[Chem. Pharm. Bull. 34(3)1228—1234(1986)]

Interaction of Indomethacin with the Vehicle Component Diisopropyl Adipate

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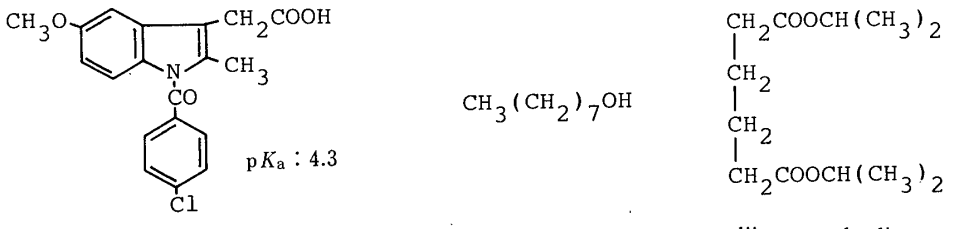
(Received May 30, 1985)

The interactions of the potent antiinflammatory agent indomethacin with diisopropyl adipate and *n*-octanol were studied under various conditions. Diisopropyl adipate has been used as a typical additive in preparations for the percutaneous absorption of indomethacin, and *n*-octanol is known to form hydrogen-bonds with various drugs. The association constants were determined from the change in the partition of indomethacin between *n*-hexane containing diisopropyl adipate or *n*-octanol and water. Both the neutral and anionic forms of indomethacin were found to associate with these additives in a 1 : 2 molar ratio, but with different association constants. The association of indomethacin with *n*-octanol was found to be dependent on pH, but that with diisopropyl adipate was independent on pH. The affinity of indomethacin for diisopropyl adipate was much smaller than that of the neutral form of indomethacin for *n*-octanol. It was also found, from an analysis of the solubilities of indomethacin in various esters, that the association constants of indomethacin with esters are about the same. The weak, but pH-independent association of indomethacin with diisopropyl adipate is suggested to be important for the induction of percutaneous absorption of indomethacin.

Keywords——partition coefficient; association constant; indomethacin; *n*-octanol; diisopropyl adipate; solvation; hydrogen-bond; vehicle component

Introduction

In order to avoid the gastrointestinal side effects of some drugs, their application through the skin has been attempted, though the percutaneous absorption of drugs is generally regarded to be very poor.[1] Recently we succeeded in making the potent antiinflammatory agent indomethacin (see Chart 1 for chemical structure) permeate rapidly through intact



indomethacin *n*-octanol diisopropyl adipate

Chart 1. Molecular Structures of Indomethacin, Diisopropyl Adipate and *n*-Octanol

human or guinea-pig skin, and penetrate deeply into the muscle.[2,3] The vehicle components should play a decisive role in the rapid penetration of indomethacin through the skin. In fact, we found that vehicle components, such as ethanol and esters, do affect the percutaneous absorption of indomethacin.[4] In particular, diisopropyl adipate (DIPA; molecular structure

in Chart 1) caused a marked increase in the solubility of indomethacin and in its penetration through the skin. Thus, the interaction of DIPA with both indomethacin and the skin should be of primary importance for understanding the mechanism of percutaneous absorption of the antiinflammatory drug.

As a first step to elucidate the role of DIPA in the percutaneous absorption of indomethacin, we studied the interaction between indomethacin and DIPA. Hydrogen-bonding is expected to be predominant in this interaction. Thus, we examined the effect of DIPA on the partition of indomethacin between the non-hydrogen-bonder *n*-hexane and water under various conditions. The effect of *n*-octanol (for molecular structure, see Chart 1) was also examined, since it is widely used as an organic solvent in the partition of drugs,[5,6] and its hydrogen-bonding with drugs was reported to facilitate the partition of drugs.[7]

Experimental

Materials——Indomethacin (J.P. grade) and DIPA (Van Dyke Co., Belleville, New Jersey, U.S.A.) were used without further purification. DIPA gave a single peak on gas-chromatography with a 3% Silicone OV-17 column at 170 °C. *n*-Octanol and *n*-hexane from Wako Pure Chemical Industries Co. (Osaka, Japan) were distilled three times before use.

Method——Partition coefficients of indomethacin between *n*-hexane and water at pH 2.5 and 9.0 were determined at 25±0.1 °C: 4 ml of *n*-hexane containing indomethacin and either *n*-octanol or DIPA was shaken with 4 ml of the aqueous phase for 18 h. Under these conditions, partition of indomethacin was confirmed to attain equilibrium. The concentration of indomethacin after equilibrium was determined either by gas-chromatography or by ultraviolet (UV) spectrophotometry as described previously.[8]

The partition coefficient was determined as the ratio of the concentration of indomethacin in the organic phase to that in the aqueous phase. The initial concentration of indomethacin was 2.24×10^{-6} M at pH 2.5 and 1.33×10^{-4} M at pH 9.0. The pH of the aqueous phase was kept constant with a mixture of 0.1 M sodium biphosphate and 0.1 M hydrochloric acid at pH 2.5 or 0.1 M sodium biphosphate and 0.1 M disodium orthophosphate at pH 9.0. Water–saturated *n*-hexane and *n*-hexane–saturated water were used as solvents in the two phases.

The association constants K_i's ($i=1$ and 2) of indomethacin with the additive *n*-octanol or DIPA were determined as follows. First the partition coefficients of indomethacin at various additive concentrations were calculated by means of the equation shown in the text with arbitrarily chosen K_i's, with the aid of a microcomputer, NEC PC 9801. Then these values were compared with the experimentally determined partition coefficients. This procedure was repeated with various sets of arbitrary K_i's until the best fit of the partition coefficients with the experimentally determined partition coefficients were obtained. The values which gave the best partition coefficients were taken as the true K_i's.

Results

The partition coefficient (P) of indomethacin between *n*-hexane and water at a certain pH is defined as the ratio of the molar concentration of indomethacin in the organic phase, $[IND]_o$, to that in the aqueous phase, $[IND]_w$, as shown in Eq. 1. The activity coefficients of these molecular species were assumed to be unity.

$$P=[IND]_o/[IND]_w \quad (1)$$

At pH 2.5, the value of P of indomethacin between *n*-hexane and water at the initial concentration of 2.24×10^{-6} M was determined as 3.55 ($\log P=0.55$). At this pH, indomethacin in the aqueous phase is considered to exist completely as a neutral form, since its pK_a was determined as 4.3.[8] Thus, the P value at this pH is the partition coefficient of the neutral form of indomethacin (true partition coefficient), and this value is much smaller than that in the *n*-octanol/water system as described below. One reason for the lower affinity of indomethacin for *n*-hexane is that the *n*-hexane can not form a hydrogen-bond with indomethacin.

The partition coefficient of indomethacin increased greatly on addition of *n*-octanol to the *n*-hexane phase. The partition coefficient in the presence of the additive is denoted as P'.

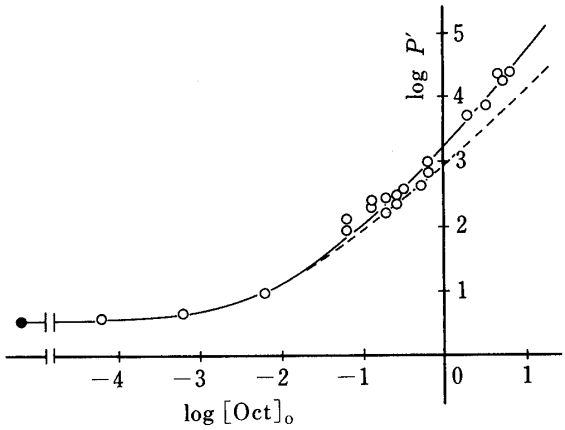


Fig. 1. Dependence of the Partition Coefficient of Indomethacin (P') at pH 2.5 on the Molar Concentration of *n*-Octanol ($[Y]_0$) in the Hexane Phase

Closed and open circles indicate the experimental values of log P and log P', respectively. Continuous and discontinuous lines were obtained by calculation of P' according to Eqs. 5 and 6, respectively, with values of $P=0.55$, $K_1=250.0$ and $K_2=0.3$.

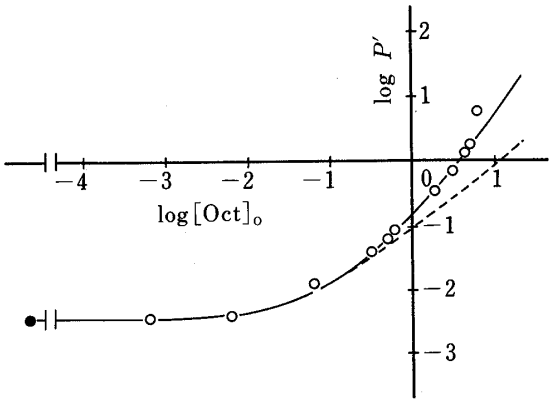


Fig. 2. Dependence of the Partition Coefficient of Indomethacin (P') at pH 9.0 on the Molar Concentration of *n*-Octanol ($[Y]_0$) in the Hexane Phase

For symbols and lines, see the legend to Fig. 1, but with $P=-2.32$, $K_1=20.0$, $K_2=0.3$.

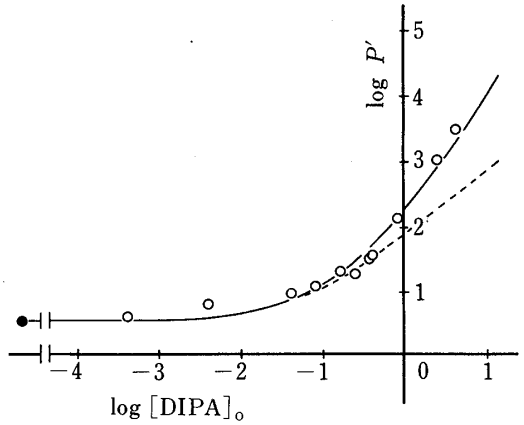


Fig. 3. Dependence of the Partition Coefficient of Indomethacin (P') at pH 2.5 on the Molar Concentration of Diisopropyl Adipate ($[Y]_0$) in the Hexane Phase

Closed and open circles indicate the experimental values of log P and log P', respectively. Continuous and discontinuous lines were obtained by calculation of P' according to Eqs. 7 and 8, respectively, with values of $P=0.55$, $K_1=9.0$, $K_2=0.5$.

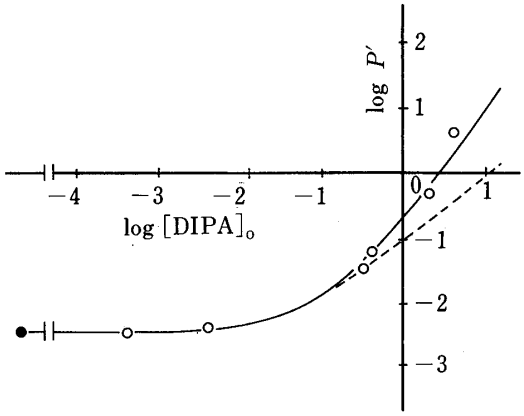


Fig. 4. Dependence of the Partition Coefficient of Indomethacin (P') at pH 9.0 on the Molar Concentration of Diisopropyl Adipate ($[Y]_0$) in the Hexane Phase

For symbols and lines, see the legend to Fig. 3, but with $P=-2.32$, $K_1=9.0$, $K_2=0.5$.

The value of P' is determined as the ratio of indomethacin concentration in the organic phase (C_o) to that in the aqueous phase (C_w) as shown in Eq. 2.

$$P'=C_o/C_w \tag{2}$$

When the organic phase consisted solely of *n*-octanol ($[Oct]_0=6.35$ M), the value of P' was determined as 2.34×10^4 ($\log P'=4.37$). The dependence of P' on the *n*-octanol concentration ($[Oct]_0=6.35\times10^{-5}$—6.35 M) in the *n*-hexane phase is shown in Fig. 1. The effect of octanol on the partition of indomethacin at low concentrations up to $\log [Oct]_0=-2.20$ M was very small, but became large above $\log [Oct]_0=-1.20$ M causing a progressive increase in P'.

Like partition at acidic pH, the partition at pH 9.0 was also affected by *n*-octanol, as shown in Fig. 2. In this case the partition coefficient of indomethacin between *n*-hexane and water (P) was determined as 4.79×10^{-3} ($\log P = -2.32$). This is the partition coefficient of the anionic form of indomethacin ion-paired with Na^+ present in the aqueous phase.[8] The partition coefficient became larger with increase in the *n*-octanol content in the organic phase, and finally, when the organic phase consisted solely of *n*-octanol, it attained a value of 5.89 ($\log P' = 0.77$).

When DIPA was added to the hexane phase, the partition of indomethacin changed markedly, as observed with *n*-octanol. The partition coefficient of indomethacin increased with increase in DIPA concentration at both pH 2.5 and 9.0 up to the values of 3.02×10^3 and 4.21, respectively, where the organic phase consisted solely of DIPA ($[DIPA]_o = 4.18$ M), as shown in Figs. 3 and 4. It is noteworthy from the results in Figs. 1—4 that in all cases $\log P'$ increased linearly with increase in the concentration of additives in the hexane phase with a slope of about 2 at high additive concentrations.

These results suggest that the interactions between the additive and indomethacin, due to whatever combination of hydrogen-bonding, ion-dipole interaction and dipole-dipole interaction is operating, facilitate the lipid solubility of indomethacin.

Suppose that indomethacin, IND, has two sites 1 and 2 for association with the additive Y to form complexes IND(1)-Y, IND(2)-Y, IND(1, 2)-Y_2 and IND(2, 1)-Y_2 in the organic phase. The association constants K can be expressed as follows:

$$K_1 = [\text{IND}(1)\text{-}Y]_o / [\text{IND}]_o [Y]_o \tag{3a}$$

$$K_2 = [\text{IND}(2)\text{-}Y]_o / [\text{IND}]_o [Y]_o \tag{3b}$$

$$K_{1,2} = [\text{IND}(1, 2)\text{-}Y_2]_o / [\text{IND}(1)\text{-}Y]_o [Y]_o \tag{3c}$$

$$K_{2,1} = [\text{IND}(2, 1)\text{-}Y_2]_o / [\text{IND}(2)\text{-}Y]_o [Y]_o \tag{3d}$$

Where brakets represent the concentration. If the associated additive does not affect the subsequent interaction between indomethacin and the additive, the following relations should hold.

$$K_{1,2} = K_2 \tag{4a}$$

$$K_{2,1} = K_1 \tag{4b}$$

The partition coefficient of indomethacin (P') is a function of the indomethacin concentration in both partition phases, as shown in Eq. 5.

$$\begin{aligned} P' &= ([\text{IND}]_o + [\text{IND}(1)\text{-}Y]_o + [\text{IND}(2)\text{-}Y]_o + [\text{IND}(1, 2)\text{-}Y_2]_o \\ &\quad + [\text{IND}(2, 1)\text{-}Y_2]_o) / [\text{IND}]_w \\ &= P(1 + K_1[Y]_o + K_2[Y]_o + 2K_1K_2[Y]_o^2) \end{aligned} \tag{5}$$

When indomethacin has only a single site for the additive Y, Eq. 5 becomes Eq. 6.

$$P' = P(1 + K_1[Y]_o) \tag{6}$$

In both cases, the increase in $\log P'$ with $\log [Y]_o$ is expected to be linear at high concentrations of Y. In the case where there are two sites, the slope of the linear portion is 2, while in the case of a single site, it is unity. It is apparent from Figs. 1—4 that $\log P'$ increased linearly at high concentrations of *n*-octanol and DIPA, and the slope was always about 2. These results suggest that indomethacin has two sites for interaction with additives.

Values of association constants between indomethacin and *n*-octanol were determined according to Eq. 5 by the method described in Experimental. It was found that in all cases K_1

TABLE I. Parameters of Association of Indomethacin with Additives under Various Conditions Determined from the Change in the Partition Coefficient between *n*-Hexane and Water on Addition of the Additives

Additive	pH	log *P*	Constants (M^{-1})	
			K_1	K_2
n-Octanol[a]	2.5	0.55	250.0	0.3
	9.0	−2.32	20.0	0.3
Diisopropyl adipate[b]	2.5	0.55	9.0	0.5
	9.0	−2.32	9.0	0.5

a) Determined by using Eq. 5. *b*) Determined by using Eq. 7.

and K_2 were required. The calculated values of P' at various additive concentrations according to Eqs. 5 and 6 are shown as continuous and discontinuous lines, respectively, in Figs. 1 and 2. The values of K_1 and K_2 as well as the partition coefficients of indomethacin in the various partition systems are summarized in Table I. In the case of DIPA, two ester groups can be regarded as participating in the interaction with indomethacin. Namely, ester groups are the sites of interaction with indomethacin. In this case, each molecule of DIPA has two sites and the concentration of sites in DIPA should be twice the concentration of DIPA. Thus, Eq. 5 becomes Eq. 7.

$$P' = P(1 + 2K_1[Y]_o + 2K_2[Y]_o + 8K_1K_2[Y]_o^2) \quad (7)$$

Furthermore, when only K_1 is effective, Eq. 7 can be reduced to Eq. 8.

$$P' = P(1 + 2K_1[Y]_o) \quad (8)$$

Calculated values of P' at various DIPA concentrations, obtained by using Eqs. 7 and 8, are shown in Figs. 3 and 4, and the values of K_1, K_2 and P are summarized in Table I.

In Table I, the value of K_1 with *n*-octanol at pH 2.5 is much greater than that at pH 9.0, suggesting that the hydrogen-bonding between the oxygen atom in *n*-octanol and the hydrogen atom of the carboxyl group in indomethacin is dominant. It is noteworthy that the interaction of DIPA with the neutral indomethacin molecule is very similar to that with the indomethacin anion, and the K values in these systems were similar to those for the anionic form of indomethacin and *n*-octanol. At present, the nature of the interactions is not clear, but ion–dipole or dipole–dipole interaction is probably important.

Discussion

In this study we examined the interaction of indomethacin with the additive *n*-octanol or DIPA, by utilizing the additive-dependent partition of the antiinflammatory drug between the non-hydrogen-bonder *n*-hexane and water. The solubility of indomethacin in a "non-interacting" inert solvent, such as *n*-hexane, should be increased on addition of esters and alcohols. When the additive Y has only one site for interaction with indomethacin, the change in the solubility can be expressed by Eq. 9 with association constants K_1' and K_2'.

$$S' = S(1 + K_1'[Y] + K_2'[Y] + 2K_1'K_2'[Y]^2) \quad (9)$$

Where, S and S' represent the solubilities of indomethacin in the absence and presence of an additive, respectively. At high concentrations of the additive Y, the last term of the right-hand side of Eq. 9 should become predominant, as observed with the partition of indomethacin

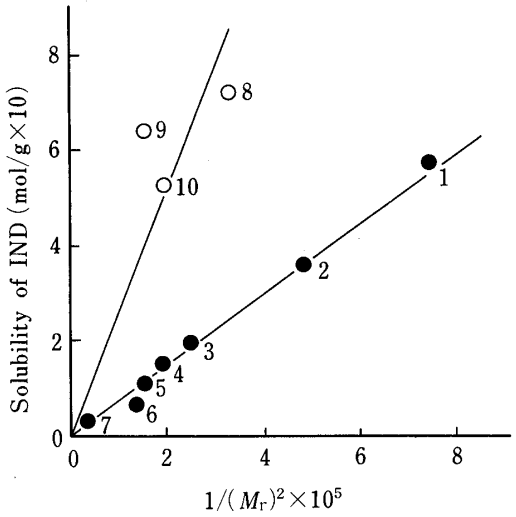


Fig. 5. Linear Relationship between Solubilities of Indomethacin in Mono- and Diesters and Reciprocals of the Squares of the Molecular Weights of Various Esters

1, ethyl butyrate; 2, ethyl caproate; 3, ethyl caprate; 4, ethyl laurate; 5, ethyl myristate; 6, isopropyl myristate; 7, octyl dodecyl myristate; 8, diethyl succinate; 9, diethyl sebacate; 10, diisopropyl adipate.

shown in Figs. 1—4.

$$S' \simeq 2SK_1'K_2'[Y]^2 \tag{10}$$

When the values of K_1' and K_2' are about the same with aditives Y in a homologous series, the solubility (S') is solely dependent on the molar concentration of the additives. Namely, the solubilities of indomethacin in the presence of the additives should increase linearly with the 2nd power of molar concentration of the additives Y. Since the molar concentration of a given amount of an additive Y is inversely related to its molecular weight (M_r), the solubility of indomethacin should be linearly dependent on the reciprocals of the squares of the molecular weights of the additives, as expressed by Eq. 11.

$$S' \propto (M_r)^{-2} \tag{11}$$

In a previous paper, we reported the solubilities of indomethacin (S') in various mono- and diesters, including DIPA.[4] The dependence of S' on $(M_r)^{-2}$ is depicted in Fig. 5. The solubilities of indomethacin in mono- and diesters are proportional to the reciprocals of the squares of the molecular weights of the esters in each series, indicating that the affinities of indomethacin for the esters in the respective series are about the same. Since diesters have two sites for indomethacin, the value of the slope with diesters should be 4-fold that with monoesters, if the esters in both series have the same affinity for indomethacin (*cf.* Eq. 7). As can be seen in Fig. 5, the slope with diesters (2.75×10^4) is indeed roughly 4-fold that with monoesters (7.53×10^3), indicating that the affinities of indomethacin for these esters are about the same. No special interaction between indomethacin and DIPA was observed.

In this study we found that both the neutral and anionic forms of indomethacin interact with *n*-octanol and DIPA. In each case, the interaction consisted of a strong primary association and a weak secondary association, though the nature of the interaction is not clear at present. It shoud be noted that the secondary association, though it is very weak, becomes very important for the interaction with additives at high concentrations.

The ester DIPA forms complexes with both neutral and anionic forms of indomethacin to similar extents, but the primary association constants are much smaller (about 1/28) than that of the neutral form of indomethacin with *n*-octanol. Such a weak, but pH-independent interaction may facilitate the penetration of indomethacin into the skin. However, it is not clear at present why, of the esters, DIPA is especially effective for induction of the penetration of indomethacin through the skin, since the association constants of various esters with indomethacin were found to be almost the same. The role of the interaction between

indomethacin and DIPA in the percutaneous absorption is currently under further study.

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