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Analysis of Structure-Permeability Correlation of Nitrophenol Analogues in Newborn Rat Abdominal Skin Using Semiempirical Molecular Orbital Calculation

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Abstract—Theoretical analysis of the permeation process in percutaneous absorption is important for the molecular design of bioavailable transdermal drugs. In the present study, we examined the difference in permeability of nitrophenols across newborn rat abdominal skin and analyzed the structure–permeability correlation by molecular orbital calculation. The permeable rate of *o-, m-, p*-nitrophenol and 2,4-, 2,5-, 2,6-dinitrophenols was 0.291, 0.212, 0.085 and 0.042, 0.109, 0.027 μmol/cm^2/h, respectively. The permeability of the nitrophenols correlated with their pK_a values, indicating the ionizing process related to the permeation through the skin. The pK_a values better correlated with the ionization potential (IP) energies than lowest unoccupied molecular orbital (LUMO) energies. Solvation free energies (dGW) of molecular form nitrophenols correlated better with permeabilities (pA) than partition coefficients (log P). In analyzing the dGW values with the permeability at pH 7.4, *o*-nitrophenol outlay from the theoretical line by *ortho*-effect. We conclude solvation free energy is a practical parameter and very useful for the molecular design of transdermal drugs.

Introduction

Transdermal therapy is very easily applied, since it is not accompanied by pain unlike an injection and does not require a cup of water, as needed for a tablet. In addition, transdermal drugs are suitable for occlusive dressing. Optimization of the permeation of therapeutic compounds through skin, which has a function to protect against external factors, is an important component in the drug design for transdermal therapeutic systems.

Analysis of the permeabilities of various chemicals through the skin and comparison of these permeabilities with molecular structural properties provides useful information for understanding the mechanism of percutaneous absorption of bioactive compounds. To examine the mechanism of association of a drug to skin epidermal cells in aqueous solution, the solvation free energy (dGW) is defined by using free-energy changes for association in the aqueous solution (dGW$_a$) and in the cell membrane (dGW$_m$) by molecular orbital calculation (MOPAC93) as follows; dGW = dGW$_a$−dGW$_m$.[1,2]

In the present study, we analyzed the molecular structure–permeability relationships of mono (*o-, m-, p-*)- and di (2,4-, 2,5-, 2,6-)-nitrophenols across the newborn rat abdominal epidermis. These compounds are of approximately equal molecular weight (M_r 139.1∼184.1), and as such, the difference of molecular sieve effect caused by these compounds can be ignored. We compared their permeabilities (pA) with their molecular properties (pK_a, log P$_{oct}$ and dGW). Moreover, we analyzed the quantitative structure–activity relationship (QSAR) between the permeability (pA) and the pK_a, log P$_{oct}$, and dGW of nitrophenols. In this case, we calculated the dGW of nitrophenols in physiological conditions (pH 7.4) by use of MOPAC (COSMO method[1]), and analyzed the correlation of the molecular structure and the permeabilities.

In general, the transdermal absorption of an ionic-form drug is very hard, and the ratio of ionic to molecular form of the compound is an important factor in the transdermal system. This ratio is dependent on the pK_a value, and relates to the ionizing mechanism of the

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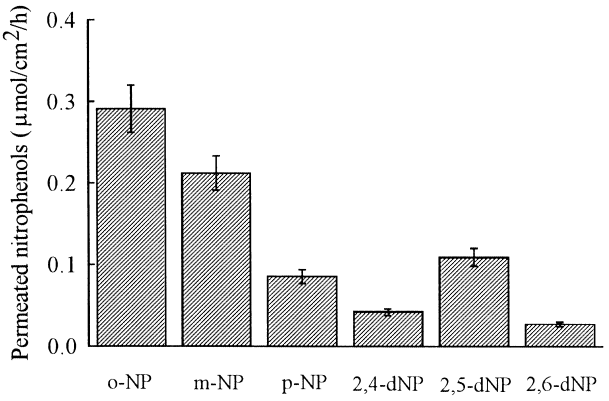

Figure 1. Permeation rate of nitrophenols across newborn rat abdominal skin at 37°C. Results are means for three determinations. *o*-NP, *m*-NP, *p*-NP and 2,4-dNP, 2,5-dNP, 2,6-dNP indicate *o-, m-, p*-nitrophenol and 2,4-, 2,5-, 2,6-dinitrophenol, respectively.

compounds. The ionizing mechanism of nitrophenols is important to the relationship between permeability and molecular structural properties. The ionization types of a molecule are classified as nucleophilic and electrophilic. The lowest unoccupied molecular orbital (LUMO) energy is useful for analyzing the ionizing mechanism in the nucleophilic reaction, while the ionization potential (IP) energy is a good indicator when analyzing the electrophilic reaction.[3,4] We calculated the LUMO and IP energies of the nitrophenols by MOPAC, and also analyzed the relationships between the pK_a values and the LUMO, IP energies.

Results

Permeations of nitrophenols through newborn rat abdominal epidermis

The permeable rates (A) of nitrophenols are compared in Figure 1. Of the nitrophenols examined, *o*-nitrophenols permeated the fastest. The permeable rate of *o-, m-* and *p*-nitrophenol was 0.291, 0.212 and 0.085 μmol/cm^2/h, respectively. Dinitrophenols permeated slower than mononitrophenols: 2,4-, 2,5- and 2,6-dinitrophenols permeated across the skin epidermis at the rate of 0.042, 0.109 and 0.027 μmol/cm^2/h, respectively.

Analysis of the correlation between permeability and molecular properties of nitrophenols

The relationship between the permeability pA (−log (permeation rate A)) and the pK_a value of nitrophenols is shown in Figure 2A. The permeability of nitrophenols (pA) across abdominal skin correlated well with pK_a, except in the case of *o*-nitrophenol. We first examined how the permeability changes with dissociation constant pK_a, as expressed by eq (1)

$$\begin{aligned} \mathrm{pA} &= 8.2176\ (\pm 0.7600) - 0.1908\ (\pm 0.1179)\ \mathrm{p}K_a \\ n &= 6,\ r = 0.9013,\ s = 0.1922,\ F = 17.32 \end{aligned} \quad (1)$$

where n, r, s and F are the numbers of compounds used for the calculation, the correlation coefficient, the standard deviation and the F-test value, respectively. The values in the parentheses are 95% confidence intervals. Hydrophobicity is an important factor for drug permeation through the skin. The correlation between the permeability (pA) and the hydrophobicity (log P (octanol/water)[5]) of nitrophenols is shown in Figure 2B and eq (2). As was the case for pK_a (Fig. 2A), *o*-nitrophenol outlay from the theoretical line.

$$\begin{aligned} \mathrm{pA} &= 9.0420\ (\pm 1.7607) - 1.1827\ (\pm 1.0243)\log \mathrm{P} \\ n &= 6,\ r = 0.8293,\ s = 0.2479,\ F = 8.812 \end{aligned} \quad (2)$$

pA showed the better correlation to pK_a than log P [eq (1) versus eq (2)]. When pA was compared both with pK_a and log P, the correlation coefficient improved to 0.9126, but the 95% confidence intervals both of pK_a and log P were larger than the mean values, respectively [eq (3)]. Thus, it was not utilized in the analysis.

$$\begin{aligned} \mathrm{pA} &= 7.6037\ (\pm 2.9493) - 0.2990\ (\pm 0.5142)\mathrm{p}K_a \\ &\quad + 0.7567\ (\pm 3.4640)\log \mathrm{P} \\ n &= 6,\ r = 0.9126,\ s = 0.2094,\ F = 7.476 \end{aligned} \quad (3)$$

To obtain a more significant correlation, we calculated the solvation free energies (dGW) for nitrophenols,

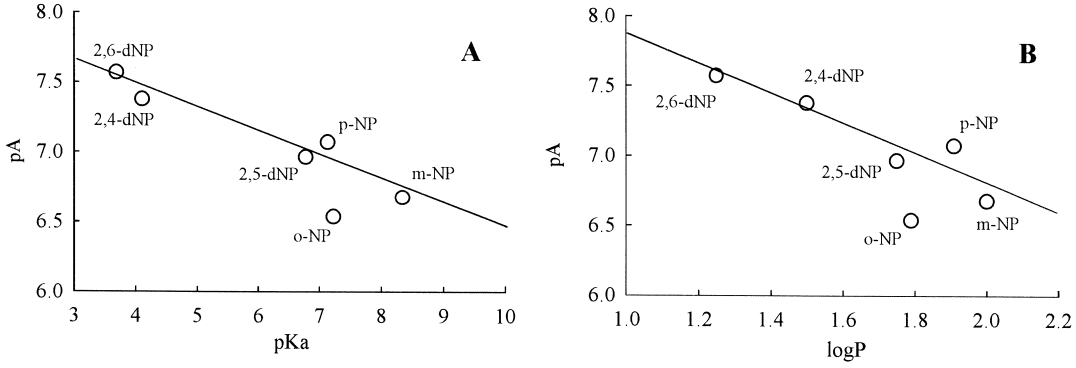

Figure 2. Relationship between the permeability pA (−log(permeation rate)), and the dissociation constant pK_a (A) and the hydrophobicity log-P(octanol/water) (B). Quantitative structure–activity relationship study was performed with a multiple regression analysis program QSAR. Abbreviations of nitrophenols are described in Figure 1. The logP values of *o-, m-, p*-nitrophenol and 2,4-, 2,5-, 2,6-dinitrophenol are 1.79, 2.00, 1.91 and 1.50, 1.75, 1.25, respectively.[5]

Table 1 The solvation free energies of nitrophenols.

	dGW (KJ)	dGW_i (KJ)	dGW_d (KJ)
o-nitrophenol	−58.1511	−308.6502	−207.6425
m-nitrophenol	−70.6964	−301.4990	−93.5687
p-nitrophenol	−74.6324	−289.7133	−213.4435
2,4-dinitrophenol	−99.1115	−287.7975	−287.7007
2,5-dinitrophenol	−93.9876	−291.5732	−253.3555
2,6-dinitrophenol	−103.2136	−304.9463	−304.9070

The energies were defined by using free-energy changes for association in the aqueous solution and cell membrane, as described in the section Experimental. dGW, dGW_i and dGW_d indicate the energies of the molecular form, ionic form and dissociated form of these compounds, respectively at pH 7.4. The values of dGW_d were calculated by Henderson–Hasselbalch equation with the pK_a values (at 25°C, in water) of *o*-nitrophenol (7.23[14]), *m*-nitrophenol (8.35[14]), *p*-nitrophenol (7.14[14]), 2,4-dinitrophenol (4.11[15]), 2,5-dinitrophenol (6.78[14]) and 2,6-dinitrophenol (3.69[16]), respectively.

which were calculated in molecular form (summarized in Table 1), and examined the correlation with the permeability pA at pH 7.4 Figure 3A and eq (4).

$$\mathrm{pA} = 5.3908\ (\pm 1.0696) - 1.9695\ (\pm 1.2597)\ \mathrm{dGW}$$
$$n = 6,\ r = 0.8953,\ s = 0.1976,\ F = 16.16 \quad (4)$$

Equation (4), gives a higher correlation coefficient than eq (2) (log P), and the solvation free energy seems to be a good indicator of permeability in the transdermal system. Permeation of nitrophenols was examined at pH 7.4, and nitrophenol molecules were expected to equilibrate between molecular and ionic form. Figure 3B and eq (5) indicate the correlation between the permeability pA and the solvation free energies at pH 7.4 (dGW_d; dissociated form), which were calculated by Hederson–Hasselbalch equation and summarized in Table 1.

$$\mathrm{pA} = 6.1042\ (\pm 0.9932)\ - 0.4089\ (\pm 0.4188)\ \mathrm{dGW_d}$$
$$n = 6,\ r = 0.7820,\ s = 0.2765,\ F = 6.299 \quad (5)$$

The *o*-nitrophenol outlay from the theoretical line (Fig. 3B), and the correlation was not significant [eq (5)]. Since the *ortho*-substituted derivatives are not explained by a single, generally applicable set of parameters due to various kinds of proximity effects, such as steric and proximity electric effects,[6,7] we compared the permeability with the solvation free energies except for *o*-nitrophenol (dGW_{d2}) at pH 7.4.

$$\mathrm{pA} = 6.2655\ (\pm 0.7073) - 0.3751\ (\pm 0.2915)\ \mathrm{dGW_{d2}}$$
$$n = 5,\ r = 0.8998,\ s = 0.1769,\ F = 12.76 \quad (6)$$

In this case the correlation was better than with eq (5) (containing *o*-nitrophenol).

When pA was compared both with pK_a and dGW, the correlation coefficient improved to 0.9428, but the confidence intervals of dGW were not utilizable [eq (7)]. Thus it is not useful to the analysis of permeation of nitrophenols.

$$\mathrm{pA} = 6.8277\ (\pm 2.7843) - 0.1082\ (\pm 0.1957)\ pK_a$$
$$- 1.0521(\pm 2.0338)\ \mathrm{dGW} \quad (7)$$
$$n = 6,\ r = 0.9428,\ S = 0.1708,\ F = 11.99$$

Lowest unoccupied molecular orbital (LUMO) and ionization potential (IP) energies of nitrophenols

To investigate the ionization mechanism of nitrophenols, we next examined the relationship between pK_a and LUMO energy (Table 2) in both aqueous solution [Fig. 4A and eq (8)] and cell membrane [Fig. 4B and eq (9)].

$$pK_a = 9.2553\ (\pm 9.0613) + 2.5967\ (\pm 7.5503)L_a$$
$$n = 6,\ r = 0.4044,\ s = 1.9168,\ F = 0.782 \quad (8)$$

$$pK_a = 1.3554\ (\pm 9.6984) + 1.5940\ (\pm 3.1225)L_m$$
$$n = 6,\ r = 0.5487,\ s = 1.7522,\ F = 1.723 \quad (9)$$

In eqs (8) and (9), L_a and L_m indicate the LUMO energy in aqueous solution and membrane, respectively. In both equations, the correlation coefficients were not significantly high and so the nucleophilic reaction does not relate to the permeation of nitrophenols.

The relationship between the pK_a and the ionization potential energy in aqueous solution [I_a in eq (10),

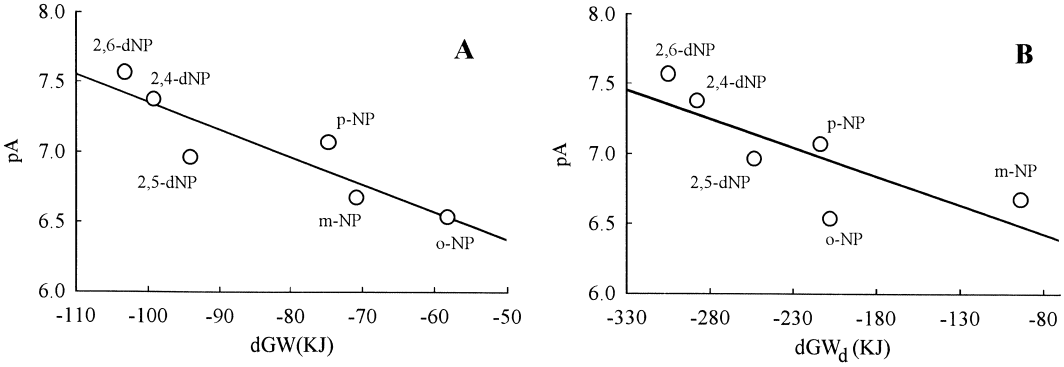

Figure 3. Analysis of the relationship between the permeability pA and the solvation free energy dGW. The solvation free energy of nitrophenols in molecular form (dGW; A) and dissociated form at pH7.4 (dGW_d; B) were calculated by MOPAC93. The correlation between pA and dGW, dGW_d were examined by the program QSAR.

Table 2 Lowest unoccupied molecular orbital (LUMO) and ionization potential (IP) energies of nitrophenos

	LUMO (EV)		IP (EV)	
	L_a	L_m	I_a	I_m
o-nitrophenol	−0.825	3.843	9.784	9.911
m-nitrophenol	−1.121	3.254	9.755	9.966
p-nitrophenol	−0.901	3.678	9.874	10.072
2,4-dinitrophenol	−1.172	2.762	10.233	10.763
2,5-dinitrophenol	−1.582	2.244	10.047	10.618
2,6-dinitrophenol	−1.420	2.517	10.140	10.659

The values of LUMO and IP energies were calculated by MOPAC93.[2] L_a, I_a and L_m, I_m indicate the LUMO, IP energies in aqueous solution and cell membrane, respectively.

Fig. 5A] and cell membrane [I_m in eq (11), Fig. 5B] was examined and summarized in Table 2.

$$\mathrm{p}K_a = 92.4091\ (\pm 51.2622) - 8.6434\ (\pm 5.1398) I_a \quad (10)$$
$$n = 6,\ r = 0.9076,\ s = 0.8799,\ F = 18.69$$

$$\mathrm{p}K_a = 47.6763\ (\pm 35.7693) - 4.0130\ (\pm 3.4602) I_m \quad (11)$$
$$n = 6,\ r = 0.8305,\ s = 1.1675,\ F = 8.891$$

The correlations between the pK_a and the ionization potential energies [eqs (10) and (11)] were better than for the LUMO energies [eqs (8) and (9)], and values for the correlation coefficients (r) improved to 0.9076 [eq (10)] and 0.8305 [eq (11)], respectively. Thus the electrophilic reaction is involved in the ionization of nitrophenols.

Discussion

It is desirable to predict the permeability ratio of drug molecules from physicochemical parameters or, ideally, from their molecular structures. In the present study, we used MOPAC to analyze the relationships between the permeation of nitrophenols through the skin and molecular structural properties.

As structural parameters, the acid dissociation constant pK_a, the partition coefficient log P and the solvation free energy dGW of nitrophenols were compared to the permeability pA. The correlation between pA and pK_a was good [eq (1)], and suggested that the permeation of nitrophenols related to the ionization process. In molecular design for transdermal drugs, the pK_a values of compounds must be determined by an experiment after chemical synthesis. The values of dGW can be predicted by calculation (MOPAC) before the synthesis, and applicable for development of the drugs. The values of pA correlated better to dGW [eq (4)] than to log P [eq (2)]. From these results we considered the dGW (molecular form) to be an important parameter in predicting the permeability of drug molecules in percutaneous

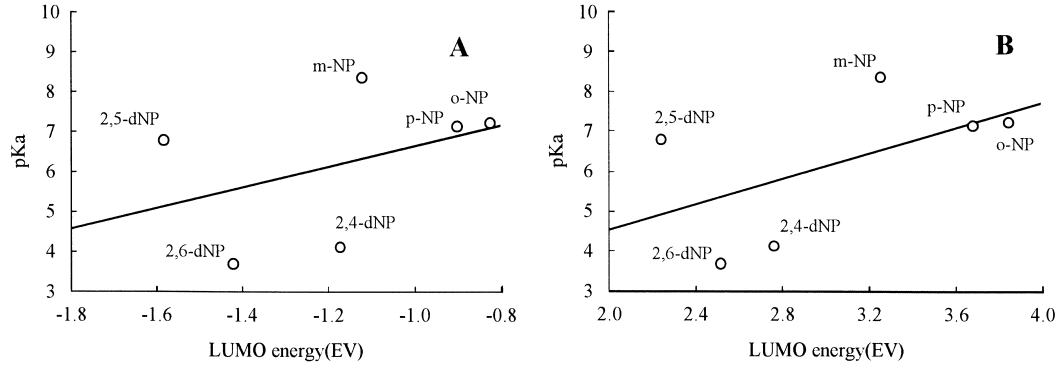

Figure 4. Correlation between the pK_a value and the lowest unoccupied molecular orbital (LUMO) energy. The LUMO energy in aqueous solution (A) and cell membrane (B) were calculated by MOPAC93. Analysis of the correlation of LUMO energy to pK_a were performed by the program QSAR, and produced eqs (8) and (9).

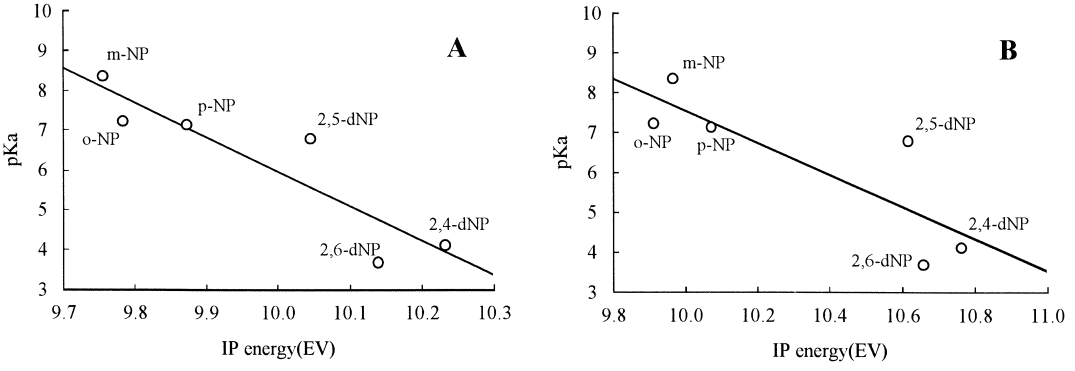

Figure 5. Relationship between the pK_a and the ionization potential (IP) energy. The IP energy in aqueous solution (A) and cell membrane (B) were determined by MOPAC93. The correlation of these parameters was investigated by program QSAR, and the results described as eqs (10) and (11).

absorption. We considered that comparison of pA with dGW or pK_a is useful for understanding of bioavailability of synthesized compounds.

Nitrophenols have a molecular and an ionic form in vivo (pH 7.4), we determined the dGW_d values (Table 1) and analyzed the relationship to the permeability pA. As shown in eq (5), the correlation was poor and *o*-nitrophenol outlay from the theoretical line (Fig. 3B). Since *ortho*-substituents are bound adjacent to the side chain, various kinds of proximity effects, such as steric and proximity electric effects, play significant roles in their reactivities.[6] Thus linear energy relationships of the Hammet type that have been used for *meta*- and *para*-substituted derivatives are not usually applicable to *ortho*-substituted compounds.[7,8] When the dGW_d value of *o*-nitrophenol was excluded from the analysis, the correlation coefficient *r* improved to 0.8998 [eq (6)], which was higher than the coefficient with log P [eq (2)]. In analysis between dGW and pA, molecular form *o*-nitrophenol did not outlay from the theoretical line (Fig. 3A). Thus, we considered that the ionic form of nitrophenol, especially *o*-nitrophenol, affected the correlation in permeation. Indeed the solvation free energy of ionic form dGW_i showed a poor correlation with the permeability pA, as follows:

$$pA = 11.8138\ (\pm 16.1503) + 1.6083\ (\pm 5.4292)\ dGW_i$$
$$n = 6,\ r = 0.3559,\ s = 0.4146,\ F = 0.58 \qquad (12)$$

indicating the ratio of ionic to molecular form of nitrophenols affected the permeability pA. Thus the ionization of nitrophenols appears an important factor in the permeation across the skin, and we next analyzed the ionizing mechanism of nitrophenols.

In the electronic interaction, an electrophile attacks the target molecule according to the eigenvalue of highest occupied molecular orbital (HOMO), while a nucleophile attacks according to the eigenvalue of lowest unoccupied molecular orbital (LUMO).[3] In this case, the eigenvalue of HOMO corresponds to the ionization potential energy.[4] The correlation between LUMO [L_a and L_m in eqs (8) and (9)] energies and pK_a were unfavorable (Fig. 4). In contrast, the IP energies [I_a and I_m in eqs (10) and (11)] well correlated to the pK_a [eq (5)]. From these results we considered that an electrophilic reaction is regarded to ionization and permeability, and we calculated the HOMO coefficients of nitrophenols with MOPAC to predict which sites are preferred by electrophiles, as shown in Figure 6.[3] Thus, the difference between the HOMO coefficients of nitrophenols predicted that the electrophiles preferably attack the phenolic hydroxy group. However the order of permeability of nitrophenols did not coincide with that of the HOMO coefficients of phenolic oxygen (the HOMO coefficients = 0.132 ~ 0.161). Furthermore, we calculated the LUMO coefficients (the values with underline) as shown in Figure 6, and we observed the significance of nitro group. From these results we considered that the electronic balance between phenolic hydroxy group and nitro group seems to be related to ionization and permeability of nitrophenols. We are currently examining the relationships between the ionizing mechanism (electrophilic, nucleophilic) and the permeability (bioavailability) of transdermal therapeutic drugs.

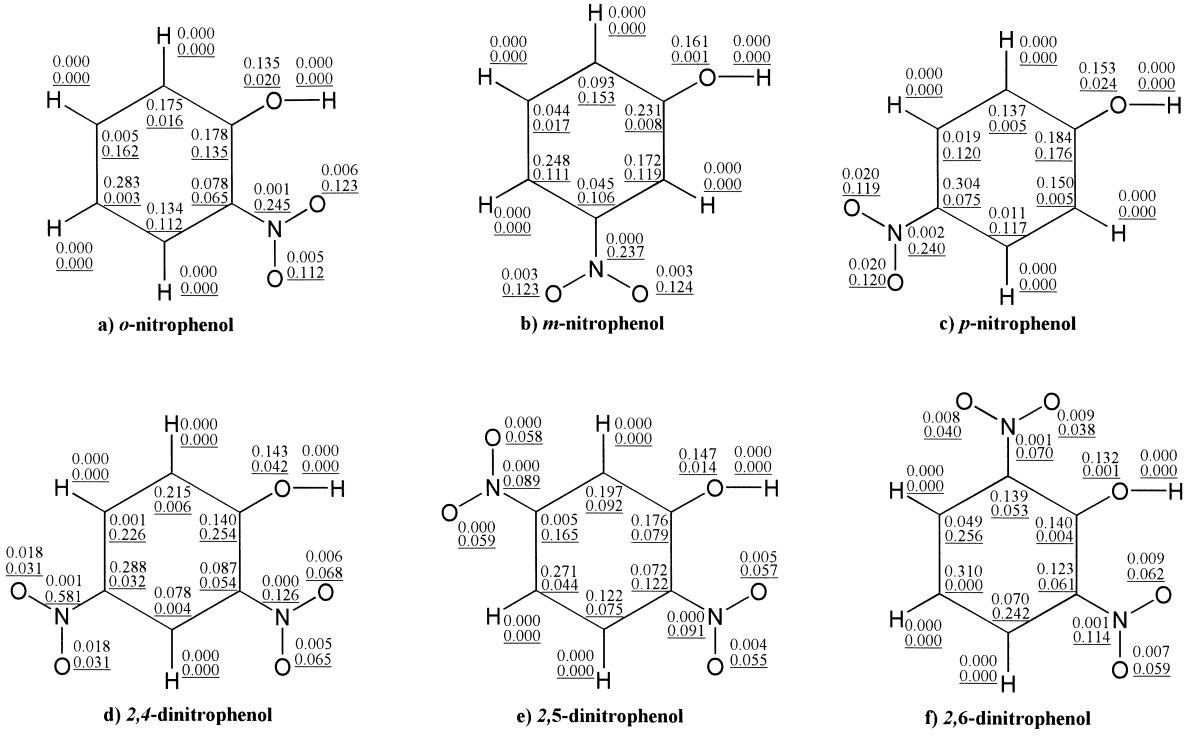

Figure 6. The HOMO and LUMO coefficients of *o*- (a), *m*- (b), *p*-nitrophenol (c) and 2,4- (d), 2,5- (e), 2,6-dinitrophenol (f). HOMO and LUMO (with underline) coefficients of nitrophenols were calculated with MOPAC 93 program on a Fujitsu S-4/10 computer, as described in the section Experimental.

In the cultured newborn rat skin basal cell layer, the nitrophenols ranked in order of permeability as follows: *p*-nitrophenol > *m*-nitrophenol > *o*-nitrophenols, the reverse to the case in intact abdominal skin.[9] Skin epidermis is constructed from various types of cells, such as basal, spinous, granular and cornified cells, and these cells are classified by their level of differentiation.[10] Then we considered that transdermal therapeutic drugs have a molecular structure suited to permeation across each of the cell layers making up the skin. From this point of view, the prediction of the permeability of transdermal drugs by their molecular dynamics should be useful for the molecular design of skin cell-type reflected therapeutic drugs. A study into this is underway.

Experimental

Reagents

The sources of the reagents used in this study were as follows: Eagle's minimum essential medium (MEM, phenol red-free), from Nissui Pharmaceutical Co., Tokyo, Japan; *o*-, *m*-nitrophenols and 2,5-, 2,6-dinitrophenols, Nacalai Tesque Inc., Kyoto, Japan; *p*-nitrophenol and 2,4-dinitrophenol, from Wako Pure Pharmaceutical Co., Osaka, Japan.

Permeation across newborn rat abdominal epidermis

The abdominal skin sheet was excised from 3 day-old Wistar rats with surgical scissors. Fat and other visceral tissue adhering to the skin were removed carefully from the under surface with forceps. The excised skin sheet was fixed with an adhesive agent to the end of a plastic cylinder of a permeation cell with its outer surface facing the inside of the cylinder; this cylinder was dipped into 50 mL of serum-free MEM (pH 7.4).[9] Then 500 μL of 7.2 mM nitrophenol solution in serum-free MEM (pH 7.4) was introduced into the cylinder. The level of solution above the abdominal skin in the cylinder was kept exactly the same as that of the lower MEM solution. The permeability of nitrophenols through the skin were monitored as the absorbance changes of *o*-, *m*-, *p*-nitrophenols and 2,4-, 2,5-, 2,6-dinitrophenols at their absorption maxima of 410, 340, 399 nm and 360, 441, 430 nm, respectively.

Computation of solvation free energies of nitrophenols and quantitative structure–activity relationship analysis

Energy calculations were performed with the Austin model 1 (AM1) Hamiltonian[11] using the MOPAC 93 program[12] on a Fujitsu S-4/10 computer at the Information Processing Center (University of Tokushima), and the stable and transient structures were initially built with general parameters of bond length, bond angle, and dihedral angle, and refined with the eigenvector following (EF) optimization method. The solvation free energy ($dGW = dGW_a - dGW_m$) of nitrophenols was defined from the free-energy changes for association in the aqueous solution (dGW_a) and in the cell membrane (dGW_m; using the dGW in vacuum, because cell membrane have hydrophobic property) by the COSMO method.[1] The dielectric constant (ε) of water and vacuum were 78.4 and 1.0, respectively. Quantitative structure–activity relationship study was performed with the multiple regression analysis program QSAR (constructed by Prof. Zenei TAIRA, Tokushima Bunri University).[13]

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