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Partition Behavior and Ion Pair Formation of Some Prostaglandins[1)]

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The pK_a and intrinsic partition coefficient (PC_0) of seven prostaglandins (PGE_1, PGE_2, PGA_1, PGA_2, PGB_1, PGB_2, and $PGF_{2\alpha}$) were determined from their pH–partition profiles. Structural changes of the prostaglandins were largely reflected in PC_0 values rather than the pK_a values. Partition of the prostaglandins from aqueous buffer (pH 7.0) to cyclohexane containing various alcohols was markedly enhanced by the addition of aliphatic amines in aqueous phase. The influences of temperature and chain length of both alcohol and amine on partition coefficients of the prostaglandins were investigated. Furthermore, extraction constants (K_e) for prostaglandin-amine ion pairs and number of alcohol molecules associated with ion pair (n) were determined to gain insight into the mechanism of enhanced extraction process.

Keywords——partition behavior of the prostaglandins; pK_a values of the prostaglandins; ion pair formation; aliphatic amines; extraction constant; alcohol solvation of ion pair; thermodynamic parameters; hydrophobic interaction

Although some naturally occurring prostaglandins such as prostaglandin E_1, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ have been successfully used for clinical application,[3)] studies on

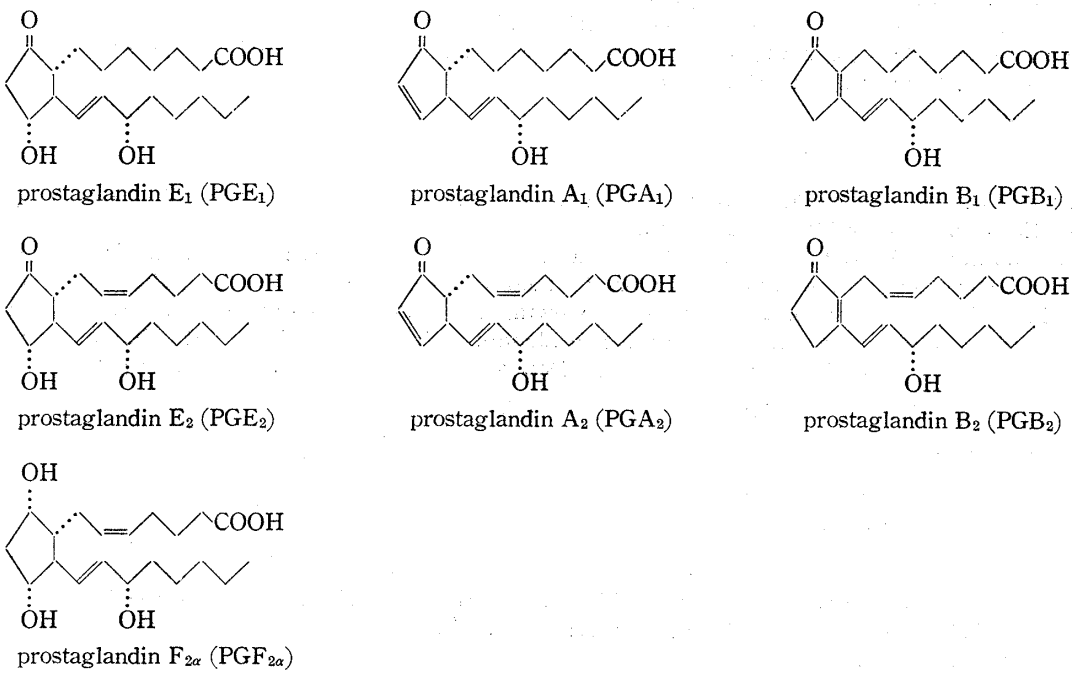

Chart 1. The Prostaglandins used in This Study

1) A part of this study was presented at the 97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1977.
2) Location: *5-1, Oe-honmachi, Kumamoto 862, Japan.*
3) E.M. Southern, ed., "The Prostaglandins: Clinical Application in Human Reproduction," Futura Publishing, Mount Kisco, N.Y., 1972.

their physicochemical properties in solution have been very limited.[4] The present study deals with the partition behavior of several prostaglandins[5] and their ion pair formation with aliphatic amines in solution. The influences of variables, such as pH, temperature, chain length of the amines on the partition coefficients of prostaglandins between aqueous buffer and cyclohexane containing various alcohols were investigated in details. This kind of knowledge will provide not only a rational basis for design of formulation but also an insight into physiological absorption pattern of the drugs, from the pharmaceutical point of view.

Experimental

Materials——All the prostaglandins used were favored from Ono Pharmaceutical Co., Ltd. Amines were commercially obtained and their purities were checked by gas chromatography.[6] All other materials and solvents were analytical reagent grade.

Procedure of Partition Study——Aqueous phase was prepared by the dissolution of the prostaglandins (1.0×10^{-4} M, in general) and amines, when necessary, in 0.1 M phosphate buffer. The pH and ionic strength ($\mu=0.2$) were adjusted to appropriate value with 0.1 M NaOH, 0.1 M H_3PO_4, and NaCl. Organic phase was prepared by the mixing of various amounts of alcohol and cyclohexane, and saturated with buffer solution to minimize the volume change due to mutual miscibility. A 5 ml of each solution was taken in glass stoppered L-shape tube and shaken for one hour in thermostated water bath ($\pm0.1°$). After separation by centrifugation, an aliquot of the aqueous phase was assayed by the procedures described previously.[7]

Partition coefficient was defined as the ratio of the equilibrium concentration in organic phase to that in aqueous phase as follows:

$$PC_o = \frac{(PGH)_o}{(PGH)_w} \tag{1}$$

$$PC' = \frac{(PGH)_o}{(PGH)_w + (PG^-)_w} \tag{2}$$

where PC_0 and PC' are intrinsic and apparent partition coefficients, $(PGH)_w$ and $(PG^-)_w$ are concentrations of unionized and ionized prostaglandin in aqueous phase, and $(PGH)_0$ is concentration of unionized prostaglandin in organic phase, respectively, in molar concentration unit.

Determination of pK_a and PC_0——These values were determined from pH–PC' profiles of the prostaglandins (see Fig. 1) according to Eq. (3):[8]

$$\frac{1}{PC'} = \frac{K_a}{PC_o}\cdot\frac{1}{(H^+)} + \frac{1}{PC_o} \tag{3}$$

Referring to Eq. (3), K_a and PC_0 were calculated from slope/intercept and 1/intercept, respectively, in the linear relationship between 1/PC' and $1/(H^+)$.

Determination of K_e and n——The calculation procedure is essentially same as that of previous paper.[9] Aqueous phase containing fixed amounts of prostaglandin (1.0×10^{-4} M) and amine (2.5×10^{-2} M) at pH 7.0 was equilibrated with organic phase composed of varying amounts of alcohol and cyclohexane. The extraction constants for ion pair, K_e, and the number of alcohol molecules associated with the ion pair, n, were evaluated from the plots of log PC_{obs} *versus* log $(M)_0$ according to following equation:[10]

$$\log PC_{obs} = n\cdot\log(M)_o + \log(A^+)_w + \log K_e \tag{4}$$

where K_e is defined as:

$$K_e = \frac{(PG^-\cdot A^+\cdot M_n)_o}{(PG^-)_w\cdot(A^+)_w\cdot(M)_o{}^n} \tag{5}$$

4) *a*) T.J. Roseman and S.H. Yalkowsky, *J. Pharm. Sci.*, **62**, 1680 (1973); *b*) T.O. Oesterling, W. Morozowich, and T.J. Roseman, *ibid.*, **61**, 1861 (1972); *c*) M.C.R. Johnson and L. Sounders, *Biochim. Biophys. Acta*, **218**, 543 (1970).
5) Chemical structures and abbreviations of the prostaglandins employed were in Chart 1.
6) I.H. Pitman, K. Uekama, T. Higuchi, and W.E. Hall, *J. Am. Chem. Soc.*, **94**, 8147 (1972).
7) K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, *J. Pharm. Sci.*, **66**, 706 (1977); K. Uekama and F. Hirayama, *Chem. Pharm. Bull.* (Tokyo), **26**, 1195 (1978).
8) A. Agren, R. Elofsson, and S.O. Nilsson, *Acta Pharm. Suecica*, **8**, 475 (1971).
9) K. Uekama, Y. Chiba, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **22**, 560 (1974); K. Tanaka, K. Uekama, T. Yotsuyanagi, and K. Ikeda, *Yakugaku Zasshi*, **98**, 1236 (1978).
10) T. Higuchi, A.F. Michaelis, T. Tan, and A. Hurwitz, *Anal. Chem.*, **39**, 974 (1967).

and PC_{obs} was experimentally determined which is defined as:

$$PC_{obs} = \frac{(PG^- \cdot A^+ \cdot M_n)_o}{(PG^-)_w} \quad (6)$$

In these equations, $(A^+)_w$ is concentration of amine cation in aqueous phase, $(M)_0$ and $(PG^- \cdot A^+ \cdot M_n)$ are concentration of alcohol and that of solvated ion pair in organic phase, respectively, in molar concentration unit.

Results and Discussion

pH-Partition Behavior of Prostaglandins

Partition behavior of seven prostaglandins[11] between cyclohexane containing *n*-octanol and aqueous buffer were investigated in the range of pH 2—7 at 25°. All the pH–PC' profiles showed a typical sigmoidal curve, as seen in Fig. 1 as an example. Then, pK_a and PC_o were determined according to Eq. (3) and are listed in Table I. It is noted that PC_o value rather

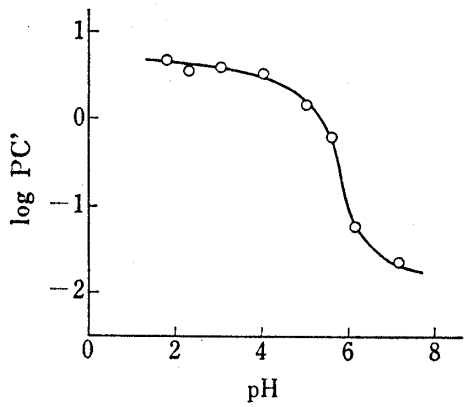

Fig. 1. The pH–PC' Profile of $PGF_{2\alpha}$ at 25°

Aqueous phase: 0.1 M phosphate buffer (μ=0.2).
Organic phase: cyclohexane+*n*-octanol (19:1 in volume).

Table I. pK_a and PC_o determined from pH-PC' Profiles of Prostaglandins

Prostaglandin	pK_a	PC_o
PGE_1	5.02	4.39
PGE_2	4.94	3.77
PGA_1	5.05	100
PGA_2	4.97	71.4
PGB_1	5.03	140
PGB_2	4.98	118
$PGF_{2\alpha}$	4.79	2.72

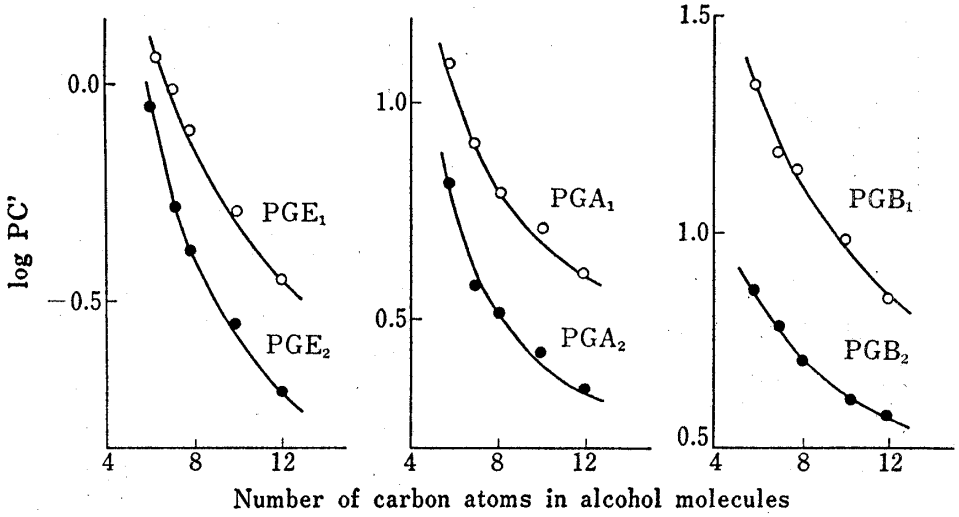

Fig. 2. Partition Coefficients of Some Prostaglandins between Cyclohexane and Aqueous Buffer (pH 7.0) in the Presence of Various Alcohols (20% in Volume) in the Organic Phase at 25°

11) Concentration of prostaglandins employed was lower than c.m.c. value (ref. 4*c*).

than pK_a value[12] was largely affected by the structural changes of the prostaglandins. The PC_o values for PGB_1, PGA_1, and PGE_1 were larger than those for PGB_2, PGA_2, and PGE_2 in corresponding order, which are in reverse of their physiological activity.[4b]

Effect of Alcohol on Partition Coefficient

Partition coefficients of prostaglandins between aqueous buffer (pH 7.0) and cyclohexane were measured in the presence of various alcohols in organic phase. As shown in Fig. 2, PC' generally decreased as increase in alcohol chain, because of the decrease in solvent polarity and/or the decrease in aqueous solubility.[13]

Table II summarizes the temperature effect on PC' in the presence of alcohol in organic phase. On the determination of thermodynamic parameters, van't Hoff plots fell fairly on

Table II. Thermodynamic Parameters[a] for Partition of $PGF_{2\alpha}$ from Cyclohexane to Aqueous Buffer (pH 7.0, $\mu=0.2$) in the Presence of Various Alcohol in Organic Phase at 25°

Alcohol added in organic phase[b]	log PC'	ΔG (cal/mol)	ΔH (cal/mol)	ΔS (cal/mol·deg)
1) *n*-Pentanol	1.25	−1710	1380	10.4
2) *n*-Hexanol	1.13	−1540	514	6.89
3) *n*-Heptanol	0.986	−1340	231	5.28
4) *n*-Octanol	0.982	−1340	−51.1	4.32
5) *n*-Nonanol	0.841	−1150	−634	1.72
6) *n*-Decanol	0.678	−925	−682	0.82
7) *n*-Dodecanol	0.761	−1040	−934	0.35

a) Accuracy of ±5%.
b) 50% in cyclohexane (v/v).

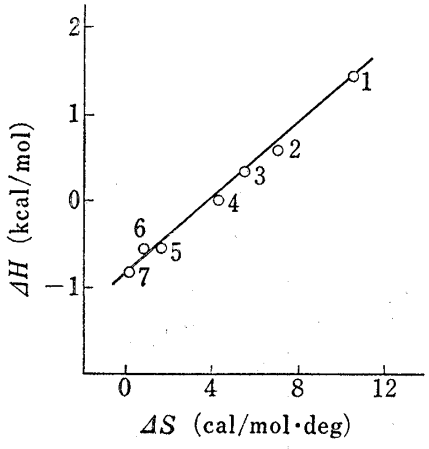

Fig. 3. Relationship between ΔH and ΔS for Partition of $PGF_{2\alpha}$ at 25°

Numbers refer to the systems in Table II.

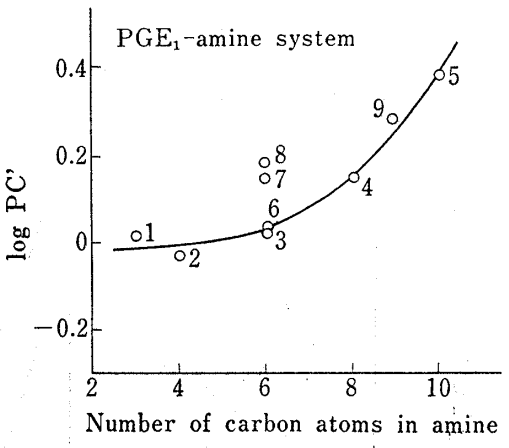

Fig. 4. Relationship between log PC' and Number of Carbon Atoms in Amine (5×10^{-2} M) at 25°

Aqueous phase: 0.05 M amine in buffer solution (pH 7.0, $\mu=0.2$).
Organic phase: cyclohexane+*n*-octanol (4: 1 in volume).
Key: 1, *n*-propylamine; 2, *n*-butylamine; 3, *n*-hexylamine; 4, *n*-octylamine; 5, *n*-decylamine; 6, diisopropylamine; 7, cyclohexylamine; 8, triethylamine: 9, tri-*n*-propylamine.

12) The pK_a value of $PGF_{2\alpha}$ obtained here, as an example, was in good accordance with that obtained by potentiometry (see ref. 4*a*).
13) J.A. Riddick and W.B. Bunger, "Organic Solvents," 3rd. ed., Wiley-Interscience, N.Y., 1970.

straight lines over the temperature range 10—40°. All the systems showed a positive entropy changes (ΔS), indicating that hydrophobic solvation may predominantly involve in the partition process. Smaller ΔS values obtained for longer alcohol systems may result from smaller aqueous solubility of the alcohols,[13] which may substantially reduce the hydrophobic association between prostaglandin and alcohol molecules in aqueous solution. In contrast, the longer the alcohol chain, the more favorable enthalpy change (ΔH) was accompanied. In fact, a linear relationship between ΔH and ΔS was obtained in Fig. 3, with a compensation temperature[14] of 223 °K.

Ion Pair Formation

The partition behaviors of prostaglandins in the presence of various amines in aqueous phase were investigated, anticipating an ion pair formation. Figure 4 shows the effects of amines on PC_{obs} of PGE_1 at pH 7.0. At this pH, both of the PGE_1 and amine are almost ionized, since their pK_a values are approximately 5 and 11,[15] respectively. It was found that PC_{obs} generally increased with increasing the chain length of amine, particularly in the series of primary amines. Comparing the various types of the amines containing same number

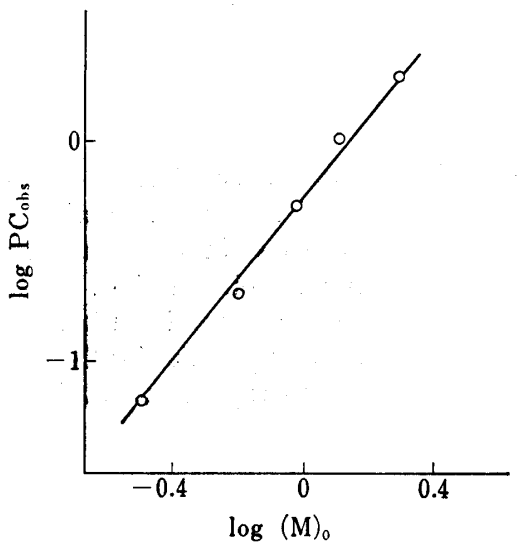

Fig. 5. Plot of log PC_{obs} *versus* log $(M)_o$ at 25°

Aqueous phase: PGE_1 (1×10^{-4} M)+*n*-hexylamine (2.5×10^{-3} M) in buffer solution (pH 7.0, $\mu=0.2$). Organic phase: cyclohexane+*n*-octanol (varied from 0.3 to 2.0 M).

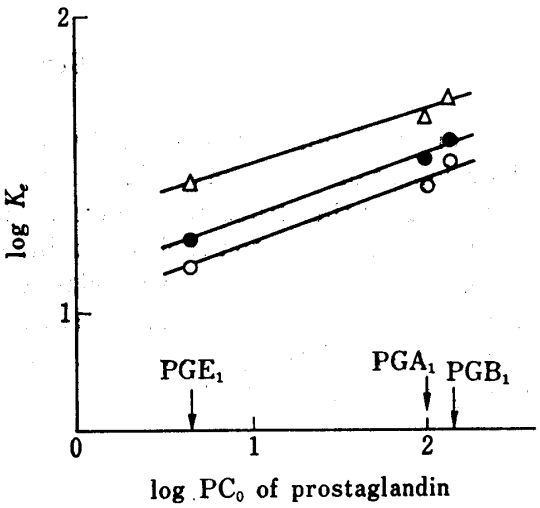

Fig. 6. Relationship between log K_e and log PC_o of Prostaglandins at 25°

Aqueous phase: prostaglandin (1.0×10^{-4} M)+amine (2.5×10^{-3} M) in buffer solution (pH 7.0, $\mu=0.2$). Organic phase: cyclohexane+*n*-octanol (1 M). ○: ethylamine, ●: *n*-butylamine, △: *n*-hexylamine.

TABLE III. K_e and n Values[a] for PGE_1–Amine Ion Pairs at 25°

Amine	K_e	n[b]
Ethylamine	13.9	1.49
n-Butylamine	17.4	1.64
n-Hexylamine	27.1	1.93
n-Octylamine	103	0.85

a) Experimental conditions were same as in Fig. 5.
b) Number of *n*-octanol molecules bound to ion pair.

14) R. Lumry and S. Rajender, *Biopolymers*, **9**, 1125 (1970).
15) G. Kortüm, W. Vogel, and K. Andrussow, "Dissociation Constant of Organic Base in Aqueous Solution," Butterworths, London, 1961.

of carbon atoms, for example C=6, PC_{obs} appears to increase in the order of amine basicity.[15] Figure 5 shows a typical plot of log PC_{obs} *versus* log $(M)_o$ according to Eq. (4) at fixed concentrations of PGE_1 and *n*-hexylamine in aqueous phase but varying concentration of *n*-octanol in organic phase. The plot was fairly on straight line with slope equal to 1.9, indicating that approximately two molecules of *n*-octanol are associated with ion pair. Similarly, K_e and n values for other amine systems were determined and are summarized in Table III. The number of *n*-octanol molecules bound per ion pair appeared to be lie in the range of 0.85 to 1.9, suggesting a steric requirement in alcohol solvation. On the other hand, K_e value increased with increasing the chain length of amine, as is expected in Fig. 4. Cho and Schnabel have recently reported similar result for PGB_2–amine system.[16] Figure 6 shows linear correlations of log K_e with log PC_o of some prostaglandins, indicating that hydrophobic nature of the prostaglandins is also responsible for the magnitude of K_e value.

Above results apparently suggest a hydrophobic ion pair formation between prostaglandin anion and amine cation. Furthermore, it was found that extraction of this ion pair was markedly enhanced by the addition of alcohol (see Fig. 5), while aprotic solvents such as ethyl acetate, *n*-octanone, *n*-butyl ether, and methyl enanthate had no effect on PC_{obs}. This implies that prostaglandin–amine ion pair may be classified to Case I type,[17] according to Higuchi *et al.*[10]

General Discussion

The prostaglandins are essentially long chain unsaturated fatty acids containing a substituted cyclopentanone moiety. It is interesting that pK_a value of the prostaglandins was little affected by the structural changes. Then, physiological activities of the prostaglandins may be rather depend upon their lipophilic nature, in physicochemical point of view. Capable formation of ion pair between prostaglandins with aliphatic amines would provide a facilitated absorption in GI lumen. Furthermore, these findings will be particularly useful for the development of dosage form, pharmaceutical analysis, partition chromatography, and evaluation of group contribution data.[16]

16) M.J. Cho and R. Schnabel, *J. Pharm. Sci.*, **64**, 1849 (1975).
17) In this case, the cation has largely lipophilic moiety and the anion carries a relatively higher negative charge density per surface area.