + 0.181D + 0.9486 \quad \text{Eq. 31b}$$
$$n=46,\ r=0.934,\ s=0.077$$

Relation of flavor enhancing activity with hydrophobic parameter π was examined in Eqs. 26 and 27. The use of squared term resulted in a statistically significant improvement in correlation ($F_{1,43}$=24.5) over the simple linear relation. By combining π^2, π, and S, Eq. 28 was obtained and the correlation was much improved. Using all of the parameters in Table III, we obtained Eq. 29a which rationalized 84.1% of the variance in the data. Eq. 29a was significant at better than 0.99 level when compared with Eq. 28 ($F_{2,40}$=9.87). Comparison of Eq. 29a with 29b, which was obtained by omitting π term from Eq. 29a, indicated that the former was not more significant over the latter ($F_{1,40}$=0.294).

In Eq. 30—31b, we examined the use of a dummy variable D which was given the value of 1.0 to the compounds having oxygen atom at the γ- or δ-position from the sulfur atom in substituents attached to the 2-position of the purine skeleton, such as 2-ethoxyethylthio-IMP. D has no effect upon other compounds. An extremely good correlation was obtained for Eq. 31a and F test indicated that π term in Eq. 31a was justified at better than 0.99 level of significance when compared with Eq. 31b ($F_{1,41}$=13.0). The optimal π value estimated from Eq. 31a was 0.76. The calculated values of activities of the 2-Substituted derivatives using Eq. 28b and 31a are given in Table VII.

In Eqs. 9—16b and 26—31b, all individual values were taken into calculation in the analysis. The advantage of this method, compared to the method using the average of individual measurements on each compound, is that all individual measurements are reflected in the regression analysis. However, when the number of measurements on each compound

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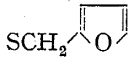
Table VII. Observed and Calculated Flavor Enhancing Activities of S-Substituted 2-Mercaptoinosine 5′-Phosphates

Substituent X	Number of measurements	C_S/C_X Obsd.[a]	C_S/C_X Calcd.[b]	C_S/C_X Calcd.[c]
	3	17.29 (0.61)[d]	17.32	19.98
$SCH_2CH_2COOC_2H_5$	3	12.06 (1.64)	9.27	11.04
$SCH_2CH_2OC_2H_5$	3	11.64 (1.34)	9.34	10.87
$SCH_2CH{=}C(CH_3)_2$	3	10.85 (1.29)	9.69	10.11
$SCH_2CH{=}CHCH_3$	3	9.35 (1.10)	9.95	10.23
$SCH_2CH{=}CH_2$	3	9.20 (0.41)	11.00	9.99
$SCH(CH_3){=}CH_2$	3	8.88 (0.49)	10.38	9.86
$SCH_2CH_2CH_3$	3	8.49 (0.50)	7.28	7.32
$SCH_2C_6H_5$	3	7.20 (0.81)	8.20	6.25
$SCH_2CH{=}CHC_6H_5$	3	6.07 (0.83)	4.50	4.81
$SCH_2CH(CH_3)_2$	3	6.95 (0.32)	5.97	6.45
$SCH_2CH_2CH(CH_3)_2$	1	5.36	5.34	5.92
$SCH_2CH\langle^{C_2H_5}_{C_4H_9}$	2	1.34 (0.04)	1.75	1.80
SCH_2CH_3	5	6.64 (1.20)	8.36	7.55
SCH_3	5	7.64 (0.60)	8.24	7.52

a) from Ref. 14; *b*) calculated by using Eq. 29b; *c*) calculated by using Eq. 31a; *d*) standard deviation

varies widely, the compounds having a small number of measurements cannot adequately contribute to the regression analysis. As an attempt to remove this contradiction, we newly adopted the reciprocals of variances of observed taste effects on each compound as a weight in the least squares fit using the average of individual measurements. By this technique, Eq. 32—35 were obtained corresponding to formerly obtained Eq. 15a, 16a, 29b, and 31a, respectively.

$$\log C_S/C_X = 0.824\sigma_m + 0.430S_{2a} + 0.4118 \qquad \text{Eq. 32}$$
$$r=0.940,\ s=0.094$$
$$\log C_S/C_X = 0.059\pi + 0.583\sigma_m + 0.450S_{2a} + 0.3553 \qquad \text{Eq. 33}$$
$$r=0.961,\ s=0.080$$
$$\log C_S/C_X = -0.074\pi^2 + 1.12S - 24.0Q + 0.287v + 0.9461 \qquad \text{Eq. 34}$$
$$r=0.921,\ s=0.114$$
$$\log C_S/C_X = -0.117\pi^2 + 0.189\pi + 2.60S_{2a} + 0.202D + 0.8579 \qquad \text{Eq. 35}$$
$$r=0.953,\ s=0.088$$

Discussion

The primary process of the taste response is a reaction in which the tasting compound interacts directly with the taste bud receptor site. This mechanism makes a strong contrast with that of many drugs which have to cross many membranes before making their way from the point of application to the site of action buried within a cellular organelle. In the case of taste response, complex processes of elimination, metabolism, and adsorption by plasma or tissue protein also deserve little consideration. It is thought, therefore, that this is one of the most suitable examples for the analysis of structure-activity relationship. In many enzymic reactions, it is apparent that a steric factor in substrates or inhibitors plays an important role.[24] In the present study on the relationship between taste effect and physico-

24) C. Hansch and E. Coats, *J. Pharm. Sci.*, **59**, 731 (1970).

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chemical parameters of ribonucleotide derivatives, good correlations were obtained using local electronic parameters and hydrophobicity without taking steric effect into account.

Analyses were made separately for two groups, 2-substituted inosine 5′-phosphates and S-substituted 2-mercaptoinosine 5′-phosphates. In the series of 2-substituted derivatives, it has been recognized that both the flavor enhancing activity synergistic with MSG and the threshold are influenced by the electrophilic superdelocalizability of atoms or atomic groups at the 2a-position of the compounds and the more increased the superdelocalizability, the stronger are the synergistic activity and the lower the threshold. Since increased superdelocalizability at the 2a-position tends to increase superdelocalizabilities and electron densities at the 1, 6a, and 7 positions, as can be seen from Table II, structure activity relationships similar to S_{2a} will be expected for parameters, S_1, S_{6a}, S_7, Q_{5a}, Q_1 or Q_7, although they are somewhatpoorer. Therefore, it is not clear whether one or more than two of these positions are concerned in the taste effect.

The threshold is also influenced by the hydrophobic parameter π, but little influenced by the electronic parameter σ_m. On the other hand, the synergistic activity is not so much influenced by the term π as by the term σ_m. Since the threshold was measured as the lowest concentration at which the induction of the stimulus on the receptor is detected by panelists, affinity of the tasting compounds with the receptor may play a major role, whereas both the affinity and intrinsic activity of the compounds will be responsible for the synergistic activity. Such a difference might result in inconsistency between structure-activity correlations of the threshold and synergistic activity. Dependence of the synergistic activity upon σ_m suggests that the electronic state on the C=O group at the 6-position plays an important role when tasting compounds from complexes with the taste bud.

The agreement between calculated and observed values in Tables IV and VI is fairly good except the synergistic activity of 2-ethoxy, 2-chloro, and 2-phenyl derivatives. Especially the values of the synergistic activity for 2-chloro and 2-phenyl derivatives calculated by using Eq. 15a deviate from the observed ones at higher than 0.95 level of significance. In 2-phenyl-IMP. there may be a question in the estimation of resonance energy between 2 and 2a-position in the calculation of electron density (0.7β used in this case). Also, the superdelocalizability at the 2a-position would be unsuitable for a parameter in this case. All of the substituents at the 2-position used in the present analysis are electron-releasing groups. The large deviation in 2-chloro-IMP will be due to the electron-attracting nature of the chlorine atom. The predicted flavor enhancing activities (C_S/C_X) of 2-halogeno-IMP and 2(*p*-substituted phenyl)-IMP in Table V were, therefore, corrected empirically for the electronic natures mentioned above.

In a series of 2-S-substituted derivatives, the electronic state on the purine moiety is little affected by the chemical structure designated by X in Table VII, and hydrophobicity, electronic state, and steric effect of the substituents themselves should be considered. Since suitable steric parameters, which cover all the substituents used in this study are unfortunately not available, we used the hydrophobic and electronic parameters in the analysis. There are, however, several compounds which have no atom at the γ- or δ-position to the sulfur atom in the substituents and, therefore, we chose the electronic state of the methylene protons in the $-CH_2S-$ group for the parameter. The results obtained from Eq . 28, 29a, and 29b indicate that the increased superdelocalizability at the methylene protons increases the synergistic activity. Combining these results with the results obtained from the analysis of the 2-substituted derivatives described before, it will be predicted that taste effects of ribonucleotide derivatives are influenced by the elctrophilic superdelocalizability around the 2a-position. A noteworthy result was obtained when a dummy variable, which represents an electron donating oxygen atom at the γ- or δ-position, was used as a parameter. The positive sign of the coefficient of the dummy variable in Eq. 30 and 31a suggests that there is an electron acceptor in taste bud receptor site which has an affinity for oxygen atom four

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or five atoms away from the purine skeleton. In this series, the hydrophobicity of the substituents plays a significant role. Where the dummy variable is considered constant, the optimal π value (π_0=0.76) estimated from Eq. 31a suggests that, in view of the hydrophobicity, 2-allylthio and 2-propylthio derivatives are the most suitable.

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