[Chem. Pharm. Bull. 34(5)1871—1880(1986)]

Conformational Studies of *N*-2-(3-Indolyl)ethyl- and *N*-2-Phenylethyl-5′-deoxy-5′-adenosineacetamides by Spectroscopic and Energy Calculation Methods, as Model Compounds for Aminoacyladenylates[1)]

TOSHIMASA ISHIDA,*[,a] HIROMI OHYABU,[a] SHINZO FUKUNARI,[a] MASATOSHI INOUE,[a] TAKUSHI KURIHARA,*[,b] HIROFUMI HAYASHI[b] and ATSUTOSHI OHTA[b]

Department of Physical Chemistry[a] and Department of Synthetic Organic Chemistry,[b] Osaka College of Pharmacy, 2–10–65 Kawai, Matsubara-shi, Osaka 580, Japan

(Received October 23, 1985)

As a part of the conformational studies of aminoacyl adenosine monophosphates, two model compounds, *N*-2-(3-indolyl)ethyl- and *N*-2-phenylethyl-5′-deoxy-5′-adenosineacetoamides, were investigated by ultraviolet, circular dichroism and ^{1}H-nuclear magnetic resonance spectroscopies, and empirical energy calculations. Aromatic ring–ring intramolecular stacking interactions were observed for both compounds. However, the stacking tendency and the ribose puckering accompanying the stacking interaction were different in the two compounds. These conformational differences may be important in the recognition of the tryptophanyl- and phenylalanyladenosine monophosphates by the respective aminoacyladenosine monophosphate synthetases.

Keywords——indole–adenine interaction; phenyl–adenine interaction; stacking interaction; model compounds for aminoacyladenylate; UV spectra; CD spectra; NMR; empirical energy calculation

Introduction

At the first step of protein synthesis, an amino acid must be transferred to the 3′-terminius of transfer ribonucleic acid (tRNA) by aminoacylation. This reaction is catalyzed by the aminoacyl-tRNA synthetase (ARS) specific for the requisite amino acid (aa). The reaction can be described as follows:

$$\text{aa} + \text{ARS} + \text{ATP} \rightleftharpoons \text{aa–AMP} \cdot \text{ARS} + \text{PPi} \quad (1)$$

$$\text{aa–AMP} \cdot \text{ARS} + \text{tRNA} \rightleftharpoons \text{aa–tRNA} + \text{AMP} + \text{ARS} \quad (2)$$

For these reactions to proceed, each ARS has to recognize accurately both the respective aa–adenosine monophosphate (AMP) and the corresponding tRNA. Therefore analysis of the preferred conformation for each aa–AMP might be important for understanding the recognition of aa–AMP by ARS. No experimental study has yet been done, because of the high lability of aminoacylesters at the nucleotide linkage.[2)] On the other hand, we have reported an improved synthetic method[3)] and a structure analysis[4)] of 5′-deoxyadenosine-acetic acid (AAA) as a model nucleotide of 5′-AMP, where CH_2CH_2COOH is replaced by $CH_2OPO_3H_2$: AAA and 5′-AMP have almost the same molecular dimensions. Therefore compounds prepared by ester or amide linkage of amino acids to the carbonyl group of AAA could be considered as model compounds of aa–AMP.

As a part of the conformational studies of aa–AMP we report here the conformational characteristics of *N*-2-(3-indolyl)ethyl- and *N*-2-phenylethyl-5′-deoxy-5′-adenosine-

acetamides, abbreviated as TRPAAA and PHEAAA, respectively, as determined by ultraviolet (UV), circular dichroism (CD) and ^{1}H-nuclear magnetic resonance (NMR) spectroscopies, and empirical energy calculations. The results, especially the conformational differences between both the molecules, may provide useful insight into the substrate specificities of tryptophanyl- and phenylalanyl-tRNA synthetases.

Experimental

Materials——The materials used for the spectroscopic studies are tryptamine (Fluka), β-phenethylamine (Nakarai), AAA and its ethylester, TRPAAA and PHEAAA. The latter four compounds were synthesized according to the literature.[3,5]

UV and CD Spectroscopies——UV absorption spectra were recorded on a Hitachi 624 spectrometer with 10-mm dual cells at 25 °C. The solutions (5.5×10^{-5} M) were prepared by dissolving the sample in 0.025 M phosphate buffer (pH = 6.8). Hypochromicity (%) was calculated as [ε(sample) − ε(summation of components)]/[ε(1 : 1 mixture) − ε(summation of components)] × 100. CD spectra were recorded with a Jasco spectrometer, model J-20. Cells with 10-mm path length were used for the measurements in the aromatic region. The sample concentrations used were 2.2×10^{-4} M, and 0.025 M phosphate buffer was used as the solvent at 25 °C.

^{1}H-NMR Spectroscopy——^{1}H-NMR spectra were measured with a Varian XL-200 (200-MHz) spectrometer equipped with a variable-temperature unit. Chemical shifts were measured *vs.* internal tetramethylsilane (Me_4Si) for $(CD)_3SO$ solution or internal 4,4-dimethyl-4-silapentanesulfonate (DSS), for D_2O solution. Samples were adjusted to about 0.05 M for $(CD_3)_2SO$ solution or 0.005 M for D_2O solution. The conformations of the TRPAAA and PHEAAA nucleoside moieties were analyzed by the conventional methods, based on the observed chemical shifts (δ) and the coupling constants (J).

1) Glycosyl Bond: Stable conformations about the glycosyl bond in many nucleosides and nucleotides exist in both the *anti* and *syn* regions. For semiquantitative evaluation of the conformational equilibrium, the following equation has been proposed:[6]

$$\delta_{obs} = P_{syn}\delta_{syn} + P_{anti}\delta_{anti}$$

where δ_{obs} is the observed chemical shift, δ_{syn} and δ_{anti} are the chemical shifts for the extreme *syn* and *anti* conformations, and P_{syn} and P_{anti} are the populations of these conformations. The conformer populations were analyzed based on the δ_{obs} of H2′, which is the most sensitive to the conformational change of *syn* (δ_{syn} = 5.02 ppm) to *anti* (δ_{anti} = 4.22 ppm) in $(CD_3)_2SO$ solution.[6]

2) Sugar Puckering: The puckering of the ribose ring can be assessed by assuming a C2′-*endo*⇌C3′-*endo* equilibrium. The percentage of C3′-*endo* can be estimated by means of the following equation.[7]

$$\text{C3′-}endo\,(\%) = 100 \times J_{3'4'}/(J_{1'2'} + J_{3'4'})$$

3) Exocyclic C4′–C5′ Bond: The conformation about the C4′–C5′ bond can be estimated using the following expressions:[8]

$$P_{gauche/gauche} = 10 \times 13 - (J_{4'5'} + J_{4'5''})$$

$$P_{gauche/trans\ \text{or}\ trans/gauche} = 100 - P_{gauche/gauche}$$

Conformational Energy Calculation——The PPF (partitioned potential energy function) method was used for energy calculations. The total energy (E) of a molecule was calculated as the sum of the following energies:

$$E = E_{nb} + E_{el} + E_t$$

where E_{nb}, E_{el} and E_t are the nonbonded, electrostatic and torsional energies, respectively. Details of the calculation procedure and the data used for calculations have presented in a previous paper.[1,9] For energy minimization, each torsion angle as a variable parameter was optimized by the Powell algorithm.[10] Minimization was carried out by parabola approximation with 4° intervals, and no angle was permitted to vary by more than 12° at each step.

All numerical calculations were carried out on an ACOS-900 computer at the Crystallographic Research Center, Institute of Protein Research, Osaka University.

Results and Discussion

Hypochromicity

The UV spectra of TRPAAA and PHEAAA are shown in Fig. 1. Both compounds showed significant hypochromicities compared with a 1 : 1 mixture of the respective com-

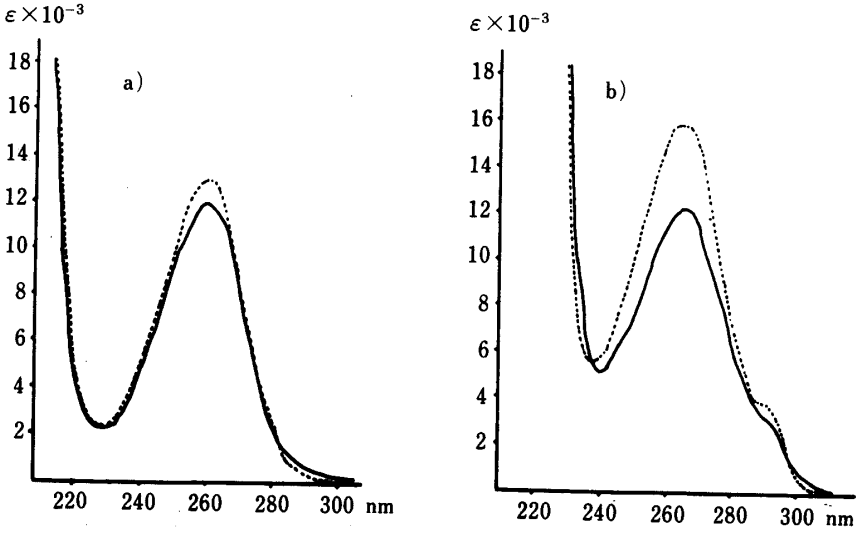


Fig. 1. UV Spectra of PHEAAA and TRPAAA in 0.025 M Phosphate Buffer

a) PHEAAA (——) and 1:1 mixture of 5′-deoxy-5′-adenosineacetic acid ethylester and β-phenethylamine (----). b) TRPAAA (——) and 1:1 mixture of 5′-deoxy-5′-adenosineacetic acid ethylester and tryptamine (----).

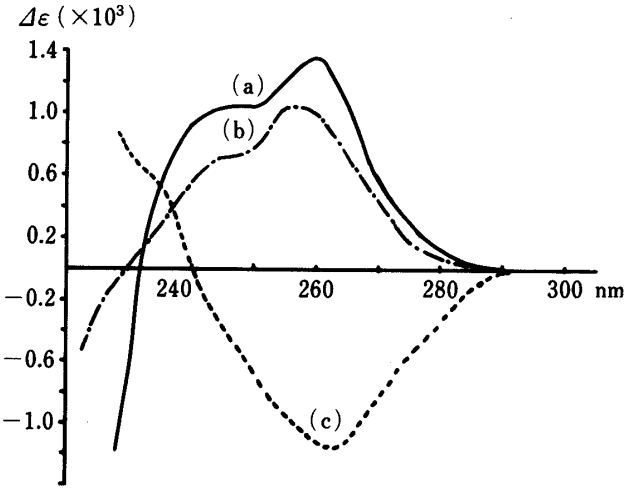


Fig. 2. The CD Spectra of TRPAAA (a), PHEAAA (b) and 5′-Deoxy-5′-adenosineacetic Acid Ethylester (c)

ponents, implying the existence of stacking interaction between the adenine base and the indole or phenyl ring. However, the degrees of hypochromicity were greatly different: 23% hypochromicity for TRPAAA (at 265 nm) and 7% for PHEAAA (at 260 nm). Furthermore, a slight red shift (emergence of a positive band at above 295 nm in the difference spectrum between the compound and the 1:1 mixture) was observed in the TRPAAA spectrum. These results could be interpreted as the result of partial charge–transfer interaction between the adenine and indole rings, while the stacking interaction between the adenine and phenyl rings is due to the normal van der Waals contacts. These UV measurements at a concentration low enough to avoid intermolecular association indicate that TRPAAA may adopt a folded conformation more readily than PHEAAA.

CD Spectra

The CD spectra of TRPAAA and PHEAAA are shown in Fig. 2, along with that of AAA ethylester. The CD band for each transition arises from the perturbation of the planar chromophore by the asymmetric sugar moiety and from the ring–ring interaction. The AAA and its ethylester both have negative bands centered at 262 nm, and these patterns are similar

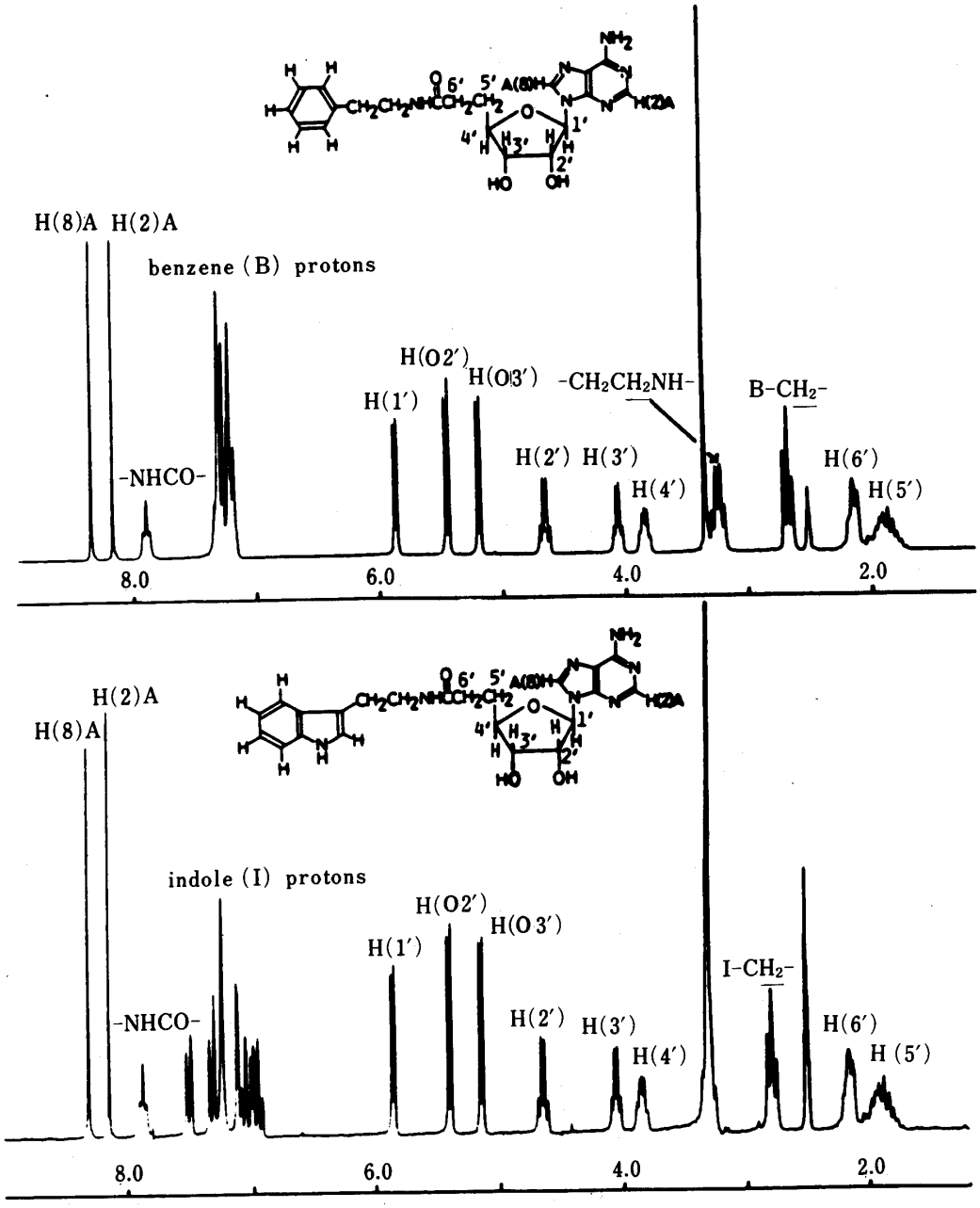


Fig. 3. The 200 MHz ^{1}H-NMR Spectra of TRPAAA and PHEAAA in $(CD_3)_2SO$ Solution

The chemical shifts (ppm) are from Me_4Si.

to those of other adenosine derivatives.[11] On the other hand, the CD patterns of TRPAAA and PHEAAA both have similar positive bands at the 230—290 nm region. Since β-phenethylamine and tryptamine have no CD spectra, this spectral difference may be due to the effect of the indole or phenyl ring on the adenosine moiety; as a result of the stacking interaction, the orientation of the adenine base with respect to the sugar moiety may be markedly different from that of AAA. The similar CD patterns of TRPAAA and PHEAAA imply that the spatial conformations involving the adenosine moiety are not greatly different from each other.

^{1}H-NMR Spectra

1) Possible Molecular Conformation in $(CD_3)_2SO$ Solution——Because of the low

TABLE I. Proton Chemical Shifts (δ),[a] Coupling Constants (J)[b] and Conformational Populations of TRPAAA and PHEAAA in $(CD_3)_2SO$ Solution at 24 °C

	TRPAAA	PHEAAA
1. Chemical shift (ppm)		
H(2)	8.15	8.14
H(8)	8.33	8.32
H(1′)	5.85	5.84
H(2′)	4.65	4.64
H(O2′)	5.46	5.48
H(3′)	4.05	4.04
H(O3′)	5.21	5.20
H(4′)	3.84	3.82
H(5′)	1.88	1.89
H(6′)	2.15	2.12
2. Coupling constant (Hz)		
$J_{1'2'}$	5.1	4.8
$J_{2'3'}$	4.3	4.7
$J_{3'4'}$	5.0	4.8
$J_{4'5'}$[c]	5.3	5.1
$J_{5'6'}$[d]	7.5	7.5
$J_{2'OH}$	5.5	5.9
$J_{3'OH}$	4.8	5.0
3. Calculated population (%) of certain conformers		
Glycosyl bond *anti*	46	47
Ribose ring C3′-*endo*	49	50
C4′–C5′ bond *gauche/gauche*	24	28
gauche/trans	76	72
(*trans/gauche*)		

a) Estimated error, 0.02 ppm. *b*) Estimated error, 0.2 Hz. *c*) $J_{4'5'} = 1/2(J_{4'5'} + J_{4'5''})$. *d*) $J_{5'6'} = 1/4(J_{5'6'} + J_{5'6''} + J_{5''6'} + J_{5''6''})$.

solubilities of TRPAAA and PHEAAA in D_2O solution, we dealt only with the NMR data in $(CD_3)_2SO$ solution. Assignment of all the proton resonances was made on the basis of homonuclear decoupling, spin multiplicities and comparison with published data.[4,12] The ^1H-NMR spectra of TRPAAA and PHEAAA are shown in Fig. 3; the NMR data are summarized in Table I. No preferential population was observed as regards the orientation about the glycosyl bond and the sugar puckering; the populations of *anti* and *syn* conformers about the glycosyl bond, as well as those of C2′-*endo* and C3′-*endo* puckerings for the sugar ring, are almost equal. On the other hand, both molecules exhibited a high preference for the exocyclic C4′–C5′ bond: *gauche*·*trans* or *trans*·*gauche* conformation. This is presumably result of the attachment of the indolylethyl or phenylethyl group to the adenosine moiety.

From the UV and CD spectra, we initially expected that both compounds would take more rigid conformations than AAA, but the NMR spectral data in $(CD_3)_2SO$ solution indicated that the conformations were nearly the same as that of AAA.[4] This may be due to the solvent used, $(CD_3)_2SO$, judging from the large conformational difference of AAA between D_2O and $(CD_3)_2SO$ solutions.[4]

2) Ring Stacking Interactions of TRPAAA and PHEAAA. Temperature Dependence of Protons in D_2O Solution——In order to investigate the ring–ring interactions between the adenine base and the indole or phenyl ring, the temperature dependence of the chemical shifts of their aromatic protons were measured in D_2O solution. Although exact analyses of all the signals were difficult because of the low solubilities of TRPAAA and PHEAAA in D_2O, the aromatic and ribose H1′ protons could be assigned. Figure 4 shows the chemical shifts of

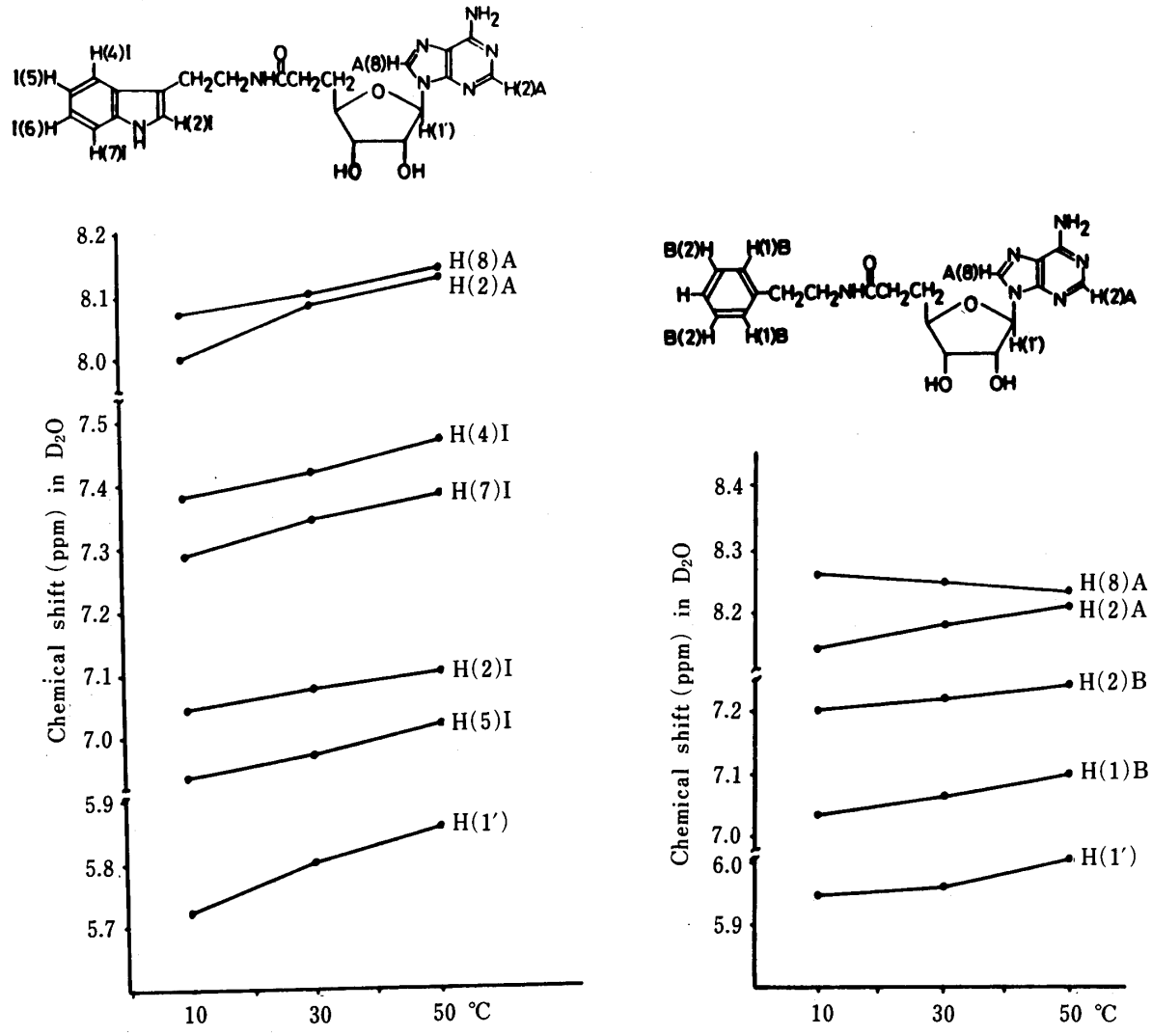


Fig. 4. The Temperature Dependence of the Chemical Shifts of the Aromatic Ring and Ribose H1′ Protons

these protons measured at 10, 30 and 50 °C. Since the protons close to the aromatic ring are subject to ring current effects, it could be expected that the signals of protons attached to one ring are displaced upfield by the stacking interaction with the second aromatic ring. On the other hand, the downfield shifts of the ring protons, as observed at higher temperature, could be interpreted as a result of the disappearance of ring–ring interaction. Since no detectable temperature dependence was observed in the 1 : 1 mixture of the components, the changes of the chemical shifts should be mainly due to the intramolecular interaction between the aromatic rings. It is conceivable from Fig. 4 that the adenine base interacts with the indole or phenyl ring at low temperature, but the mode and degree of stacking interaction appear to be different from each other in the two compounds; the indole ring of TRPAAA is stacked on the whole of the adenine base, while the stacking interaction of PHEAAA is rather weak, and the binding site of the phenyl ring is the pyrimidine moiety of the adenine base. Weak stacking interaction in PHEAAA, as compared with that in TRPAAA, was also suggested from the UV measurements.

The changes of H1′ coupling constant ($J_{1'2'}$) accompanying the temperature variation are shown in Fig. 5. The $J_{1'2'}$ of TRPAAA showed a linear temperature dependence, while that of PHEAAA was less variable. Since the value of $J_{1'2'}+J_{3'4'}$ is known to be constant

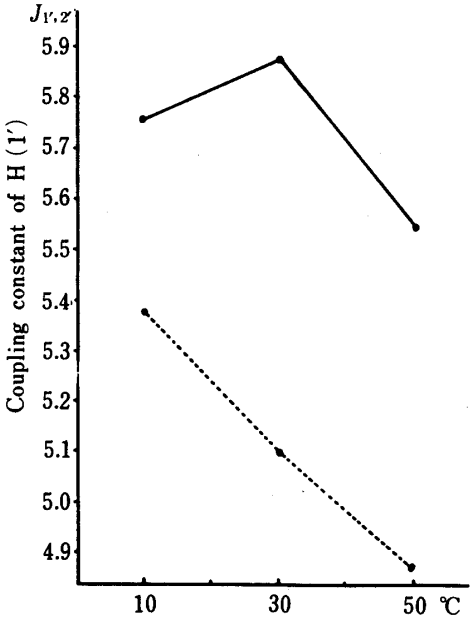
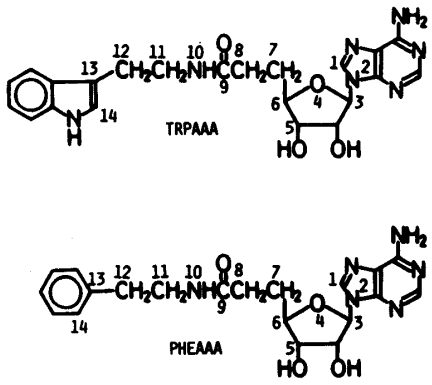


Fig. 5. The Temperature Dependence of the Coupling Constant (Hz) of Ribose H1′ Proton

——, PHEAAA; ----, TRPAAA.



ribose ring:	C2′-*endo*, C3′-*endo*
ω1 (1–2–3–4):	30°, −150°
ω2 (5–6–7–8):	60°, 180°, −60°
ω3 (6–7–8–9):	60°, 180°, −60°
ω4 (7–8–9–10):	60°, 180°, −60°
ω5 (9–10–11–12):	60°, 180°, −60°
ω6 (10–11–12–13):	60°, 180°, −60°
ω7 (11–12–13–14):	90°, −90°

Fig. 6. Notation of the Torsion Angles and Their Starting Sets

In PHEAAA, a torsion angle of $\omega 7 = 90°$ gives the same conformation as $\omega 7 = -90°$.

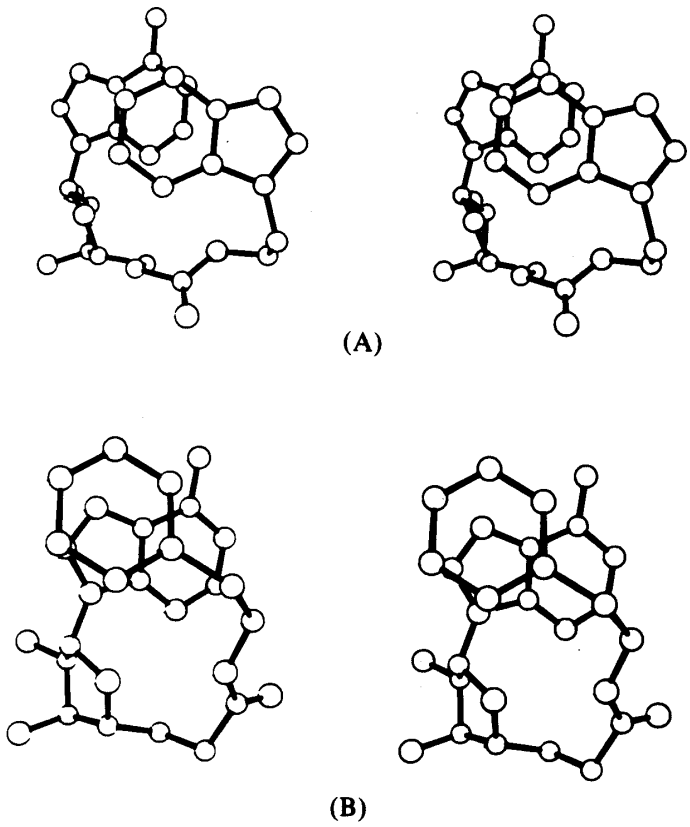


Fig. 7. Stereo Views of the Most Energetically Stable Conformations of TRPAAA (A) and PHEAAA (B)

(=10.1 Hz),[13] the increase of $J_{1'2'}$ value at lower temperature in TRPAAA implies the predominance of S-conformer (C2′-*endo* sugar puckering). Therefore it can be deduced from these NMR data that the TRPAAA molecule prefers the S-conformer with stacking between the indole ring and adenine base in aqueous solution.

TABLE II. Starting and Refined Torsion Angles (°) and Their Energies

Order	Starting angle							Refined angle							Energy kcal/mol	Sugar pucker
	ω1	ω2	ω3	ω4	ω5	ω6	ω7	ω1	ω2	ω3	ω4	ω5	ω6	ω7		
								TRPAAA								
1	−150	180	180	60	−60	−60	−90	−150	180	178	51	−70	−65	−94	−13.66	C2′-*endo*
2	30	180	180	60	60	−60	−90	23	195	190	96	86	−33	−84	−12.91	C2′-*endo*
3	−150	180	180	60	60	60	90	−142	194	191	86	78	73	81	−12.52	C2′-*endo*
4	−150	180	180	60	180	−60	−90	−166	191	183	67	184	−43	−25	−12.36	C2′-*endo*
5	−150	180	180	180	180	−60	−90	−158	181	181	155	173	−67	−90	−12.00	C2′-*endo*
6	30	180	180	180	60	60	90	36	189	186	211	79	50	94	−11.99	C2′-*endo*
7	−150	180	180	−60	−60	60	−90	−154	193	189	−86	−61	48	−73	−11.82	C2′-*endo*
8	−150	180	180	180	60	−60	−90	−159	189	191	194	82	−56	−96	−11.75	C3′-*endo*
9	−150	180	180	60	−60	−60	−90	−168	187	194	33	−78	−70	−98	−11.17	C3′-*endo*
10	−150	180	180	−60	60	−60	−90	−144	189	185	−93	84	−59	−93	−10.63	C2′-*endo*
								PHEAAA								
1	−150	180	−60	60	180	−60	90	−134	180	−96	89	182	−75	89	−25.17	C2′-*endo*
2	30	180	−60	60	60	180	90	77	183	−64	95	77	181	88	−23.86	C2′-*endo*
3	−150	180	−60	60	180	−60	90	−155	164	−99	92	190	−69	86	−23.55	C3′-*endo*
4	−150	180	−60	60	60	180	90	−141	190	−73	89	71	183	91	−23.20	C2′-*endo*
5	−150	180	−60	−60	−60	−60	90	−174	198	−83	−46	−57	−60	93	−22.96	C3′-*endo*
6	−150	180	−60	−60	−60	−60	90	−144	209	−58	−58	−50	−56	91	−22.94	C2′-*endo*
7	−150	−60	60	−60	180	60	90	−126	−64	70	43	169	52	88	−22.36	C2′-*endo*
8	−150	180	−60	60	180	180	90	−133	185	−94	92	174	191	88	−21.92	C2′-*endo*
9	30	180	−60	60	180	−60	90	30	167	−93	95	185	−70	90	−21.55	C3′-*endo*
10	30	180	−60	180	60	60	90	35	204	−61	153	64	59	89	−21.43	C2′-*endo*

Conformational Energy Calculations

To elucidate what stacking conformation is energetically stable, we carried out energy calculations for various conformers by using a minimization technique. The requisite structural parameters for TRPAAA and PHEAAA were obtained by model building on the basis of the X-ray studies of AAA,[4] tryptamine[14] and β-phenethylamine.[15] The nomenclature of the torsion angles and their possible starting angles for minimization are shown in Fig. 6. These starting angles were determined on the basis of various X-ray structural results. Of 1944 different sets ($2\times2\times3\times3\times3\times3\times3\times2$) for each of TRPAAA and PHEAAA, some conformers had abnormally short steric contacts and were excluded from the energy calculations. The starting and refined torsion angles of the ten energetically most stable conformers are listed in Table II, along with their energy values (kcal/mol).

It is important to note that the stable conformers exist overwhelmingly in the *syn* orientations ($\omega1 = -150°$) about the glycosyl bond and with the C2′-*endo* ribose puckerings. However, this tendency appears to be more prominent for TRPAAA than PHEAAA. Among the variable torsion angles, $\omega2$ and $\omega3$ defining the relative orientation between the adenosine and indolylethyl or phenylethyl moieties appear to play an important role in determining the energetically preferred conformation. As is clear from Table II, the combinations of (180°, 180°) for TRPAAA and (180°, −60°) for PHEAAA as the ($\omega2$, $\omega3$) starting set give energetically stable conformers; the conformations of the exocyclic C4′–C5′ bond ($\omega2$) are all in the *trans·gauche* region, in agreement with the ^{1}H-NMR results in $(CD_3)_2SO$ solution.

The most energetically stable conformations for both compounds are shown in Fig. 7. The stacking interaction between aromatic rings can be seen in both conformations. These stacking modes are different from those expected from the ^{1}H-NMR data: the stacking degree of TRPAAA is rather less than that of PHEAAA, while the ^{1}H-NMR data suggested the reverse. Based on CPK model buildings using normal van der Waals radii, however, conformers which are not in conflict with the NMR data can be built from these energetically stable conformers without large changes of torsion angle, and have the energy values of *ca.* −10 kcal/mol for TRPAAA and −20 kcal/mol for PHEAAA.

The lower energy values of PHEAAA (−21 – −25 kcal/mol) than TRPAAA (−10 – −13 kcal/mol) imply that the former compound has various conformations that are more stable than those of the latter. An extended conformer of PHEAAA (C2′-*endo*, $\omega1 = -140°$, $\omega2 = 195°$, $\omega3 = -61°$, $\omega4 = -86°$, $\omega5 = 167°$, $\omega6 = -51°$ and $\omega7 = 89°$) had an energy of −20.18 kcal/mol, and the energy difference from the most stable conformer is about 5 kcal/mol. In the case of TRPAAA, this difference is about 10 kcal/mol: the most stable extended conformation has an energy of −3.86 kcal/mol: (C3′-*endo*, $\omega1 = -154°$, $\omega2 = 190°$, $\omega3 = 187°$, $\omega4 = 87°$, $\omega5 = 177°$, $\omega6 = 182°$ and $\omega7 = -91°$). Since these energy calculations did not take into account any solvent effect and treated the molecule as isolated, it is difficult to draw any definite conclusions. However, it appears that TRPAAA takes stacked conformations preferentially, while PHEAAA is, to some extent, at equilibrium between the folded and extended conformers.

The results of the present study may be summarized as follows.

1) The ring–ring stacking interactions of TRPAAA and PHEAAA were evaluated by UV, ^{1}H-NMR and conformational energy calculations.

2) The tendency to take the stacking conformation is stronger for TRPAAA than PHEAAA.

3) The ribose ring of TRPAAA predominantly shows C2′-*endo* puckering with stacking.

These conformational differences should provide clues for understanding the substrate-specificity of aminoacyl-AMP synthetases.

References and Notes

1) This work is part XV of "Structural Studies of the Interaction between Indole Derivatives and Biologically Important Aromatic Compounds." Part XIV: T. Ishida, M. Itoh, M. Horiuchi, S. Yamashita, M. Doi, M. Inoue, Y. Mizunoya, Y. Tona and A. Okada, *Chem. Pharm. Bull.*, **34**, 1853 (1986).
2) P. Berg, *J. Biol. Chem.*, **233**, 608 (1958).
3) T. Kurihara, A. Ota, H. Hayashi, H. Urata, M. Inoue and T. Ishida, *Chem. Pharm. Bull.*, **31**, 2126 (1983).
4) T. Ishida, T. Miyazaki, M. Inoue, A. Ota, T. Kurihara and M. Sugiura, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1325.
5) T. Kurihara, A. Ota, H. Hayashi, M. Inoue and T. Ishida, *J. Heterocycl. Chem.*, **21**, 259 (1984).
6) R. Stolarski, A. Pohorille, L. Dudycz and D. Shugar, *Biochim. Biophys. Acta*, **610**, 1 (1980).
7) D. B. Davies and S. S. Danyluk, *Biochemistry*, **13**, 4417 (1974).
8) D. J. Wood, F. E. Hruska, R. J. Mynott and R. H. Sarma, *Can. J. Chem.*, **51**, 2571 (1973).
9) Y. Miyamoto, T. Ishida and M. Inoue, *Chem. Pharm. Bull.*, **29**, 3427 (1981).
10) M. J. D. Powell, *Comput. J.*, **7**, 155 (1964).
11) W. C. Brunner and M. F. Maestre, *Biopolymers*, **14**, 555 (1975).
12) S. P. Hiremath and R. S. Hosmane, *Advan. Heterocycl. Chem.*, **15**, 277 (1973).
13) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
14) M. Inoue, T. Sakaki, A. Wakahara and K. Tomita, *Biochim. Biophys. Acta*, **123**, 543 (1978).
15) T. Ishida, K. Yamamoto and M. Inoue, *Bull. Chem. Soc. Jpn.*, **56**, 955 (1983).