Activation Thermodynamic Quantities for the Release of 3-Aminopyridine (3AP) from Fatty Acid–3AP Equimolar Complexes

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The rates of release of 3-aminopyridine (3AP) from fatty acid (FA)−3AP complexes, FA–3AP, were determined at various temperatures, and the thermodynamic quantities of the release of 3AP were estimated. The results were compared with previous results obtained for FA–nicotinamide (NAA) complexes, FA–NAA.

The values of activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($\Delta S^{\ddagger}$) for the release of 3AP from FA–3AP were positive and negative, respectively, indicating that the release of 3AP is disadvantageous from not only enthalpic but also entropic viewpoints. The plots of $\Delta H^{\ddagger}$ against the carbon number (n) in the constituent FA showed a zig-zag line with an upward convex at odd-numbered positions and the plots of the absolute values of $|-\Delta S^{\ddagger}|$ showed a zig-zag line with a downward convex at odd-numbered positions, though the positive value of $\Delta H^{\ddagger}$ increased and the negative ralue of $\Delta S^{\ddagger}$ decreased with an increasing n for either even-numbered or odd-numbered FA. It was found that the release of 3AP from FA–3AP formed with odd-numbered FA is more disadvantageous enthalpically but more advantageous entropically as compared with that from FA–3AP formed with even-numbered FA. Furthermore, it is suggested that FA–3AP is formed by at least van der Waals forces and hydrophobic interactions.

It was found that FA–3AP has similar physicochemical properties to FA–NAA and that the variation in physicochemical properties of FA–3AP owing to the difference in n is smaller than that of FA–NAA. This is considered to be due to the fact that the packing of 3AP into the FA host cavity is looser than that of NAA.

Keywords 3-aminopyridine; complex; release; fatty acid; kinetics; activation thermodynamic quantity

It has been found that fatty acids (FA) form crystalline complexes with water-soluble drugs in 1,2-dichloroethane[1]: for example, a thiamine disulfide (TDS) complex $(FA)_6(TDS)$[1a] whose molar ratio of FA to TDS is 6:1, and a nicotinamide (NAA) complex FA–NAA[1b] whose molar ratio of FA to NAA is 1:1. The usefulness and applicability of FA–drug complexes in the pharmaceutical field have already been reported.[2] The release rates of TDS from $(FA)_6(TDS)$ and of NAA from FA–NAA have already been determined in an aqueous medium.[3,4] In the release studies,[3,4] the plots of the release rate constants (k) of TDS and NAA against the carbon number of the constituent FA (n) showed a zig-zag line which was represented by alternative convexes at odd-numbered positions, and k decreased rather regularly with an increase of n for either even-numbered or odd-numbered FA. $(FA)_6(TDS)$ and FA–NAA are considered to be inclusion compounds.[5,6] Furthermore, their release behaviors are suggested to be caused by the difference in the host structure, composed of odd-numbered FA or even-numbered FA.[5]

Recently, we have found that 3-aminopyridine (3AP) forms the crystalline complex FA–3AP with FA.[7] The stoichiometry of FA–3AP was found to be 1:1 by elemental analysis and differential scanning calorimetry, the details of which were presented in the previous paper.[7] Furthermore, it is suggested from the IR spectra[1b,7] that FA–3AP has a similar structure to FA–NAA and that FA–3AP also may be an inclusion compound. The only difference in FA–3AP from $(FA)_6$ (TDS) and FA–NAA is its melting point[7]: the melting point of FA–3AP is lower than that of the constituent FA, while the melting points of $(FA)_6(TDS)$ and FA–NAA are higher than that of the constituent FA. It will be very interesting to see whether the zig-zag release patterns caused by the host structure composed of odd-numbered FA or even-numbered FA, as observed for $(FA)_6(TDS)$ or FA–NAA, will be seen for FA–3AP.

From these points of view, the release rates of 3AP from FA–3AP will be measured at various temperatures and the activation thermodynamic quantities for the release will be estimated. Furthermore, the results obtained for FA–3AP will be compared with the results[4] obtained for FA–NAA.

Experimental

Materials 3AP, tetradecanoic acid (C14), pentadecanoic acid (C15), hexadecanoic acid (C16), heptadecanoic acid (C17) and octadecanoic acid (C18) were the same as those used for the previous studies.[7,8] FA–3AP was prepared as follows: FA and 3AP were dissolved in warm 1,2-dichloroethane, and the solution was set aside to crystallize.[7] The purity of each FA–3AP was examined by measuring the melting point of FA–3AP.[7] After it had been confirmed that no extra free FA and/or 3AP was present, crystals of FA–3AP were passed through 48 and 60 mesh sieves, and the particles of 48—60 mesh[8] were taken for the release test.

Measurement of the Release of 3AP from FA–3AP The release of 3AP from FA–3AP was determined in a JP XII dissolution test apparatus (paddle method) in 500 ml of JP XII disintegration test medium No. 1 (pH 1.2) as described in the previous paper.[8] About 17—20 mg of FA–3AP was used in each test. Experiments were carried out not only at 37 °C but also at 7, 17 and 27±0.2 °C. All experiments were carried out in triplicate and the results were highly reproducible.

Quantitative Analysis of 3AP The concentration of released 3AP was determined spectrophotometrically as previously described.[8]

Results

Release Behavior of 3AP from FA–3AP The release behaviors of 3AP from C14–3AP at the four temperatures are shown in Fig. 1 as a relationship between the percentage of released 3AP and time. The percentages of released 3AP were calculated with respect to the theoretical total concentration of 3AP contained in the 1:1 complex, C14–3AP. A similar release behavior was observed for the four other FA–3AP, although the other results are not given here. The equilibrium percent of released 3AP was 94—98 under various conditions. The release rate of 3AP is faster under higher temperatures.

Rate Constants for the Release of 3AP from FA–3AP

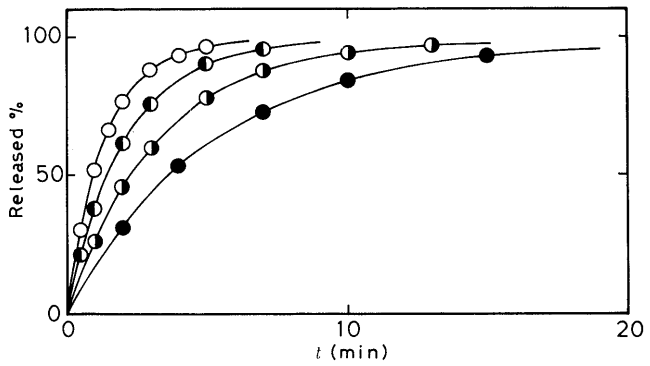

Fig. 1. Effect of Temperature on the Release Behavior of 3AP from C14–3AP

Temperature: ●, 7 °C; ◑, 17 °C; ◐, 27 °C; ○, 37 °C. Particle size: 48—60 mesh.

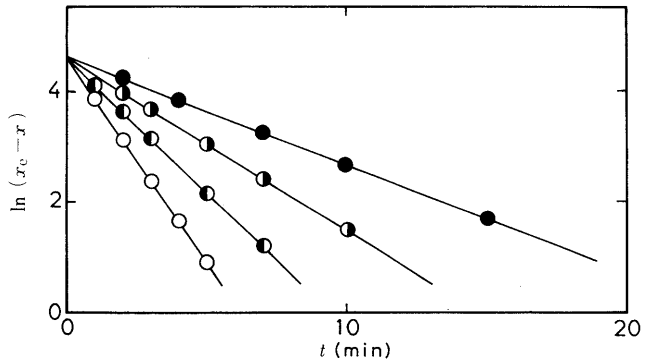

Fig. 2. Effect of Temperature on the Release of 3AP from C14–3AP, $\ln(x_e-x)$ *vs.* Time

Symbols are the same as in Fig. 1.

FA–3AP, FA and 3AP are in equilibrium in an aqueous solution (pH 1.2) as follows:

$$\text{FA–3AP solid} \rightleftharpoons \text{FA solid} + 3\text{AP}$$

where released 3AP is dissolved in the test medium. FA and FA–3AP were almost insoluble in an aqueous acidic medium, and the solubilities of FA–3AP and FA were negligible under the experimental conditions. In the equilibrium equation, it is assumed that the concentration change of FA is negligible because of its insolubility. Furthermore, it is assumed that the formation of FA–3AP by a reversed reaction is negligible because the concentration of FA–3AP formed by the reversed reaction is sufficiently lower than that of released 3AP. According to the assumptions, the reaction of pseudo first order can be applied in this system. The rate constant for the release of 3AP was defined as follows:

$$\ln(x_e-x)=\ln x_e-kt \tag{1}$$

where k is the rate constant of release, x is the percentage of 3AP released from FA–3AP during time t, and x_e is the equilibrium percent of released 3AP. Plots of $\ln(x_e-x)$ *vs.* t, calculated from the values shown in Fig. 1, are presented in Fig. 2. As can be seen in Fig. 2, good linear relationships were obtained at various temperatures. Furthermore, the four other FA–3AP yielded similar good linearity, although the figures are not given here. The values of log k obtained for C14–3AP, C15–3AP, C16–3AP, C17–3AP and C18–3AP at four temperatures are plotted against n

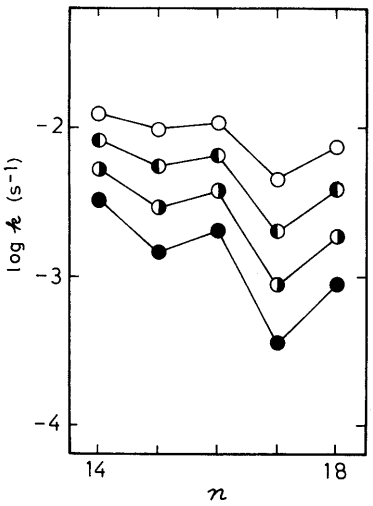

Fig. 3. Effect of FA on the Release Rate Constants (k) of 3AP from FA–3AP

Symbols are the same as in Fig. 1.

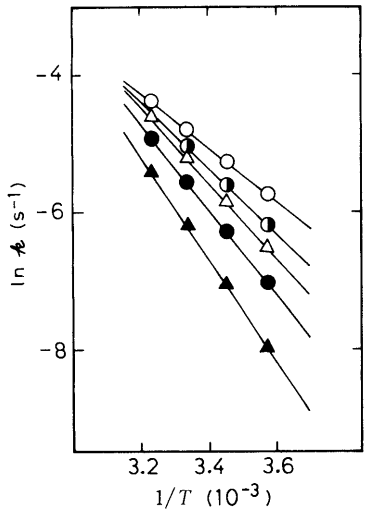

Fig. 4. Arrhenius Plots

Carbon numbers in FA: ○, 14; △, 15; ◑, 16; ▲, 17; ●, 18.

of the constituent FA in Fig. 3. As can be seen in Fig. 3, the plots of log k against n showed zig-zag lines with a downward convex at odd-numbered positions.

Discussion

Activation Energy for the Release of 3AP from FA–3AP

The values of k were found to depend to depend on temperature. The activation energy ($E^{\ddagger}$) for the release can therefore be calculated from the values of k. According to the theory of Arrhenius, the relationship between k and the absolute temperature (T) is represented as follows:

$$\ln k=-\frac{E^{\ddagger}}{R}\cdot\frac{1}{T}+\ln A \tag{2}$$

where R is the gas constant and A is a constant which is called a frequency factor. Plots of $\ln k$ *vs.* $1/T$ based on Eq. 2 are shown in Fig. 4. As is clear in Fig. 4, the relationship between $\ln k$ and $1/T$ can be represented by a single line which depends on the alkyl chain length of FA. Therefore, the values of $E^{\ddagger}$ were obtained from the values of the

Table I. Activation Energies for the Release of 3AP from FA–3AP

	C14–3AP	C15–3AP	C16–3AP	C17–3AP	C18–3AP
$E^{\neq}$ (kJ mol^{-1})	32.3	45.9	39.8	61.2	50.9

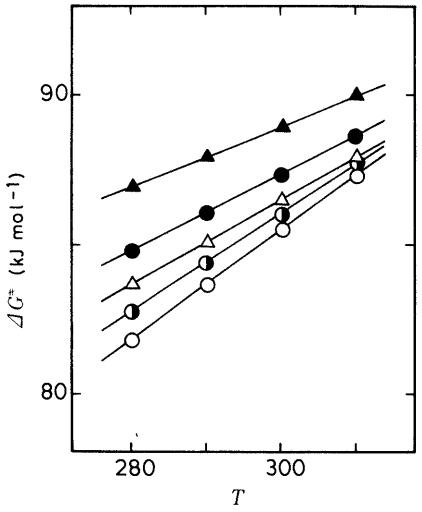

Fig. 5. Relationship between Activation Gibbs Energy and Temperature
Symbols are the same as in Fig. 4.

slopes, and the results were summarized in Table I. The positive value of $E^{\neq}$ for FA–3AP formed with odd-numbered FA was larger than that for FA–3AP formed with even-numbered FA whose alkyl chain length is one more carbon number longer, though $E^{\neq}$ increased rather regularly with an increase in n for both even-numbered and odd-numbered FA. The slower release rate of 3AP from FA–3AP formed with odd-numbered FA, which is shown in Fig. 3, could be explained by the relationship between $E^{\neq}$ and n. In addition, the "odd-even effect" is considered to be due to the difference in the crystal structure of FA–3AP: the crystal structures of FA–3AP formed with odd-numbered FA is slightly different from that of FA–3AP formed with even-numbered FA. This has already been made clear from the IR spectra of FA–3AP.[7]

Activation Thermodynamic Quantities for the Release of 3AP from FA–3AP The activation Gibbs energy, $\Delta G^{\neq}$, for the release of 3AP from FA–3AP can be represented in terms of the release rate constant, k, as follows:

$$\Delta G^{\neq} = -RT\ln k + RT\ln(k_B T/h) \quad (3)$$

where k_B and h are the Boltzmann constant and Planck constant, respectively. The values of $\Delta G^{\neq}$ were estimated from the values of k shown in Fig. 3. The values of $\Delta G^{\neq}$ were graduated at the ordinate in Fig. 5. All the values of $\Delta G^{\neq}$ are positive.

Next, we investigated which activation thermodynamic parameter contributes to the positive value of $\Delta G^{\neq}$. $\Delta G^{\neq}$ is related to activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) as follows:

$$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq} \quad (4)$$

According to Eq. 4, the values of $\Delta G^{\neq}$ were plotted against T, and the relationship is shown in Fig. 5. As can be seen in Fig. 5, the relationship between $\Delta G^{\neq}$ and T can be

Table II. Activation Thermodynamic Quantities for the Release of 3AP from FA–3AP

	$\Delta G^{\neq}$ (kJ mol^{-1})[a]	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})
C14–3AP	83.8	29.8	−186
C15–3AP	85.3	43.5	−144
C16–3AP	84.6	37.3	−163
C17–3AP	88.1	58.8	−101
C18–3AP	86.2	48.5	−130

a) At 290.15 K.

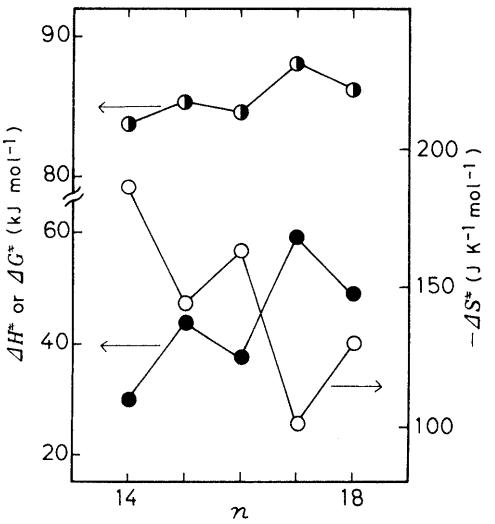

Fig. 6. Effect of FA on the Activation Thermodynamic Quantities Regarding the Release of 3AP from FA–3AP
Thermodynamic parameter: ◑, $\Delta G^{\neq}$; ●, $\Delta H^{\neq}$; ○, $\Delta S^{\neq}$.

represented by a single line for each complex with a different chain length of FA. The values of $\Delta S^{\neq}$ were therefore obtained from the values of the slope. $\Delta H^{\neq}$ was approximately estimated from the intercept, which agreed well with the value from the slope of $\Delta G^{\neq}/T$ *vs.* $1/T$ and nearly agreed with the approximate evaluation, $E^{\neq} - RT$. The results are summarized in Table II. As can be seen in Table II, the value of $\Delta H^{\neq}$ is positive while the value of $\Delta S^{\neq}$ is negative. This indicates that the release of 3AP from FA–3AP is disadvantageous not only enthalpically but also entropically. These are the same tendencies as the results obtained for FA–NAA.[4]

Effect of FA on Activation Thermodynamic Quantities The values of $\Delta G^{\neq}$ at 290.15 K, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the release of 3AP from FA–3AP are represented graphically against n in Fig. 6. As can be seen in Fig. 6, the plots of the positive values of $\Delta H^{\neq}$ *vs.* n indicate a zig-zag line with an upward convex at an odd-numbered position, while the plots of the negative values of $\Delta S^{\neq}$ *vs.* n indicated a zig-zag line with a downward convex at an odd-numbered position. This indicates that the release of 3AP from FA–3AP formed with odd-numbered FA is more disadvantageous enthalpically but more advantageous entropically as compared with that formed with even-numbered FA. Furthermore, the positive value of $\Delta H^{\neq}$ increased and the negative value of $\Delta S^{\neq}$ decreased with an increasing n for either even-numbered or odd-numbered FA. This indicates that the release of 3AP from FA–3AP with a longer alkyl

Table III. Comparison of the Magnitude of $|\Delta H^{\neq}|$ with $|T\Delta S^{\neq}|$

C14–3AP	$\|\Delta H^{\neq}\| < \|T\Delta S^{\neq}\|$
C15–3AP	$\|\Delta H^{\neq}\| \fallingdotseq \|T\Delta S^{\neq}\|$
C16–3AP	$\|\Delta H^{\neq}\| < \|T\Delta S^{\neq}\|$
C17–3AP	$\|\Delta H^{\neq}\| > \|T\Delta S^{\neq}\|$
C18–3AP	$\|\Delta H^{\neq}\| > \|T\Delta S^{\neq}\|$

chain of FA is more disadvantageous enthalpically but more advantageous entropically. However, the positive value of $\Delta G^{\neq}$ increased with an increasing n for either even-numbered or odd-numbered FA, and the plots of $\Delta G^{\neq}$ *vs.* n indicate a zig-zag line with an upward convex at odd-numbered positions.

Next, in order to investigate which activation thermodynamic parameter controls the release of 3AP from FA–3AP, a comparison of the magnitude of $|\Delta H^{\neq}|$ with $|T\Delta S^{\neq}|$ was summarized in Table III. $|\Delta H^{\neq}|$ was larger than $|T\Delta S^{\neq}|$ for FA–3AP whose $n \geqq 17$, while $|\Delta H^{\neq}|$ was nearly equal to $|T\Delta S^{\neq}|$ or smaller than $|T\Delta S^{\neq}|$ for FA–3AP whose $n \leqq 16$. Namely, the release of 3AP from C17–3AP or C18–3AP is an activation enthalpically controlled reaction, and the release of 3AP from C14–3AP or C16–3AP is an activation entropically controlled reaction. This tendendy of FA–3AP is roughly similar to FA–NAA.[4] In addition, this tendency of FA–3AP is different from the results[3] that $|\Delta H^{\neq}|$ is larger than $|T\Delta S^{\neq}|$ for all the $(FA)_6$ (TDS). The equimolar complex FA–NAA is composed of m molecules of NAA and $(FA)_m$[4] in contrast to $(FA)_6$ (TDS), which is composed of one molecule of TDS and $(FA)_6$. A similar tendency observed between FA–3AP and FA–NAA leads to the conclusion that FA–3AP is composed at least in part of m molecules of FA and m molecules of 3AP, where m is not unity ($m > 1$).

Regarding the formation of complexes, it has been reported[9] that complexes formed by hydrophobic interactions have an approximate zero $\Delta H^{\neq}$ and a positive value for $\Delta S^{\neq}$, and that complexes formed by van der Waals forces have a negative value of $\Delta H^{\neq}$ and a negative $\Delta S^{\neq}$ value. Taking into account the report[9] and the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ shown in Table II, it is suggested that FA–3AP is formed at least in part by van der Waals forces and hydrophobic interactions.

Comparison of k and $\Delta G^{\neq}$ between FA–3AP and FA–NAA In order to make a comparison between the release behaviors of 3AP from FA–3AP and NAA from FA–NAA, the previously obtained values of k[5] at 37 °C for FA–NAA were shown by open circles in Fig. 7a together with the values of k for FA–3AP, where the measurement of k for FA–NAA was done under the same conditions applied in this paper. As can be seen in Fig. 7a, the absolute values of $\log k$ for FA–3AP and FA–NAA formed with odd-numbered FA are larger than those formed with even-numbered FA, which have an alkyl chain length one carbon number longer. The plots of log k *vs.* n show a similar zig-zag pattern in the cases of both FA–3AP and FA–NAA. However, the variation of log k for FA–3AP owing to the difference in n is smaller than that for FA–NAA. Namely, the absolute value of log k for FA–3AP is larger compared with that for FA–NAA in the case of $n = 14$ and 16, while the absolute value of log k for FA–3AP is smaller than that for FA–NAA in the case of $n = 17$ and 18.

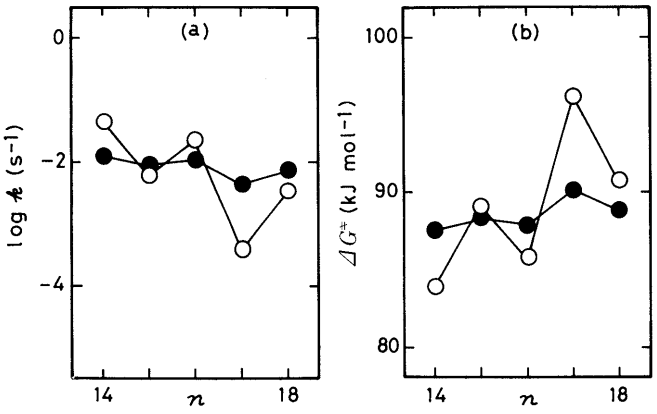

Fig. 7. Comparison of k and $\Delta G^{\neq}$ between FA–3AP and FA–NAA
(a) Plots of k *vs.* n. b) Plots of $\Delta G^{\neq}$ *vs.* n. Complex: ●, FA–3AP; ○, FA–NAA. Temperature: 37 °C.

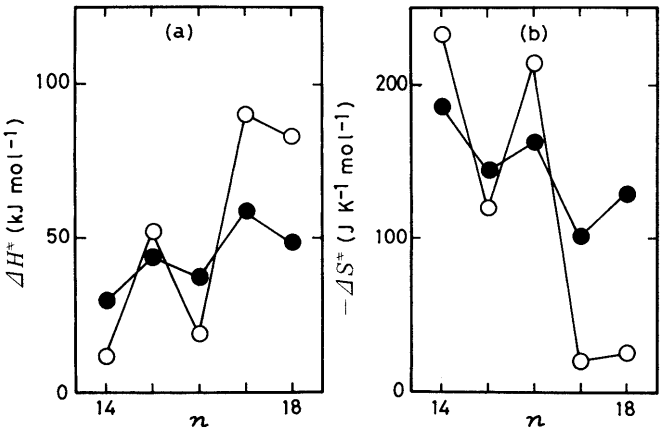

Fig. 8. Comparison of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ between FA–3AP and FA–NAA
(a) Plots of $\Delta H^{\neq}$ *vs.* n. (b) Plots of $\Delta S^{\neq}$ *vs.* n. Complex: ●, FA–3AP; ○, FA–NAA.

On the other hand, the previously obtained values[5] of $\Delta G^{\neq}$ at 37 °C for the release of NAA from FA–NAA were shown by open circles in Fig. 7b, together with the values of $\Delta G^{\neq}$ for FA–3AP. As can be seen in Fig. 7b, the positive values of $\Delta G^{\neq}$ for FA–3AP and FA–NAA formed with odd-numbered FA are larger than those formed with even-numbered FA, which have an alkyl chain length one carbon number longer. The plots of $\Delta G^{\neq}$ *vs.* n show a similar zig-zag pattern in the cases of both FA–3AP and FA–NAA. Furthermore, the variation of $\Delta G^{\neq}$ for FA–3AP owing to the difference in n is smaller than that for FA–NAA, and the same is true for variation of k.

Comparison of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ between FA–3AP and FA–NAA The values of $\Delta H^{\neq}$ and $-\Delta S^{\neq}$ for FA–3AP are shown by closed circles in Fig. 8, together with the values[4] for FA–NAA, which are shown by open circles. The plots of $\Delta H^{\neq}$ *vs.* n show zig-zag lines with an upward convex at odd-numbered positions. It is found that the relationship between $\Delta H^{\neq}$ and n for FA–3AP is similar to that for FA–NAA. However, the variation of $\Delta H^{\neq}$ for FA–3AP owing to the difference in n is smaller than that for FA–NAA. Namely, the positive value of $\Delta H^{\neq}$ for FA–3AP is larger compared with that for FA–NAA in the case of $n = 14$ and 16, while the value of $\Delta H^{\neq}$ for FA–3AP

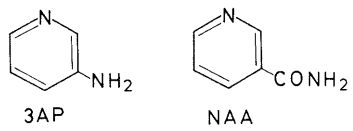

Chart 1

is smaller than that for FA–NAA in the case of $n \geqq 17$.

On the other hand, the plots of $\Delta S^{\neq}$ *vs.* n show zig-zag lines with a downward convex at odd-numbered positions. The relationship between $\Delta S^{\neq}$ and n for FA–3AP is similar to that for FA–NAA. The variation of $\Delta S^{\neq}$ for FA–3AP owing to the difference in n is smaller than that for FA–NAA. Namely, the negative value of $\Delta S^{\neq}$ for FA–3AP is smaller compared with that for FA–NAA in the case of $n = 14$ and 16, while the negative value of $\Delta S^{\neq}$ for FA–3AP is larger than that for FA–NAA in the case of $n \geqq 17$. As described above, it is evident that FA–3AP has physicochemical properties similar to FA–NAA, and that the variation in physicochemical property of FA–3AP owing to the difference in n is smaller than that of FA–NAA.

FA–NAA seems to be an inclusion compound.[5,6] The similar physicochemical properties between FA–3AP and FA–NAA shown in this paper and the previous paper[7] suggest that FA–3AP has a similar structure to FA–NAA, and may be an inclusion compound as well. The variations of physicochemical parameters for FA–3AP owing to the difference in n were smaller than those for FA–NAA. Taking into account the smaller variation of physicochemical parameters and the lower melting points of FA–3AP, and also the structure formulas of 3AP and NAA shown in Chart 1, the fitness of 3AP into the FA host structure may be inferior to that of NAA.

Conclusion

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the release of 3AP from FA–3AP were positive and negative, respectively. The plots of $\Delta H^{\neq}$ *vs.* n showed a zig-zag line with an upward convex at odd-numbered positions, while the plots of $\Delta S^{\neq}$ *vs.* n showed a zig-zag line with a downward convex at odd-numbered positions. These phenomena were similar to those observed for FA–NAA.

In comparing $\Delta H^{\neq}$ and $\Delta S^{\neq}$ among FA–3AP with various alkyl chains, it is suggested that FA–3AP is formed by at least van der Waals forces and hydrophobic interactions. Furthermore, it is suggested that FA–3AP may be an inclusion compound, similar to FA–NAA, and that the packing of 3AP is looser than that of NAA into the FA host cavity.

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