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Conformational Analysis of Prostaglandins. III.[1] Study on Active Sites and Conformation-Action Relationship[2]

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A physicochemical and mathematical model of the mechanism of physiological action was assumed, and the model was applied on prostaglandins. A conformation-action relationship was studied using the model, and high values of correlation coefficients were obtained. Biological activities of prostaglandins on rat uterus, guinea pig ileum, and rabbit jejunum were explained using the model. Active sites of prostaglandins were derived from the mathematical model. They are the oxygen atom bonded to the carbon atom C_1, the oxygen atom bonded to C_9, the oxygen atom bonded to C_{15}, and the carbon atom C_5 or C_{20}.

Keywords——active site; conformation; conformation-activity relationship; conformational analysis; conformational energy; prostaglandins; receptor; computer experiment

Introduction

Many studies on conformation-action relationship have been carried out to study the mechanism of the action of physiologically active compounds. In most cases, a conformational information obtained by X-ray diffraction studies has been used in these studies. But the conformation estimated by X-ray diffraction studies is the conformation in the crystalline state. Physiologically active compounds act not only in a crystalline state but in a liquid phase, so that the conformation estimated by X-ray diffraction study do not always afford informations adequate to the studies on the mechanism of action of the compounds. In a liquid phase, the compounds may not have only one conformation but many kinds of conformations, especially in the case of flexible molecules such as prostaglandins studied in this paper.

The main purpose of this paper is to study the mechanism of the action of physiologically active compounds. The most simple physicochemical and mathematical model of action was assumed for the aforementioned purpose, using which the conformation-action relationship was quantitatively interpreted. The conformation and the conformational information used in this study were obtained by the method of computer experiments, of which details were described in our previous papers.[1,4]

Prostaglandins were chosen as an example of physiologically active compounds. The prostaglandins used in this paper were PGE_1, 15-epi PGE_1, 11-epi PGE_1, 11,15-epi PGE_1, PGA_1, 15-epi PGA_1, $PGF_{1\alpha}$, $PGF_{1\beta}$, and PGB_1. These structural formulae and the numbering of the carbon atoms in prostaglandin molecules were given in the previous paper.[1]

Model

The assumed physicochemical model for the action of prostagladins is described in equation (1).

1) Part II: A. Murakami and Y. Akahori, *Chem. Pharm. Bull.* (Tokyo), **25**, 2870 (1977).
2) A part of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.
3) Location: *2–1, Oshika 2-chome, Shizuoka 422, Japan.*
4) A. Murakami and Y. Akahori, *Chem. Pharm. Bull.* (Tokyo), **22**, 1133 (1974).

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$$\mathbf{P} + \mathbf{R} \longrightarrow \mathbf{P}\cdots\mathbf{R} \longrightarrow \mathbf{P}' + \mathbf{R}, \quad (1)$$

where **P** is a prostaglandin, **R** a receptor for the prostaglandin, **P**···**R** an intermediate comprising a prostaglandin and its receptor, and **P**′ a metabolite. The intermediates **P**···**R** are responsible for pharmacological actions of prostaglandins. Some factors, *e.g.* inhibition by metabolites were neglected in this case to simplify the model, but the model might be sufficient to consider approximately the mechanism of action of prostaglandins.

If intermediates **P**···**R** cause pharmacological actions, there would be some quantitative relationships between potency of prostaglandins and concentration of **P**···**R**. It is, however, very difficult to know the concentration of **P**···**R** for each prostaglandin directly, because there have been obtained very poor information about receptors. The first stage of the action of prostaglandins is interaction between a prostaglandin **P** and a receptor **R**. Prostaglandins take suitable conformations to interact with receptors when the prostaglandins approach the receptors. Therefore, the concentration of suitable conformations of a prostaglandin is the most important factor to exhibit the activity.

A mathematical model was introduced for further and quantitative discussion of conformation–action relationship of the prostaglandins. Not all atoms within a physiologically active compound is responsible for pharmacological actions, so that there are some active sites comprising one or some atoms in the compound. The active sites in the compound interact or bond weakly with active sites of a receptor. The other atoms within the compound, called non-active sites, are not responsible for pharmacological actions when the physiologically active compound acts. Therefore, the active sites must be in sterically particular positions one another, but non-active sites must not be in specific positions.

These concepts introduce a mathematical description of prostaglandin (2). In equation (2), the concept of set and some popular symbols of set are used, and in the following equations the symbols of set are also used.

$$\boldsymbol{PG} = \{a_i, n_j;\ i=1, \cdots, k,\ j=1, \cdots, 20\text{-}k\}, \quad (2)$$

where a_i is the ith active site centered on the ith carbon atom, n_j the jth non-active site centered on jth carbon atom. The active site a_i comprises the carbon atom C_i and O or H atoms bonded to C_i. Active sites are classified as main active sites or subactive sites according as they bond weakly or do not directly bond to active sites of receptors. Therefore, equation (2) is rewritten in the following equation.

$$\boldsymbol{PG} = \{am_i, as_l, n_j;\ i=1, \cdots, q,\ l=1, \cdots, k\text{-}q,\ j=1, \cdots, 20\text{-}k\}, \quad (3)$$

where am_i is the ith main active site and as_l is the lth subactive site. As described above, the non-active site n_j is not responsible to exhibit activities of prostaglandins, so that non-active sites can be neglected. Therefore, prostaglandins are described by equation (4),

$$\boldsymbol{PG} = \{a_i;\ i=1, \cdots, k\} = \{am_i, as_l;\ i=1, \cdots, q,\ l=1, \cdots, k\text{-}q\}. \quad (4)$$

For further detailed discussion, Cartesian space of three dimension is introduced in the description. The center of the ith active site is given as a point within the space, and given by the following equation,

$$\boldsymbol{R}^3 = \boldsymbol{R} \times \boldsymbol{R} \times \boldsymbol{R}, \quad (5)$$

$$a_i = (x_1{}^i, x_2{}^i, x_3{}^i) \qquad (x_1{}^i, x_2{}^i, x_3{}^i \in R), \quad (6)$$

where $\boldsymbol{R}^3$ is Cartesian space, $\boldsymbol{R}$ a set of real numbers, and $(x_1{}^i, x_2{}^i, x_3{}^i)$ coordinates of the point a_i in the space $\boldsymbol{R}^3$. The distance between a_i and a_j of $d^{(3)}(a_i, a_j)$ can be calculated by equation (7), using these description.

$$d^{(3)}(a_i, a_j) = \sqrt{(x_1{}^i - x_1{}^j)^2 + (x_2{}^i - x_2{}^j)^2 + (x_3{}^i - x_3{}^j)^2}, \quad (7)$$

where $d^{(3)}$: $\boldsymbol{R}^3 \times \boldsymbol{R}^3 \rightarrow \boldsymbol{R}$ is a distance function in the space $\boldsymbol{R}^3$. The same description can be given on a main active site *am* and a sub-active site *as*.

The conformation of prostaglandins responsible for pharmacological actions are given by the following equation.

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$$\boldsymbol{PG}^* = \{a_i;\ i=1, \cdots, k, \bigcap_{i=1}^{k} U(a_i;\varepsilon) \supset R_i\}, \tag{8}$$

where R_i is the ith active site in a receptor and $U(a_i;\ \varepsilon)$ is ε-neighborhood around the ith active site in the prostaglandin. The ε-neighborhood $U(a_i;\ \varepsilon)$ is given in a metric space of $(\boldsymbol{R}^3,\ d^{(3)})$ by the following equation.

$$U(a_i;\varepsilon) = \{x; x \in \boldsymbol{R}^3, d^{(3)}(x,a_i) < \varepsilon\}. \tag{9}$$

As described above, main active sites bond weakly or interact with active sites of receptors, and sub-active sites have no intermolecular steric hindrance between receptors. Therefore, equation (8) is rewritten in the following form

$$\boldsymbol{PG}^* = \{am_i,\ as_j;\ i=1, \cdots, q,\ j=1, \cdots, k-q,$$
$$\bigcap_{i=1}^{q} U(am_i;\ \varepsilon) \supset R_i,\ \bigcap_{j=1}^{k-q} U(as_j;\ \varepsilon) \not\supset R_s (s=1, \cdots, m)\}, \tag{10}$$

where

$$\boldsymbol{RE} = \{R_s;\ s=1, \cdots, m\} \tag{11}$$

represent receptors.

Natural prostaglandins and their *enantiomers* show quite different potency.[5] To explain this property, four points at least are required as active sites; three main active sites and one sub-active site. In this model, prostaglandins are classified as natural types or their *enantiomers* by the arrangement of the four active points, just as right or left configurations are distinguished from four different atoms or fuuctional groups bonded to an asymmetric carbon atom. Therefore, the most simple description of prostaglandins is given by the following equation,

$$\boldsymbol{PG} = \{a_i;\ i=1,2,3,4\} = \{am_i,\ as_j;\ i=1,2,3,\ j=1\} \tag{12}$$

The prostaglandin responsible for a pharmacological action is described in the form

$$\boldsymbol{PG}^* = \{am_i,\ as_j;\ i=1,2,3,\ j=1,\ \bigcap_{i=1}^{3} U(am_i;\ \varepsilon) \supset R_i,$$
$$U(as_1;\ \varepsilon) \not\supset R_s (s=1, \cdots, i, \cdots, m)\}. \tag{13}$$

Only very poor informations of a molecular structure is known about receptors, but the molecular weight of receptors may be larger than that of prostaglandins. Therefore, receptors are more sterically stable than prostaglandins, and the interatomic distances between active sites in receptors would be non-variable. Each interatomic distance between four active sites of the prostaglandin responsible for pharmacological action must take a specific value.

The conformation of prostaglandins described by equation (12) should be sterically allowed conformations calculated by the computer experiment described in our previous paper.[4] The conformations responsible for pharmacological actions described by equation (13) are collected from sterically allowed conformations of the prostaglandin, and the conformations have the active sites at specific distance from one another. The population of each conformation is calculated from statistical mechanics using conformational energy, and this calculation is carried out on each conformation of a prostaglandin.

A quantitative conformation-action relationship was calculated by comparing potencies with total population of the conformations responsible for pharmacological action of each prostaglandin. Not only one conformation but all sterically allowed conformations were considered, so that this paper offers more valuable informations than the other studies. Polar functional groups in prostaglandins should be main active sites, because main active sites interact with or are weakly bonded to receptors. Three polar groups being common to all prostaglandins were assumed as the main active sites, and two of other atoms were assumed as sub-active sites. These active sites are illustrated in Fig. 1. The calculations were carried out using the assumed main active sites, one of the two sub-active sites, and equation (13).

5) P.W. Ramwell, J.E. Shaw, E.J. Corey, and N. Andersen, *Nature* (London), **221**, 1251 (1969).

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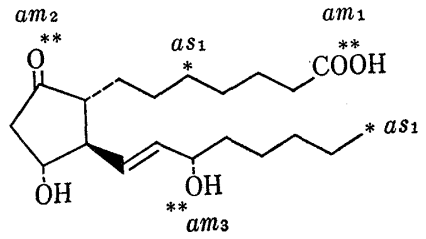

Fig. 1. Assumed Main Active Sites and Sub-active Sites

**: main active site.
*: sub-active site.

Pharmacological potencies of prostaglandins were reported by Ramwell, *et al.*[5] In our paper, the potencies of original paper were converted into potencies expressed relative to Natural PGE_1 (=1.00) using another data reported in the same paper; the potencies of PGA_1 group were divided by 10, and the potencies of $PGF_{1\alpha}$ group were divided by 2. The converted potencies of prostaglandins are listed in Table I, and these values were used to be compared with populations calculated by the computer experiment.

The corrlation coefficient between population of the conformation and potencies of the prostaglandins is affected by the value of ε, and ε=0.3 Å was used in this paper. Affection by the value of ε was also studied.

Table I. Cross Assay of Authentic and Synthetic PGE, PGF, and PGA Compounds[a]

Prostaglandins	Rat uterus	Guinea pig ileum	Rabbit jejunum	Rat blood pressure
Natural PGE_1	1.00	1.00	1.00	1.00
Rac-PGE_1	0.44	0.5	0.4	0.57
Ent-PGE_1	0.001	—	0.0013	—
Natural 15-epi PGE_1	0.008	0.02	—	0.05
Rac-15-epi PGE_1	0.04	0.06	—	0.05
Rac-11-epi PGE_1	0.13	0.036	0.12	0.05
Rac-11,15-epi PGE_1	1.00	0.1	5.4	0.04
Natural PGA_1	0.10	—	0.05	1.0
Rac-PGA_1	0.073	—	0.027	1.0
Natural 15-epi PGA_1	0.042	—	—	0.16
Rac-15-epi PGA_1	0.10	—	0.10	0.16
Natural $PGF_{1\alpha}$	0.50	0.005	0.50	—
Rac-$PGF_{1\alpha}$	0.225	0.003	0.20	—

Potency expressed relative to Natural PGE_1 (=1.00).
a) P.W. Ramwell, J.E. Shaw, E.J. Corey, and N. Andersen, *Nature*, **221**, 1251 (1969).

Experimental

The equations and the values of parameters used in the calculation of conformational energies were all the same as in our previous paper.[4] The computation was programed in FORTRAN for use with a NEAC 2200-375 electronic computer (Nihondenki, Tokyo) at Computing Center of Shizuoka Prefectural Government.

Results

The sterically allowed conformations of prostaglandin E_1 (PGE_1) were grouped into ten different conformational groups from spatial arrangements of three main active sites and a sub-active site on the carbon atom C_5, and were grouped into twenty different groups when C_{20} was used as a sub-active site. These conformational groups and some typical interatomic distance between the active sites are listed in Table II and III.

Less calculation is required by the method in this paper to study a conformation-action relationship than other methods, because the method in this paper classifies the sterically allowed conformations into small number of conformational groups.

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Table II. Typical Interatomic Distance and Correlation Coefficient Used C_5 as Sub-active Site

Conformational Group	Interatomic distance				Correlation coefficient			
	am_1–am_2	am_1–am_3	am_2–am_3	am_3–as_1	Rat uterus	Guinea pig ileum	Rabbit jejunum	Rat blood press.
1	8.533	10.621	8.090	8.585	−0.061	0.619	−0.299	0.506
2	9.707	9.323	8.090	7.911	0.030	0.553	−0.176	0.079
3	10.253	13.153	8.090	8.585	−0.060	0.619	−0.299	0.514
4	11.103	12.236	8.090	7.911	−0.022	0.511	−0.218	0.280
5	9.437	13.046	8.090	8.585	0.019	0.691	−0.228	0.536
6	10.273	12.019	8.090	7.911	0.027	0.544	−0.176	0.074
7	9.852	13.810	8.090	8.585	−0.060	0.618	−0.299	0.513
8	11.246	11.817	8.090	7.911	0.028	0.547	−0.176	0.076
9	7.790	11.807	8.090	8.585	−0.063	0.618	−0.301	0.506
10	9.951	8.473	8.090	7.911	0.030	0.554	−0.176	0.079

Interatomic distance of each conformational group expressed interatomic distance of PGE_1.

Table III. Typical Interatomic Distance and Correlation Coefficient Used C_{20} as Sub-active Site

Conformational group	Interatomic distance				Correlation coefficient			
	am_1–am_2	am_1–am_3	am_2–am_3	am_1–as_1	Rat uterus	Guinea pig ileum	Rabbit jejunum	Rat blood press.
1	8.533	10.621	8.090	11.442	−0.200	0.482	−0.378	0.385
2	8.533	10.621	8.090	15.162	−0.146	0.507	−0.343	0.421
3	9.707	9.324	8.090	5.214	0.048	0.649	−0.182	0.118
4	9.707	9.324	8.090	8.194	0.034	0.644	−0.191	0.106
5	10.253	13.153	3.090	12.326	−0.020	0.580	−0.236	0.461
6	10.253	13.153	8.090	16.507	−0.073	0.544	−0.288	0.456
7	11.103	12.236	8.090	8.113	0.014	0.621	−0.214	0.334
8	11.163	12.236	8.090	11.840	0.004	0.611	−0.221	0.371
9	9.437	13.046	8.090	12.103	0.031	0.636	−0.201	0.266
10	9.437	13.046	8.090	16.076	−0.020	0.580	−0.236	0.461
11	10.273	12.019	8.090	8.620	0.063	0·653	−0.172	0.130
12	10.273	12.019	8.090	12.540	0.063	0.653	−0.172	0.130
13	9.852	13.810	8.090	13.444	0.031	0.636	−0.201	0.266
14	9.852	13.810	8.090	17.357	−0.073	0.544	−0.288	0.456
15	11.246	11.817	8.090	7.641	0.063	0.653	−0.172	0.130
16	11.246	11.817	8.090	11.888	0.063	0.653	−0.172	0.130
17	7.790	11.807	8.090	13.199	−0.123	0.540	−0.321	0.182
18	7.790	11.807	8.090	16.531	−0.155	0.504	−0.349	0.416
19	9.951	8.473	8.090	4.000	0.063	0.653	−0.172	0.130
20	9.951	8.473	8.090	8.298	0.063	0.653	−0.172	0.130

Interatomic distance of each conformational group expressed interatomic distance of PGE_1.

a) Result using C_5 as the Sub-active Site

The correlation coefficients between the population of each conformational group and potencies of various biological activities of prostaglandins are listed in Table II. Table II shows that the population of the group 1,3,5,7 and 9 is correlated with the biological potency of prostaglandins on guinea pig ileum, and the correlation coefficients are 0.619, 0.619, 0.691, 0.618, and 0.618, respectively. Interatomic distance am_3-C_5 of all conformers classified into the conformational group 1,3,5,7 and 9 is 8.585 Å, and it suggests that C_5 of sub-active site is responsible for the pharmacological action on guinea pig ileum. The feature of the conformational group 5 is illustrated in Fig. 2.

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b) Result using C_{20} as the Sub-active Site

The correlation coefficients between the population of each 20 conformational group and potencies of biological activities of prostaglandins are listed in Table III. Table III suggests that the population of conformational groups listed in Table III is correlated with the potency of guinea pig ileum of prostaglandins, but interatomic distance reponsible for the pharmacological actions was not found in this case.

As described above, good conformation-action relationship was obtained about the biological activity of prostaglandins on guinea pig ileum, but poor relationship was found about other biological activities of prostaglandins. The sterically allowed conformations of each prostaglandin were compared with 10 or 20 conformational groups listed in Table II or III, and conformations different from the conformations listed in Table II or III were rejected by the restriction of the electronic computer used in this study. These conformations would be responsible for the action of prostaglandins on rat uterus, *etc.* Therefore, the following studies were also carried out.

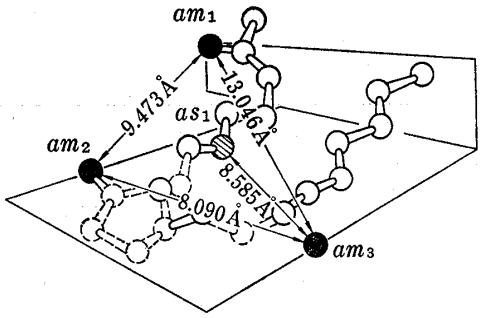

Fig. 2. View of the Conformation Responsible for the Pharmacological Action of Prostaglandins

Active sites of am_2, am_3 and as_1 lie on the same plane.

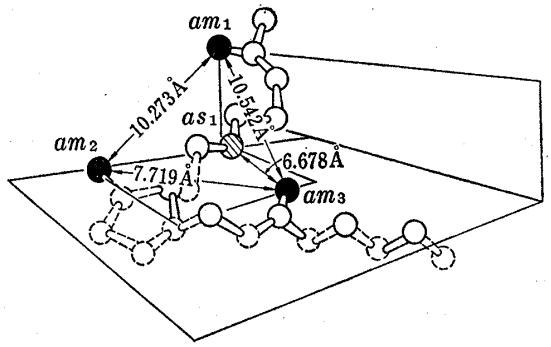

Fig. 3. View of the Conformation Responsible for the Pharmacological Action of Prostaglandins

Active sites of am_2, am_3 and as_1 lie on the same plane.

TABLE IV. Typical Interatomic Distance and Correlation Coefficient Used C_5 as Sub-active Site

Conformational group	Interatomic distance				Correlation coefficient			
	am_1–am_2	am_1–am_3	am_2–am_3	am_3–as_1	Rat uterus	Guinea pig ileum	Rabbit jejunum	Rat blood press.
1	8.533	10.778	7.719	7.949	0.418	−0.081	0.563	−0.348
2	9.707	7.365	7.719	6.678	0.663	−0.012	0.793	−0.314
3	10.253	12.755	7.719	7.949	0.411	−0.018	0.557	−0.348
4	11.103	10.550	7.719	6.678	0.658	−0.036	0.794	−0.329
5	9.437	12.416	7.719	7.949	0.409	−0.083	0.555	−0.348
6	10.273	10.542	7.719	6.678	0.669	−0.011	0.799	−0.314
7	9.852	13.408	7.719	7.949	0.413	−0.082	0.559	−0.348
8	7.790	11.910	7.719	7.949	0.418	−0.081	0.564	−0.348
9	9.951	6.906	7.719	6.678	0.662	−0.012	0.792	−0.315

Interatomic distance of each conformational group expressed interatomic distance of 15-epi PGE_1.

c) Classification centered on 15-epi PGE_1

Sterically allowed conformations of 15-epi PGE_1 were classified into nine conformational groups, and these groups and four typical interatomic distance between the active sites were listed in Table IV. The correlation coefficients between the population of each conforma-

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tional group and the potencies of prostaglandins were also listed in Table IV. The atom C_5 was used as the sub-active site.

Table IV shows that the population of the conformational group 2,4,6 and 9 is correlated with the potencies of prostaglandins on rat uterus and rabbit jejunum, especially highly correlated with the potency on rabbit jejunum. The values of correlation coefficients of conformational group 2,4,6 and 9 on rat uterus are 0.663, 0.658, 0.669, and 0.662, respectively. The values of correlation coefficients of conformational group 2,4,6 and 9 on rabbit jejunum are 0.793, 0.794, 0.799, and 0.792, respectively. Interatomic distance am_3-C_5 of all conformations classified into groups 2,4,6 and 9 are 6.678 Å, and it suggests that C_5 of sub-active sites is responsible for the action on rat uterus and rabbit jejunum. The feature of conformational group 6 is shown in Fig. 3.

d) Dependency of the Correlation Coefficients on the Value of ε

The correlation coefficient is affected by the value of ε. Change of correlation coefficients by the value of ε is listed in Table V.

Table V. Dependency of the Correlation Coefficient on the Value of ε

ε	Rat uterus	Guinea pig ileum	Rabbit jejunum	Rat blood press
$\varepsilon=0.3$	0.658	−0.036	0.794	−0.329
$\varepsilon=0.4$	0.480	−0.067	0.624	−0.345
$\varepsilon=0.5$	0.475	−0.068	0.620	−0.345
$\varepsilon=1.0$	0.362	−0.193	0.538	−0.028

Conformation: am_1-am_2=11.103 Å,
am_1-am_3=10.550 Å,
am_2-am_3= 7.719 Å,
am_3-as_1 = 6.678 Å.

Discussion

Many studies have been already carried out on prostaglandins, but there is no case in which high values of correlation coefficients were found. We have already studied on structure-action, energy-action, and other relationship of prostaglandins, but no relationship has been found, having higher correlation coefficients than conformation-action relationship studied in this paper. The method described in this paper is more suitable than other method to study active sites or mechanism of actions of physiologically active compounds especially prostaglandins. It was shown that three main active sites and two sub-active sites assumed in this paper are reasonable.

It is necessary to study other factors *e.g.* electronic structure, solubility of prostaglandins, *etc.*, but prostaglandins exhibit pharmacological activities in very low concentration,[6] and the concentration of prostaglandins in a neighborhood of receptors might be far lower than the saturated concentration. Prostaglandins exhibit quite different pharmacological activities between a natural compound and its *enantiomer*, although they have the same electronic structure. These evidences suggest the importance of conformations. Therefore, the conformational investigation in this paper would be one of the most important approach to discuss the actions of physiologically active compounds.

The population of sterically allowed conformations are affected by their conformational energies. The conformational energies contain electrostatic and nonbonded energies, while

6) M. Sakamoto and M. Kigawa, *Igaku-no-Ayumi*, **81**, 237 (1972).

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hydrogen bonding energies and electronic energies were neglected in this paper. The reason to neglect these energies was described in our previous paper.[4]

Some further discussions would be necessary to make the method described in this paper more useful. It is necessary to study more suitable active sites especially sub-active sites, values of ε, and parameters used in the calculation of conformational energies. The values of van der Waals radii used in the calculation of nonbonded energy were determined by experiment, in a nonpolar solution of carbon tetrachloride using model compounds which have the same functional groups as prostaglandins.[7] Accordingly, it is necessary for further discussions to obtain suitable values of van der Waals radii in an aqueous solution.

Acknowledgement The authors wish to thank Computing Center of Shizuoka Prefectural Government for performance of our calculation.

7) A. Murakami, Z. Shiba, N. Murakami, and Y. Akahori, unpublished data, in preparation.

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