Ring Stacking Interaction on the Thiamin–Tryptophan Systems

Toshimasa Ishida,*[1a] Miyuki Matsui,[1a] Masatoshi Inoue,[1a] Hiroshi Hirano,[1b] Mayumi Yamashita,[1b] Kazuaki Sugiyama,[1b] Makiko Sugiura,[1c] and Ken-ichi Tomita[1d]

Contribution from the Department of Physical Chemistry, Osaka College of Pharmacy, 2-10-65 Kawai, Matsubara, Osaka 580, Japan, the Department of Synthetic Organic Chemistry, Osaka College of Pharmacy, Kobe Women's College of Pharmacy, 4-19-1 Motoyama kita, Higashinada, Kobe 658, Japan, and the Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka, Suita, Osaka 565, Japan. *Received January 31, 1984*

Abstract: In order to investigate the specific interaction mode, at the atomic level, between thiamin coenzyme and tryptophanyl residues in apoenzymes, three model compounds, thiamin indole-3-propionate (PTI), 5-[2-(indol-3-ylpropionyloxy)ethyl]-3,4-dimethylthiazolium iodide (TI), and 4-amino-5-[(indol-3-ylpropionylamino)methyl]-2-methylpyrimidine (PI), were synthesized, and the specific ring–ring stacking interactions were determined in these compounds by the spectroscopic and X-ray crystallographic measurements. UV and ^{1}H NMR spectra indicated π–π stacking interactions between the indole and pyrimidine rings and between the indole and thiazolium rings. X-ray analyses of these three compounds showed three kinds of π–π stacking modes: indole–pyrimidinium (PTI), indole–thiazolium (TI), and indole–pyrimidine (PI). These ring–ring interactions are all characterized by nearly parallel alignments of both aromatic rings and by the interplanar spacing of 3.4–3.6 Å. An important result obtained from both the spectroscopic and X-ray crystallographic studies is that the π–π stacking interaction between an indole ring and a pyrimidine ring or between indole and thiazolium rings is significantly strengthened when the pyrimidine and thiazolium rings are linked by a methylene group, as in the thiamin molecule. On the other hand, empirical energy calculations for 5-(3-indol-3-ylpropyl)-3,4-dimethylthiazolium (TC3I), 4-amino-5-(3-indol-3-ylpropyl)-2-methylpyrimidine (PC3I), and 4-amino-5-(3-indol-3-ylpropyl)-2-methyl-1*H*-pyrimidinium cation (PHC3I) showed that the stacking interactions between the indole and pyrimidine rings are nearly equal to those between the indole and thiazolium rings, although protonation of the pyrimidine N1 atom makes the stacking interaction with the indole ring energetically unstable. The biological implications of these findings are considered.

Thiamin, as its pyrophosphate form, is an important cofactor of several enzymes which catalyse the decarboxylation of α-keto acids such as pyruvic acid and α-ketoglutaric acid and the transfer of aldehyde or acyl groups.[2] It has been shown in many enzymes such as pyruvate oxidase (*Escherichia coli*)[3] and transketolase (bakers' yeast)[4] that a tryptophanyl residue in these apoenzymes may play an important role in binding with the thiamin coenzyme and the subsequent catalytic reaction. In these observations, spectroscopic studies by Sable et al.[5] showed that thiamin can form molecular complexes with indole derivatives, and such complexes may be responsible for the binding of coenzyme with apoenzymes. A detailed knowledge of the molecular geometry of tryptophan–thiamin complexes may, therefore, be helpful in understanding the roles of all the components of the thiamin coenzyme and the tryptophanyl residue in the catalytic reaction.

To our knowledge, no crystallographic study on the tryptophan–thiamin interaction has been reported. But X-ray studies on thiamin picrolonate[6] and thiamin pyrophosphate 1,10-phenanthroline aqua copper[7] complexes indicated stacking between the pyrimidine moiety of thiamin and the indole ring of tryptophan. This interaction is somewhat different from that proposed from spectroscopic studies in solution,[5] i.e., the tryptophanylindole ring centers upon the positively charged quarternary nitrogen atom of thiamin, whereas both the pyrimidine and thiazolium rings are involved in π–π charge-transfer interactions with the indole ring. Therefore, these association modes largely depend on the three stable conformations of thiamin molecule, namely, the F, S and V conformations,[8] determined in more than a dozen crystal structures.

Since the specific structure of the thiamin molecule apparently influences its catalytic properties as a coenzyme, it is of great importance to elucidate the conformation of thiamin in its interactive state with the tryptophanyl residue. In an effort to observe how these species interact at the atomic resolution, we have attempted to obtain complexes suitable for X-ray crystallographic and various spectroscopic studies by using the model compounds shown in Figure 1. We discuss here the ring–ring interaction mode in thiamin–tryptophan systems, based on the absorption spectroscopy, ^{1}H NMR spectroscopy, X-ray crystallographic studies, and conformational energy calculations. The preliminary X-ray results for thiamin indole-3-propionate (PTI) perchlorate[9] and 5-[2-(indole-3-propionyloxy)ethyl]-3,4-dimethylthiazolium (TI) iodide[10] have been already reported.

Experimental Section

Materials. Thiamin, L-tryptophan, and L-tryptophan methyl ester hydrochloride were obtained from Wako Pure Chemical Co. (Japan) and indole-3-propionic acid from Sigma Co. (U.S.A.). PTI, TI, 4-amino-5-[(indole-3-propionylamino)methyl]-2-methylpyrimidine (PI), 5-(2-hydroxyethyl)-3,4-dimethylthiazolium (T), and 4-amino-5-(aminomethyl)-2-methylpyrimidine (P) were chemically synthesized from commercially available reagents. Melting points (uncorrected) were determined on a Yanagimoto micromelting piont apparatus, and microanalyses were done in the microanalytical laboratory of our college.

Synthetic Procedures: PTI. A mixture of thiothiamin (6 g)[11] and indole-3-propionic anhydride (10.9 g) in pyridine (750 mL) was allowed to stand for 48 h at room temperature. After concentration under reduced pressure, the residue was extracted with ethyl acetate and was washed successively with 5% sodium hydrogen carbonate solution and

(1) (a) Department of Physical Chemistry, Osaka College of Pharmacy. (b) Department of Synthetic Organic Chemistry, Osaka College of Pharmacy. (c) Kobe Women's College of Pharmacy. (d) Pharmaceutical Sciences, Osaka University.

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Table I. Summary of Crystal Data and Intensity Collections

	PTI	TI	PI
formula	$C_{23}H_{25}N_5O_2S^+ \cdot ClO^- \cdot HClO_4 \cdot CH_3OH$	$C_{18}H_{21}N_2O_2S^+ \cdot I^-$	$C_{17}H_{19}N_5O$
F_w	667.49	456.34	309.37
crystal system	triclinic	monoclinic	monoclinic
space group	$P\bar{1}$	$P2_1/c$	$P2_1/c$
cell constants			
a, Å	14.960 (4)	7.663 (2)	4.945 (2)
b, Å	14.170 (3)	10.870 (4)	22.416 (9)
c, Å	7.436 (2)	23.100 (9)	14.372 (6)
α, deg	101.79 (2)	90.00	90.00
β, deg	102.39 (2)	105.20 (3)	95.47 (4)
δ, deg	94.15 (2)	90.00	90.00
vol, Å^3	1496.1 (6)	1856.8 (11)	1585.9 (11)
Z	2	4	4
density, Mg/m^3			
calcd	1.484	1.632	1.296
obsd	1.469 (3)	1.613 (2)	1.287 (1)
crystal dimens, mm	0.2 × 0.05 × 0.1	0.3 × 0.1 × 0.05	0.3 × 0.1 × 0.2
linear abs coeff, cm^{-1}	37.71	149.31	6.95
take-off angle, deg	2.0	2.0	2.0
scan speed (in 2θ/min), deg/min	4.0	4.0	4.0
data limit, deg	<130.0	<130.0	<130.0
bkgd counts, s	5.0	5.0	5.0
scan range in $A + B \tan \theta$			
A, deg	2.0	1.2	1.2
B, deg	0.15	0.15	0.15
data collected	$\pm h,k,\pm l$	$h,k,\pm l$	$h,k,\pm l$
no. of obsd reflections	5109	3155	2690
unique data with $F_o^2 > 3\sigma(F_o)^2$	4477	2825	2045
coeff used in refinements (see text)			
a	0.0	0.0	0.30469
b	−0.21625	−0.24381	−0.18025
c	0.01121	0.065	0.093
R in $[F_o^2 > 3\sigma(F_o)^2]$	0.100	0.065	0.093
R_w in $[F_o^2 > 3\sigma(F_o)^2]$	0.117	0.102	0.092

then with water. Concentrated over anhydrous sodium sulfate, thiothiamin-indole-3-propionic acid ester (7 g) was obtained as crude crystals in 74% yield, mp 103–110 °C. To the above thiothiamin derivatives (2.3 g) without further purification suspended into an ice-cooled ethanolic solution (45 mL) containing concentrated sulfuric acid (0.12 g), 30% hydrogen peroxide (1.6 g) was added dropwisely with stirring. After continuous stirring for 2 h, the reaction mixture was allowed to stand overnight at room temperature. The resulting resinous substance was washed with methanol and recrystallized in dimethyl sulfoxide–methanol mixture to obtain PTI hydrogen sulfate (1.0 g) as pale yellow crystalline powders in 40% yield, mp 203–205 °C. Anal. Calcd for $C_{23}H_{27}N_5O_6S_2$: C, 51.77; H, 5.10; N, 13.12. Found: C, 51.30; H, 5.36; N, 13.43.

Sodium perchlorate (0.26 g) was added to an aqueous solution (5 mL) of PTI hydrogen sulfate (0.5 g), and a resinous crude product (0.25 g), after washing with ethyl acetate, was dissolved in methanol–ethyl acetate. By slow evaporation at room temperature, it gives PTI perchlorate hydroperchlorate as pale yellow platelets, mp 135–141 °C. Anal. Calcd for $C_{23}H_{27}C_{12}N_5O_{10}S$: C, 43.41; H, 4.28; N, 11.00. Found: C, 43.21; H, 4.46; N, 11.17.

PI. A mixture of P[12] (0.66 g) and indole-3-propionic anhydride (1.72 g) in pyridine (4 mL) was allowed to stand overnight at room temperature, and the resulting solution was evaporated to dryness under reduced pressure. The residue was washed extensively with 5% aqueous sodium hydrogen carbonate solution, and the colorless needle crystals of PI were obtained from ethanol solution, mp 213–215 °C, yield 0.85 g (57.7%). Anal. Calcd for $C_{17}H_{19}N_{50}$: C, 66.00; H, 6.19; N, 22.64. Found: C, 66.29; H, 6.22; N, 22.77.

TI. T[13] (0.68 g) was added to a cooled mixture of indole-3-propionic acid (0.9 g) and dicyclohexylcarbodiimide (0.98 g) in anhydrous pyridine (3 mL) with stirring for 30 min. After standing overnight at room temperature, and by addition of ethyl acetate to the reaction mixture, the precipitate (dicyclohexylurea) was removed by filtration. The filtrate was evaporated to dryness under reduced pressure, and the resulting residue gave pale yellow needle crystals of TI iodide after recrystallization from n-hexane–benzene, mp 105–107 °C, yield 1.35 g (66.7%). Anal. Calcd for $C_{18}H_{21}N_2O_2SI$: C, 47.38; H, 4.64; N, 6.14. Found: C, 47.10; H, 4.70; N, 6.44.

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Spectroscopic Measurements. The electronic absorption spectra at a given temperature were recorded on a Hitachi 624 spectrometer with 10-mm dual cells. For quantitative measurements, the solutions of 1.5 × 10^{-4} M (hypochromic measurements) and 0.03 M (charge-transfer band measurements) concentrations were prepared by dissolving the sample in 0.025 M phosphate buffer containing 10% ethanol (pH 6.9 at 25 °C). Spectra were measured twice and averaged. The ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) spectrometer equipped with fast FT and temperature-controlled units (the error: within ±1 °C). The solutions with 0.05 M concentration were prepared as 50% CD_3OD–D_2O solution. Chemical shifts were measured with 3-(trimethylsilyl)-d_4 propionate (TSP) as an internal standard.

Crystal Structure Determination of PTI Perchlorate Hydroperchlorate, TI Iodide, and PI: Data Collection. Crystals were obtained by slow evaporation at room temperature (ca. 21 °C) from ethyl acetate–methanol (1:1, v/v) mixture (for PTI) or 50% aqueous ethanol (for TI and PI). Crystal data for these compounds are summarized in Table I. Intensities for each crystal were collected in a similar manner: a single crystal was mounted on a goniometer and intensity data collections were performed with Cu Kα radiation (λ = 1.54184 Å) monochromatized with a graphite crystal on a Rigaku AFC-5 computer-controlled diffractometer at 40 kV and 180 mA. The unit-cell dimensions were refined with a least-squares procedure of setting angles of 25 strong reflections (30° < 2θ < 60°). The θ–2θ scan technique was employed for intensity recording. Peak counts were corrected with background counts at both ends. Intensities were also corrected for Lorentz and polarization effects. The data for PTI and TI were further corrected for absorption effects by using an empirical correction based on ϕ scans.

Structure Determinations and Refinements. The structures of PTI and TI were solved by heavy-atom method and successive Fourier syntheses: the positions of chlorine (for PTI) or iodine (for TI) atoms were determined by interpretation of a three-dimensional Patterson map, and all the remaining non-hydrogen atoms were positioned by successive Fourier syntheses. On the other hand, the structure of PI was solved by direct method (program MULTAN 78[14]): a phase set of 212 $|E|$'s (>1.80) with

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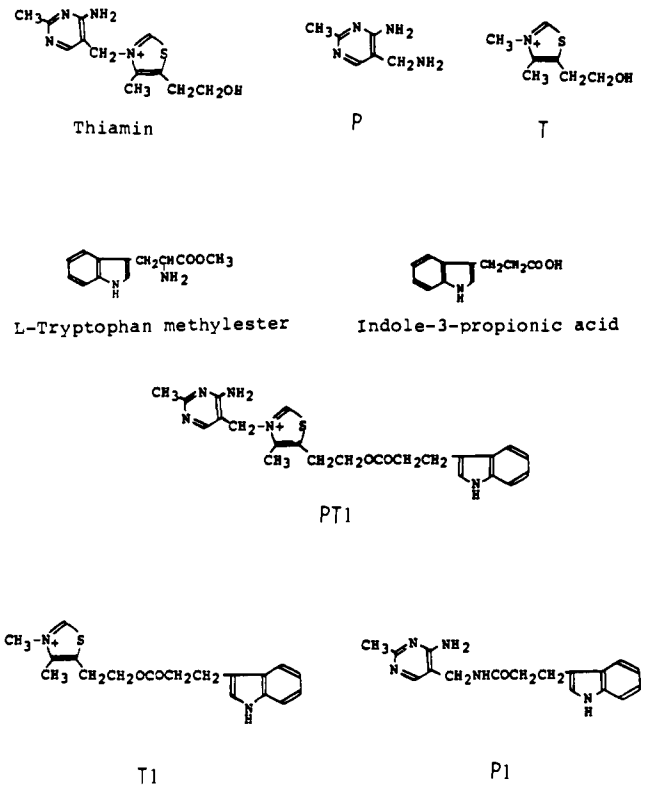

Figure 1. Chemical structures and abbreviated names of model compounds used for studies of thiamin–indole interaction.

the highest figure of merit gave an E map in which all non-hydrogen atoms could be reasonably located. Refinement of each structure was done in a similar manner: positional parameters of non-hydrogen atoms were refined by a full-matrix least-squares method with isotropic thermal parameters and then by a block-diagonal least-squares method with anisotropic ones. Geometrically reasonable hydrogen atom positions were determined on a difference map and included in subsequent refinements with isotropic temperature factors. The function minimized was $\sum w(|F_o| - |F_c|)^2$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes. The used weighting scheme is as follows: $w = a$, for $F_o = 0.0$, and $w = 1.0/[\sigma(F_o)^2 + b|F_o| + c|F_o|^2]$ for $F_o > 0.0$, where $\sigma(F_o)^2$ is the standard deviation of the intensity based on counting statistics and the coefficients, a, b, and c, are given in Table I. In the final refinements, none of the positional parameters shifted more than their standard deviations. Final R ($\sum||F_o| - |F_c||/\sum F_o$) and R_w ($\sum w(|F_o| - |F_c|)^2/\sum wF_o^2$) are tabulated. The atomic parameters of non-hydrogen atoms with their standard deviations are given in Table II. For all crystallographic computations, the UNICS programs[15] and the atomic scattering factors by Cromer and Weber[16] were used.

Conformational Energy Calculations of 5-(3-Indol-3-ylpropyl)-3,4-dimethylthiazolium (TC3I), 4-Amino-5-(3-indol-3-ylpropyl)-2-methylpyrimidine (PC3I), and 4-Amino-5-(3-indol-3-ylpropyl)-2-methyl-1*H*-pyrimidinium (PHC3I). The PPF (partitioned potential energy function) method was used for energy calculation; the total energy (E_{tot}) of a molecule can be represented by $E_{tot} = E_{nb} + E_{el} + E_t$, wheere E_{nb}, E_{el}, and E_t are the nonbonded, electrostatic, and torsional energies, respectively. These quantities, in units of kilocalories/mole can be obtained by the following equations:

$$E_{nb} = \sum_{i>j}\sum(-A_{ij}R_{ij}^{-6} + B_{ij}R_{ij}^{-12}) \quad (1)$$

$$E_{el} = \sum_{i>j}\sum 332.0Q_iQ_jR_{ij}^{-1}e^{-1} \quad (2)$$

$$E_t = \sum_{k=1}^{N} 0.5\ V_k(1.0 + \cos X\,\theta k) \quad (3)$$

In eq 1–3, R_{ij} is the distance between atoms i and j in angstroms; A_{ij} and B_{ij} are the coefficients in the Lennard-Jones "6–12" potential function.[17]

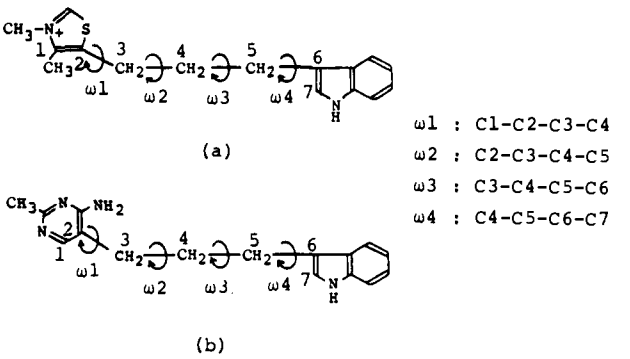

Figure 2. Variable torsion angle notations of TC3I (a) and PC3I (b).

Q_i is the Coulombic charge on atom i, calculated by the CNDO/2 method;[18] e is the dielectric constant and was taken as 4.0, close to the experimental values for biomolecules in polar media[17] V_k is the barrier potential for the internal rotation about the kth torsion angle (θk) and was taken as 2.5 kcal/mol.[17] X is the periodicity for the barrier [=6.0 for C(aliphatic)–C(aromatic) and 3.0 for C(aliphatic)–C(aliphatic)], and N is the number of variable torsion angles (=4). The notations of torsion angles for TC3I and PC3I are shown in Figure 2.

For energy minimization, each torsion angle as a variable parameter was optimized by the Powell algorithm.[19] 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 the numerical calculations were carried out on an ACOS-900 computer at the Computation Center of Osaka University.

Results and Discussion

Ring-Stacking Interaction of Tryptophan–Thiamin Systems in Aqueous Solution. Hypochromicity and Charge-Transfer Bands. A method for assessing ring–ring interaction is the measurement of hypochromicity, which is mainly dependent upon the degree and orientation of intra-and/or intermolecular stacking.[20] In order to estimate the extent of interaction between the indole ring and the thiazolium and/or pyrimidine ring in the model compounds, their electronic absorption spectra were quantitatively compared with the sum of spectral intensities for individual constituents. The averaged percentages of hypochromicity (at λ_{max}) at 5, 25, and 45 °C are given in Table III. The negative bands at 257–294 nm for PTI and at 249–296 nm for TI are due to the hypochromic effects, indicating the presence of prominent stacking interaction between the indole ring and thiazolium and/or pyrimidine ring. Since the spectra were measured with the solution at its concentration low enough to avoid intermolecular association, these effects are apparently due to the intramolecular ring–ring interaction accompanied by the folded conformation of PTI or TI molecule. The fact that these hypochromicities increase at lower temperature implies that the folded conformer is energetically more stable than the extended one. As suggested by yellowish colorations of PTI and TI solutions, the characteristic positive broad bands at 295–380 nm can be assigned as the charge-transfer bands[21] resulting from the electronic transitions from the indole ring to the thiazolium and/or pyrimidine rings. A similar positive broad band at 310–350 nm is also observable in the thiamin–tryptophan system.[5] λ_{max} and ϵ_{max} for respective bands at 25 °C are as follows: for PTI, λ_{max} = 315 nm, ϵ = 533.3; for TI, λ_{max} = 313 nm, ϵ = 253.3; for thiamin-L-tryptophan methyl ester, λ_{max} = 312 nm, ϵ = 29.5. The intensities of these charge-transfer bands also increase at lower temperature up to the similar extent as

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Table II. Atomic Coordinates ($\times 10^4$) of Non-Hydrogen Atoms and Their Estimated Standard Deviations in Parentheses

$$B_{eq} = \frac{4}{3}(a^2B_{11} + b^2B_{22} + c^2B_{33} + abB_{12}\cos\gamma + acB_{13}\cos\beta + bcB_{23}\cos\alpha)$$

atom	x	y	z	B_{eq}, Å	atom	x	y	z	B_{eq}, Å
		PTI					TI		
S(1)	3150 (1)	7948 (1)	11744 (2)	5.8	S(1)	9290 (3)	1679 (1)	6651 (1)	4.8
C(2)	2015 (4)	7785 (4)	11335 (8)	5.0	C(2)	10292 (10)	2898 (7)	7037 (3)	4.9
N(3)	1632 (3)	8230 (3)	10062 (6)	4.2	N(3)	10236 (7)	3863 (5)	6692 (2)	4.0
C(4)	2225 (4)	8753 (4)	9374 (7)	4.5	C(35′)	11079 (10)	5062 (6)	6937 (4)	5.5
C(4α)	1883 (5)	9325 (5)	7934 (9)	6.1	C(4)	9358 (8)	3673 (5)	6096 (3)	3.8
C(5)	3091 (4)	8682 (4)	10142 (8)	5.1	C(4α)	9169 (11)	4685 (7)	5641 (3)	5.7
C(5α)	3960 (5)	9107 (5)	9757 (10)	6.4	C(5)	8746 (8)	2499 (6)	5992 (3)	4.1
C(5β)	4179 (5)	8570 (5)	7983 (11)	6.7	C(5α)	7790 (10)	1931 (6)	5408 (3)	4.8
O(5γ)	4243 (3)	7580 (3)	8181 (6)	6.1	C(5β)	9142 (10)	1585 (6)	5056 (3)	4.6
C(35′)	591 (4)	8178 (4)	9375 (8)	4.9	O(5γ)	8282 (6)	1040 (4)	4492 (2)	4.3
N(1′)	−855 (3)	7610 (3)	12602 (7)	4.7	N(1)I	5890 (8)	−447 (5)	1662 (2)	4.5
C(2′)	−922 (4)	6630 (4)	12282 (7)	4.2	C(2)I	6488 (9)	−124 (5)	2263 (3)	4.1
C(2′α)	1403 (4)	6152 (4)	13464 (9)	5.4	C(3)I	6143 (8)	1100 (5)	2330 (3)	3.8
N(3′)	−547 (3)	6132 (3)	11047 (6)	4.3	C(4)I	4614 (9)	2731 (6)	1519 (3)	4.4
C(4′)	−66 (4)	6585 (4)	10058 (7)	4.4	C(5)I	3880 (9)	2830 (7)	923 (3)	5.0
N(4′α)	298 (4)	6047 (3)	8833 (7)	6.3	C(6)I	3725 (10)	1849 (6)	525 (3)	5.0
C(5′)	47 (4)	7630 (4)	10404 (7)	4.4	C(7)I	4377 (8)	709 (6)	727 (3)	4.5
C(6′)	−374 (4)	8088 (4)	11680 (8)	4.8	C(8)I	5120 (8)	578 (6)	1328 (3)	3.8
N(1)I[a]	3863 (5)	3051 (5)	7865 (9)	8.3	C(9)I	5287 (8)	1552 (5)	1755 (3)	3.7
C(2)I	4076 (6)	3915 (6)	7344 (10)	7.3	C(10)I	6590 (10)	1832 (5)	2906 (3)	4.4
C(3)I	3851 (4)	4657 (5)	8523 (8)	5.7	C(11)I	7075 (9)	1065 (6)	3455 (3)	4.0
C(4)I	3076 (5)	4657 (5)	11364 (9)	6.4	C(12)I	7661 (9)	1776 (5)	4025 (3)	4.0
C(5)I	2761 (6)	4059 (6)	12375 (11)	7.7	O(12)I	7679 (7)	2892 (4)	4078 (2)	4.3
C(6)I	2783 (6)	3069 (6)	11903 (13)	8.7					
C(7)I	3143 (6)	2642 (6)	10434 (13)	8.5	iodide	2316 (1)	3331 (0)	8729 (0)	4.3
C(8)I	3474 (5)	3245 (5)	9417 (10)	7.0			PI		
C(9)I	3458 (4)	4255 (5)	9847 (8)	5.7	N(1′)	4894 (5)	1398 (1)	4183 (2)	3.8
C(10)I	3956 (5)	5722 (5)	8494 (9)	5.9	C(2′)	4271 (6)	992 (1)	4811 (2)	3.4
C(11)I	4382 (5)	5940 (5)	6918 (9)	6.3	C(2′α)	5895 (7)	977 (2)	5727 (3)	4.5
C(12)I	4426 (5)	6959 (6)	6716 (9)	6.3	N(3′)	2252 (5)	592 (1)	4667 (2)	3.3
O(12)I	4622 (4)	7237 (4)	5411 (8)	8.7	C(4′)	819 (6)	579 (1)	3832 (2)	3.2
					N(4′α)	−1229 (5)	182 (1)	3713 (2)	3.8
C1P	1578 (1)	6522 (1)	5129 (2)	4.8	C(5′)	1459 (6)	962 (1)	3101 (2)	3.2
O(1)P	2419 (5)	6782 (7)	4731 (10)	12.7	C(6′)	3480 (7)	1364 (1)	3344 (2)	3.8
O(2)P	1175 (6)	5629 (5)	4001 (11)	13.8	C(5′α)	22 (6)	933 (1)	2135 (2)	3.7
O(3)P	1790 (4)	6482 (4)	7069 (6)	7.2	N(5′β)	468 (5)	359 (1)	1683 (2)	3.5
O(4)P	978 (4)	7226 (4)	4817 (8)	9.0	N(1)I	1183 (5)	−2569 (1)	676 (2)	4.2
C1′P	1400 (2)	631 (1)	3452 (2)	6.9	C(2)I	1100 (8)	−2112 (2)	1327 (2)	4.6
O(1′)P	1834 (6)	1413 (6)	4853 (13)	14.7	C(3)I	−954 (7)	−1724 (1)	1043 (2)	4.0
O(2′)P	484 (7)	463 (7)	3764 (18)	17.5	C(4)I	−4416 (7)	−1759 (1)	−434 (2)	4.0
O(3′)P	1340 (9)	713 (7)	1668 (12)	17.0	C(5)I	−5099 (7)	−2090 (2)	−1226 (2)	4.3
O(4′)P	1807 (9)	−188 (7)	3661 (11)	16.0	C(6)I	−3612 (7)	−2591 (2)	−1443 (3)	4.6
C(1)M	6076 (13)	9380 (12)	4874 (42)	27.7	C(7)I	−1434 (7)	−2784 (1)	−842 (3)	4.3
O(1)M	5537 (10)	8789 (9)	4165 (19)	20.5	C(8)I	−777 (6)	−2462 (1)	−36 (2)	3.5
					C(9)I	−2186 (6)	−1942 (1)	175 (2)	3.5
					C(10)I	−1614 (8)	−1155 (2)	1538 (3)	4.9
					C(11)I	−721 (7)	−605 (2)	1015 (3)	4.4
					C(12)I	−1510 (6)	−35 (1)	1468 (2)	3.7
					O(12)I	−3868 (4)	52 (1)	1653 (2)	5.4

[a] The suffixes I, P, and M in the atomic designations refer to indole moieties of PTI, TI, and PI molecules and perchloride and methanol solvent molecules, respectively.

Table III. Averaged Percentage Hypochromicity of the λ_{max} Absorption Band at Different Temperatures

compd	λ_{max}	temp, °C	H, %[a]
PTI	273	5	22.3
		25	15.8
		45	13.7
TI	268	5	14.1
		25	9.5
		45	8.8
PI	275[b]	5	1.9
		25	0.5
		45	0.0

[a] These values are internally reproducible within 0.5%. [b] Hypochromicity at different λ_{max}: 275 nm for PI and 279 nm for the summation of P and indole-3-propionic acid.

hypochromicity. This also implies that the formation of a charge-transfer complex is more favorable at lower temperature as well as in many donor–acceptor systems.[21] It is worthwhile to note that the charge-transfer band for PTI is significantly larger than that for TI molecule. Although the interaction between the indole and pyrimidine rings in PI molecule was relatively weak as already found in the related systems[22] it is obvious that the pyrimidine moiety certainly participates in the formation of charge-transfer complex between the thiamin and tryptophan molecules; in other words, the effective formation of the charge-transfer complex may require two aromatic rings involved in thiamin molecules and pyrimidine and thiazolium rings.

^{1}H NMR Spectra. NMR is a very sensitive and useful technique for investigating a mutual orientation between complexed molecules in solution, because the signals of nuclei close to the aromatic ring give rise to observable changes in their chemical shifts by ring current effects. It was obvious with UV spectroscopic measurement that the molecular associations between thiamin and tryptophan derivatives are mainly due to π–π stacking interactions between the aromatic rings. In the case of the intermolecular stacking complex, therefore, it could be expected that the signals

(22) Farzami, B.; Mariam, Y. H.; Jordan, F. *Biochemistry* **1977**, *16*, 1105.

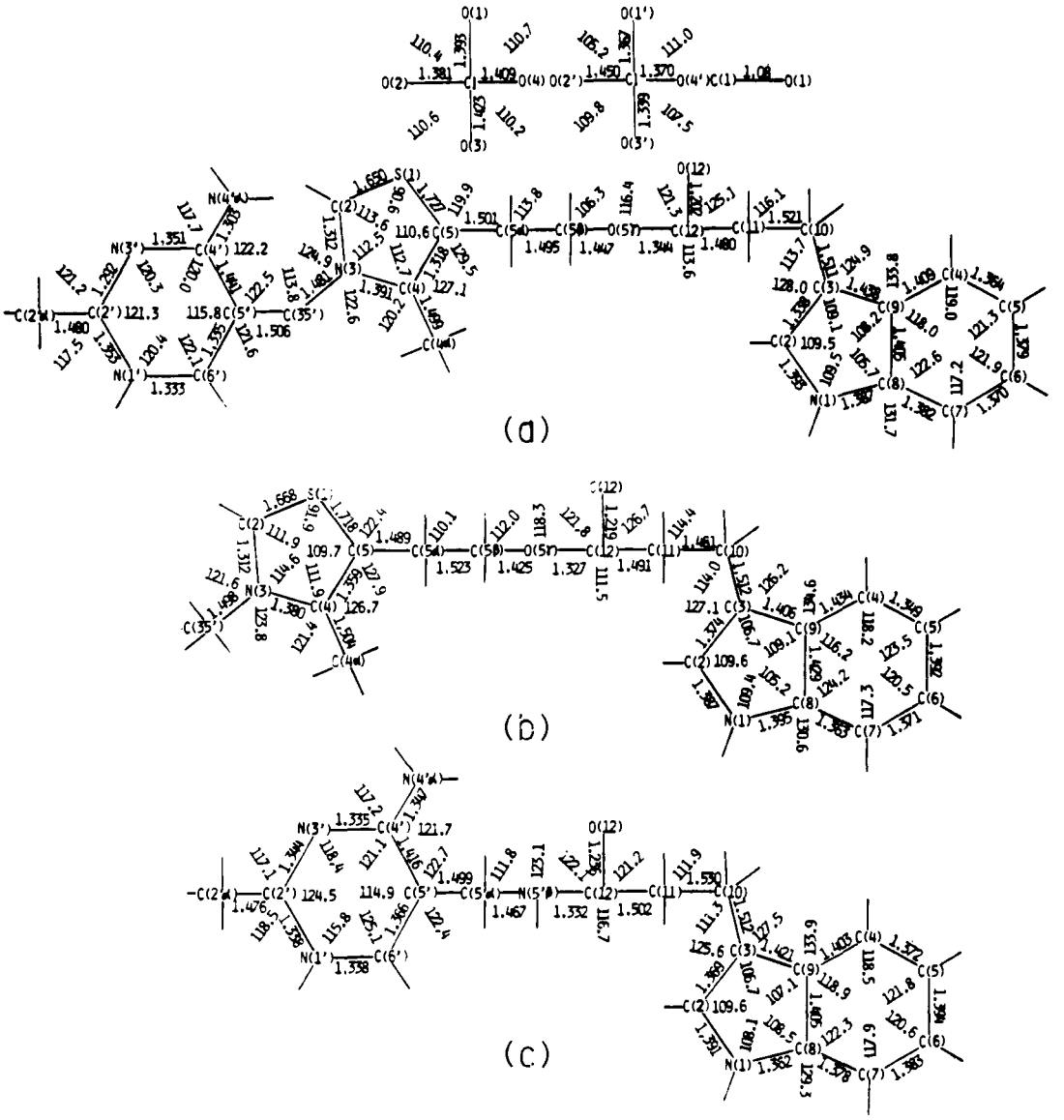

Figure 3. Bond lengths (in angstroms) and angles (in degrees) between the non-hydrogen atoms, together with the atomic numbering used in this work.

of protons attached to one ring are displaced to upfield by ring current effects of the second aromatic ring plane. On the other hand, downfield shifts of the ring protons, as observed at higher temperature, could be expected as the result of disappearance of intramolecular ring-ring interaction. Thus, we measured the ^1H NMR spectra in 50% CD_3OD-D_2O solution at three different temperatures. Although ^1H NMR studies on intermolecular complex of thiamin with indole derivatives have been already carried out,[5] we reexamined for thiamin-L-tryptophan methyl ester system as comparison with the intramolecular compounds, PTI, TI, and PI. All the proton resonances were assigned by homonuclear decouplings, spin multiplicities, and in comparison with previously published data.[5] The chemical shifts varied with the temperatures (5, 20, and 40 °C) and are given in Table IV, where the spectra for thiazolium C2 proton are absent because of its fast H-D exchange in D_2O.

As was expected, all the protons in the thiamin molecule were shifted to upfield upon mixing with L-tyrptophan methyl ester. These upfield shifts decreased in proportion to the increase of temperature. The $\Delta\delta/\Delta T$ values of pyrimidine protons in thiamin (C2′ methyl and C6′ protons) are almost the same as those of the thiazolium protons (C4 methyl and C5 α protons), implying approximately equal stacking interactions of both of the two rings of thiamin with the indole ring of tryptophan. These results, together with the significantly large $\Delta\delta/\Delta T$ value of the C35′ proton, led us to the following idea on the most probable association modes between thiamin and tryptophan molecules: (1) the indole ring stacks just upon the C35′ atom of thiamin by assuming the V conformer, as was already supposed by Biaglow et al. (see Figure 6 of ref 5b) and (2) the nearly equal two stacking states of indole-pyrimidine and indole-thiazolium rings exist at equilibrium, where the C35′ atom is involved in the stacking interaction with the indole ring in both states.

Similar stacking modes could be also supposed for PTI when considered from respective $\Delta\delta/\Delta T$ values, representing the degree of stacking interaction; the value of H35′, 4.0, is significantly larger than those of pyrimidine and thiazolium ring protons. On the other hand, the $\Delta\delta/\Delta T$ values of the thiazolium ring protons in TI are somewhat larger than those of the pyrimidine ring in PI, suggesting that the indole ring can stack with the former ring more preferentially than the latter one, as agreed with the UV results. This supposition was further substantiated by the $\Delta\delta/\Delta T$ values of the indole ring protons of the respective compounds: The values in TI (0.63 on the average) are larger than those in PI (0.35 on the average), and a most important evidence is that the indole ring protons in PTI show the largest $\Delta\delta/\Delta T$ values (1.02 on the average). This corresponds to two strong stacking interactions of the indole ring with pyrimidine and with the thiazolium ring linked by a methylene group, and it is also compatible with the UV results.

Descriptions of Molecular Dimensions of PTI, TI, and PI Compounds. Figure 3 shows the bond lengths and angles for the

Table IV. Changes of Chemical Shifts, δ (in ppm), upon Temperature Variation[a]

(a) PTI Sulfate, TI Iodide, and PI (at T = 5, 20, 40 °C)

	PTI sulfate				TI iodide				PI			
proton	5	20	40	$\Delta\delta/\Delta T$[b] ($\times 10^3$)	5	20	40	$\Delta\delta/\Delta T$ ($\times 10^3$)	5	20	40	$\Delta\delta/\Delta T$ ($\times 10^3$)
H2′(methyl)	2.55	2.58	2.61	1.7					2.38	2.40	2.43	1.4
H6′	7.91	7.93	7.95	1.1					7.69	7.72	7.76	2.3
H35′	5.30	5.34	5.44	4.0	3.87[c]	3.90	3.93	1.7	4.05[d]	4.06	4.10	1.4
H4(methyl)	2.33	2.35	2.38	1.4	2.20	2.24	2.27	2.0				
H5	3.06	3.08	3.10	1.1	3.00	3.02	3.06	1.7				
H2I	7.05	7.06	7.08	0.9	7.02	7.04	7.05	0.9	7.05	7.06	7.06	0.3
H4I	7.33	7.35	7.38	1.7	7.39	7.40	7.41	0.6	7.36	7.37	7.38	0.6
H5I	7.06	7.07	7.11	1.4	7.17	7.18	7.18	0.3	7.12	7.13	7.13	0.3
H6I	6.99	7.01	7.02	0.9	7.04	7.04	7.05	0.3	7.01	7.01	7.02	0.3
H7I	7.48	7.50	7.51	0.9	7.46	7.48	7.50	1.1	7.54	7.55	7.55	0.3
H10	2.76	2.77	2.77	0.3	2.75	2.76	2.77	0.6	2.63	2.63	2.64	0.3

(b) 1:1 Mixture of L-Tryptophan Methyl Ester HCl and Thiamin HCl (at T = 5, 20, 40 °C)

	5			20			40		
proton	1:1 mix	compnent	$\Delta\delta$	1:1 mix	compnent	$\Delta\delta$	1:1 mix	compnent	$\Delta\delta$
H2′(methyl)	2.58	2.59	0.01	2.58	2.58	0.0	2.57	2.57	0.0
H6′	8.12	8.15	0.03	8.11	8.12	0.01	8.10	8.09	0.01
H35′	5.48	5.55	0.07	5.51	5.56	0.05	5.55	5.57	0.02
H4(methyl)	2.63	2.65	0.02	2.64	2.65	0.01	2.65	2.65	0.0
H5	3.16	3.19	0.03	3.17	3.19	0.02	3.18	3.19	0.01

[a] Estimated standard error of chemical shift is ±0.005 ppm. The used solution is 50% CD_3OD–D_2O, and the concentration is 0.05 M. The pH values were measured on a Horiba pH meter, and the pD was then added to the observed one by 0.4. The respective pD values are as follows: 6.4 for PTI sulfate, 6.2 for TI iodide, 6.4 for PI, 5.8 for L-tryptophan methyl ester HCl, 5.4 for thiamin HCl, and 5.4 for their 1:1 mixture. [b] These values (in ppm/°C) were calculated by using the difference between the chemical shifts at 5 and 25 °C. [c] The value corresponds to the protons of CH_3–N. [d] The value corresponds to the protons of pyrimidine–CH_2–N.

non-hydrogen atoms of PTI, and PI molecules. The averaged standard deviations for lengths and angles are 0.008 Å and 0.4° for PTI, 0.009 Å and 0.6° for TI, and 0.005 Å and 0.3° for PI, respectively. Although some values are a little different to one another for the same chemical groups in these three compounds, the quoted values seem to be all normal compared with the other thiamin[6-8] and indole[23] compoounds.

We considered first that the pyrimidine N(1′) atom of PTI exists in a nonprotonated state because of the positional ambiguity of hydrogen atom attached to N(1′) on a difference Fourier map. But the dimensional comparison of nonprotonated thiamin derivatives (PI, thiamin nitgrate,[24] thiamin chloride,[25] and thiamin picrolonate[6]) with other many protonated ones led us to the conclusion that PTI is a protonated form. The protonation at N(1′) causes a longer C(2′)–N(1′)–C(6′) angle (120°–122°) than that of the nonprotonated one (115°–117°). Moreover the shortening of C(5′)–C(6′) and C(2′)–N(3′) bond lengths might be induced by protonation: the corresponding averaged values are 1.370 and 1.332 Å for the neutral base and 1.349 and 1.308 Å for the pronated one, respectively. The ring planes are roughly planar with small but significant deviations: least-squares planes and dihedral angles are available from the supplementary material. The C(2′α) atom deviates significantly from the pyrimidine or pyrimidinium ring plane (0.109 (9) Å for PTI and 0.116 (5) Å for PI). This tendency may be intrinsic and so frequently observed in other thiamin derivatives. The perspective drawings of molecules are given in Figure 4. The table of the selected torsion angles is deposited as the supplementary material. The dihedral angle between pyrimidine and thiazolium rings in PTI is 72.1 (2)°, and the torsion angles of ϕ_T and ϕ_P are 5.4 (8)° and −76.3 (7)°, respectively. These conformational characteristics, as already mentioned in the introduction, show that the thiamin moiety, irrespective of the attachment of the indole-3-propionyl group to the O(5γ) atom, takes a F conformation, being inherently observed in thiamins with no substituent at C(2) atom. The similar conformation around C(5)–C(5)α)–C(5β)–O(5γ) linkage is also

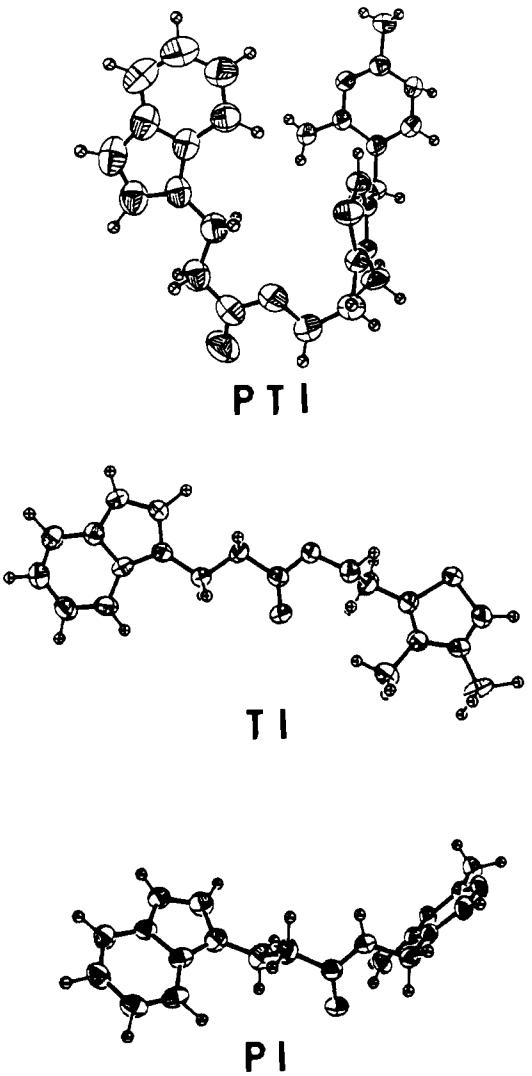

Figure 4. Perspective views of PTI, TI, and PI molecules observed in their crystal structures.

(23) Ishida, T. Dissertation, Faculty of Pharmaceutical Sciences, Osaka University, 1979, p 125.

(24) Ishida, T.; Tanaka, A.; Inoue, M. *Acta Crystallogr., Sect. C* **1984**, *C40*, 437.

(25) Pletcher, J.; Sax, M.; Sengupta, S.; Chu, J.; Yoo, C. S. *Acta Crystallogr., Sect. B* **1972**, *B28*, 2928.

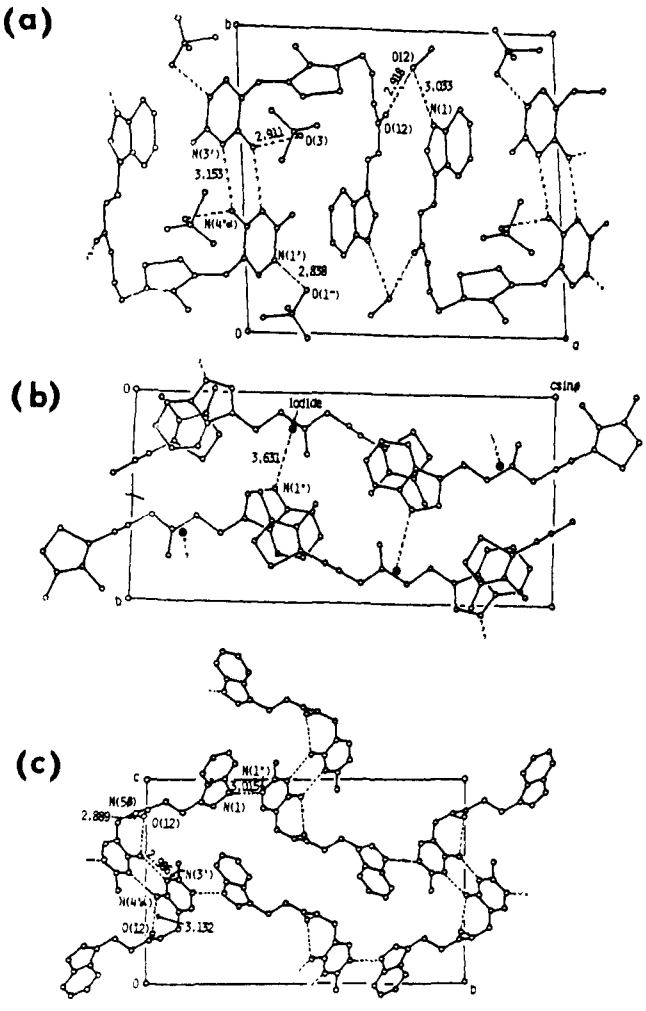

Figure 5. Crystal packings of the PTI (a), TI (b), and PI (c) molecules. Dashed lines represent possible hydrogen bonds.

frequently found in related thiamins, although relatively large variations for $\phi_{5\alpha}$ ±60° to ±90°) and (within 10° from ±60°) were observed. The molecular conformation of PTI is characterized by cis connection at the C(11)I-C(12)I bond in two trans zigzag chains of C(5α)-C(5β)-O(5γ)-C(12)I-C(11)I and C(3)I-C(10)I-C(11)I-C(12)I, and thus the planar but curved conformation by C(5α)-C(5β)-O(5γ)-C(12)I-C(11)I-C(10)I-C(3)I was formed.

Both TI and PI molecules take open conformations with the nearly extended chains connecting two aromatic rings. The torsion angle $\phi_{5\alpha}$ in TI is similar to that usually observed in thiamins, but $\phi_{5\beta}$ deviates significantly from the normal range. This would be resulted from attachment of a fully extended trans zigzag indole-3-propionyl group. On the other hand, the conformation of the PI molecule is stabilized by an intramolecular hydrogen bond formation between N(4′α) and O(12)I atoms: N(4′α)-O(12)I = 3.132 (4) Å, H(4′α)-O(12)I = 2.27 (3) Å, and ∠N(4′α)-H(4′α)-O(12)I = 153 (3)°.

Out of two torsion angles $\chi^{1,2}$ and $\chi^{2,1}$ defining the indole side chain conformation, the cis orientation of the latter angle found in PTI or TI is very unusual,[26] and it may arise from a prominent interaction of indole ring with the neighboring pyrimidinium or thiazolium ring (discussed later), while the former angles of these three molecules are in the trans region commonly observed in other trytophan derivatives.

Descriptions of Crystal Packings and Hydrogen Bonds. The possible hydrogen bonding modes are shown in Figure 5 by the dashed lines; these parameters and short contacts are summarized in table which is deposited as the supplementary material. The most remarkable feature of the PTI structure is the dimer formation by stacking interaction between the indole and pyrimidinium rings and by hydrogen bond formations via the perchlorate ion or solvent methanol (see Figure 6), in which the respective molecules are related to each other by centrosymmetric operation ($-x$, $1-y$, $2-z$): two independent perchlorate ions located near the thiamin moiety form two hydrogen bonds with the pyrimidinium N(1′) and N(4′α) atoms, and many short contacts were found (supplementary material). The oxygen atom of solvent methanol forms a bifurcated hydrogen bond with the indole N(1) and ester O(12)I atoms. The dimeric hydrogen bonds formed between the pyrimidinium N(3′) and N(4′α) atoms related by a center of symmetry at $-x$, $1-y$, $2-z$ stabilize the molecular packing along the c axis. The thiazolium sulfur atom has short contacts with the neighboring O(5γ), O(12)I, and O(1) atoms.

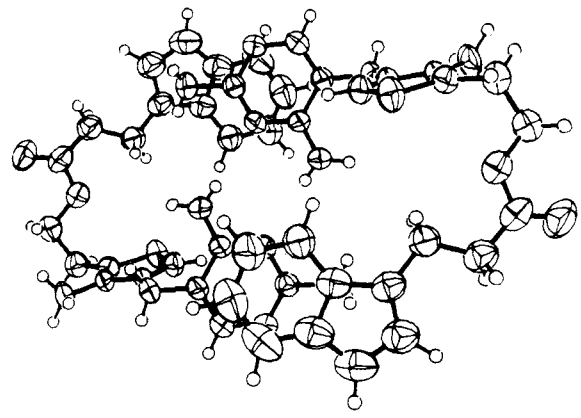

Figure 6. Perspective view of PTI dimer.

Fully extended TI molecules packed along the c axis and prominent layer structures are formed by stacking of the alternating indole and thiazolium rings which are piled up to the a axis. This hydrogen bond between the indole N(1) atom and iodine atom and the short contact between the polar atoms stabilize the molecular packing along the b axis.

On the other hand, the spirally curved and extended PI molecules are packed nearly perpendicular to the c axis. These molecules are stabilized by three hydrogen bonds and van der Waals contacts between the neighboring polar atoms; the N(4′α) atom forms a bifurcated hydrogen bond with the O(12)I atom of the same molecule and the N(3) atom of neighboring molecule related by a center of symmetry. The latter hydrogen bond is also observed in PTI as well as thiamin compounds. The N(5β) and N(1)I atoms are linked to the neighboring O(12)I and N(1) atoms, respectively, stabilizing the molecular packing along the a and b axes.

Stacking Interactions between Indole and Aromatic Rings in Thiamin. Figure 7 illustrates the stacking modes between the indole and pyrimidinium, indole and thiazolium, or indole and pyrimidine rings as observed in PTI, TI, and PI crystals; this figure also shows the vectors of respective aromatic dipole moments represented by arrows above the respective figures (I, indole; P^+, pyrimidinium; T^+, thiazolium; P, pyrimidine). Parameters concerning these stacking modes are summarized in Table V.

All the values involving the dipole moments in Table V were calculated by the CNDO/2 method using 3-methylinole, 4-amino-2,5-dimethyl-1*H*-pyrimidinium cation, 3,4,5-trimethylthiazolium, and 4-amino-2,5-dimethylpyrimidine as the most simplest stacking model compounds. These atomic coordinates were derived from the present X-ray results.

The respective stacked pairs can be built up between the original (x, y, and z) and the nearest-neighboring molecules translated by centrosymmetry (PTI: $-x$, $1-y$, $2-z$), glide (TI: x, $0.5-y$, $0.5+z$ and $1+x$, $0.5-y$, $0.5+z$)8 or diad screw (PI: $-x$, $0.5+y$, $0.5-z$) operation. The indole ring is stacked with the pyrimidinium, thiazolium, and pyrimidine rings, respectively; dihedral angles between aromatic rings are ranged from 3.4 (3) to 11.7 (1)°. The average interplanar spacings are in the range

(26) This angle is usually in the range of ±90 ° to ±10°: Bakke, O.; Mostad, A. *Acta Chem. Scand. Ser. B* **1980**, *B34*, 559.

Table V. Stacking Parameters for Thiamin–Indole Ring Interactions

compound	ring pair	interaction mode	interplanar spacing, Å	dihedral angle, deg	electrostatic energy[a]	stabilization energy[b]
PTI	indole–pyrimidinium	Figure 7a	3.338	9.2 (3)	12.24	−33.51
TI stack I	indole–thiazolium	Figure 7b	3.498	3.4 (3)	15.64	−34.20
TI stack II	indole–thiazolium	Figure 7c	3.759	3.4 (3)	15.60	−30.12
PI	indole–pyrimidine	Figure 7d	3.578	11.7 (1)	−2.89	−31.37

Electrostatic and Orbital Interactions for Short-Contact Atomic Pairs

atomic name			atomic charge					
acceptor	indole	distance, Å	acceptor	indole	electrostatic interaction[c]	atomic LUMO (acceptor)	coeff HOMO (indole)	orbital coupling[d]
				PTI (less than 3.8 Å)				
N(1′)	C(7)	3.662 (10)	−0.095	−0.043	−	0.4074	−0.3499	−
C(2′)	C(4)	3.799 (9)	0.327	0.007	−	−0.4228	−0.3811	+
C(2′)	C(8)	3.785 (9)	0.327	0.103	−	−0.4228	0.0604	−
C(2′)	C(9)	3.794 (8)	0.327	−0.015	+	−0.4228	0.2641	−
C(2′α)	C(3)	3.646 (9)	−0.115	−0.022	−	0.0017	0.2619	+
C(2′α)	C(8)	3.635 (10)	−0.115	0.103	+	0.0017	0.0604	+
C(2′α)	C(9)	3.430 (9)	−0.115	−0.015	−	0.0017	0.2641	+
N(3′)	C(4)	3.799 (9)	−0.236	0.007	+	−0.0624	−0.3811	+
N(3′)	C(5)	3.672 (10)	−0.236	−0.018	−	−0.0624	−0.0654	+
				TI Stack I (less than 3.7 Å)				
S(1)	C(4)	3.574 (7)	−0.007	−0.013	−	−0.5140	−0.3367	+
S(1)	C(9)	3.687 (7)	−0.007	−0.0098	−	−0.5140	−0.1720	+
C(2)	C(3)	3.588 (10)	0.0921	−0.0296	+	0.5126	0.4659	+
C(4)	C(8)	3.522 (9)	0.1996	0.0930	−	−0.1083	−0.2574	+
C(5)	C(4)	3.687 (9)	−0.0266	−0.0103	−	0.2989	−0.3367	−
C(5)	C(9)	3.694 (9)	−0.0266	−0.0098	−	0.2989	−0.1720	−
C(5α)	C(5)	3.511 (10)	−0.0233	−0.0074	−	0.0033	0.0287	+
C(5α)	C(6)	3.461 (11)	−0.0233	0.0	0	0.0033	0.3660	+
				TI Stack II (less than 3.8 Å)				
C(3′)	C(8)	3.787 (11)	0.0401	0.0930	−	0.0189	0.2574	+
C(3′)	C(9)	3.790 (11)	0.0401	−0.0098	−	0.0033	0.3660	+
				PI (less than 3.7 Å)				
N(1′)	C(7)	3.571 (4)	−0.241	−0.037	−	−0.2146	0.1476	−
N(1′)	C(8)	3.557 (4)	−0.241	0.101	+	−0.2146	−0.1487	+
C(2′)	C(7)	3.478 (5)	0.250	−0.037	+	0.5617	0.1476	+
C(2′α)	C(6)	3.587 (5)	−0.075	0.0	0	0.1733	0.1951	+
C(2′α)	C(7)	3.561 (5)	−0.075	0.037	−	0.1733	0.1476	+
C(6′)	N(1)	3.693 (4)	0.145	−0.114	+	−0.3265	−0.3244	+

[a] Electrostatic energy (kcal/mol) was computed by 332.0 $\sum_{i>j}\sum q_i q_j / r_{ij}$, where r_{ij} is the distance (Å) between atom i of the acceptor ring and j of the indole ring and q_i is the Coulombic charge on atom i. [b] Stabilization energy was calculated by using the following equation and the total energy (E) of the respective molecule: stabilization energy = E(stacked pair) − E(individual).

of 3.338–3.759 Å, of which those for the stacked pairs of TI and of PI are out of the van der Waals separation (3.4 Å). The stacking pair of PTI, however, having parallel stacking and short interplanar spacing (=3.338 Å) indicates that two aromatic rings was partly associated by the π–π charge transfer form the occupied orbital of the indole ring to the unoccupied one of the pyrimidinium ring in their ground states, because of the yellowish colorations of crystals, as well as in their solution states.

In order to elucidate the factors controlling the mutual orientation between indole and the pyrimidinium, thiazolium, or pyrimidine ring in PTI, TI, and PI crystals, respectively, we calculated the direction and magnitude of permanent dipole moment of the individual ring. However, this interaction is not so strong in each stacked pair: as shown in Figure 7, the directions of their dipole moments are almost at a right angle or parallel to each other, indicating little dipole–dipole interaction. An alternative factor, electrostatic interaction, is favorable for the neutral pyrimidine–indole stacked pair as shown in Table V, but not for the indole–pyrimidinium or indole–thiazolium pair because of the protonations on the latter ring.

On the other hand, stabilization energies of these four stacking pairs, resulting rather from the stacking interaction than the electrostatic ones, could be determined by the interaction between the HOMO (highest occupied molecular orbital) of the indole ring and the LUMO (lowest unoccupied molecular orbital) of the pyrimidinium, thiazolium, or pyrimidine ring: the coefficients of the atomic orbital for the paired atoms in short contact have the same signs and they are interactive to each other by the coupling of their orbitals (see Table V). Similar HOMO–LUMO interactions have been observed between the indole ring and the aromatic rings such as the adenine,[27] adeninium,[27] flavin,[28] and pyridinium[29] rings.

The present X-ray results clearly show that the indole ring can interact with both of the pyrimidine (neutral or cationic) and thiazolium rings of the thiamin molecule with nearly the same stacking forces. Furthermore, it appears to be important to note that, when the neutral or cationic pyrimidine and thiazolium rings are linked by a methylene bridge, the indole ring forms a stacked complex with the neutral ring more than with the cationic ring. Although even at present it is not clear whether this stacking tendency is due to the intrinsic character of thiamin molecule or is incidentally resulted from the crystal packing, the same stacking pattern has also been observed between the neutral pyrimidine ring of thiamin and the planar picrolonate molecule.[6] Certain answer, for this interesting problem must be awaited until further X-ray stuudies on thiamin–tryptophan systems have been done.

Conformational Analyses of TC3I, PC3I, and PHC3I. Spectroscopic and X-ray crystallogrpahic studies on tryptophan–thiamin systems showed the prominent π–π stacking interactions of the indole ring with the pyrimidinium and thiazolium rings of which the indole–pyrimidine stacking is somewhat weak. On the other hand, it was supported by the spectroscopic studies[6,22] that

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Table VI. Starting and Refined Angles and Their Conformational Energies

order	starting angle, deg				refined angle, deg				energy, kcal/mol	type
	ω_1	ω_2	ω_3	ω_4	ω_1	ω_2	ω_3	ω_4		
					TC3I					
1	-30.0	-60.0	60.0	90.0	-33.7	-74.6	66.7	83.6	-15.79	A
2	-90.0	-60.0	60.0	30.0	-83.2	-59.2	74.6	30.8	-15.65	A
3	-90.0	-60.0	60.0	-30.0	-82.9	-57.0	80.7	33.9	-14.94	A
4	30.0	60.0	-60.0	-90.0	33.0	73.2	-72.0	-84.1	-14.57	A′
5	-150.0	-60.0	60.0	-30.0	-87.2	-41.8	84.2	34.5	-14.34	A
6	90.0	-60.0	60.0	30.0	99.0	-45.0	77.6	34.0	-13.98	B
7	90.0	60.0	-60.0	-30.0	82.9	47.6	-77.8	-32.1	-13.74	A′
8	30.0	60.0	-60.0	90.0	41.8	76.4	-45.2	97.2	-13.55	B
9	-30.0	-60.0	60.0	30.0	-71.7	-62.7	74.9	43.5	-13.52	A
10	-90.0	60.0	-60.0	-30.0	-100.3	44.3	-76.3	-35.0	-13.19	B′
					PC3I					
1	-90.0	-60.0	60.0	30.0	-85.5	-52.7	70.1	35.8	-15.86	A
2	90.0	60.0	-60.0	-30.0	83.7	48.8	-72.1	-36.0	-15.81	A′
3	90.0	-60.0	60.0	30.0	96.6	-56.8	71.9	35.3	-15.72	B
4	150.0	-60.0	60.0	90.0	96.1	-59.6	71.7	37.8	-15.58	B
5	30.0	60.0	-60.0	-90.0	36.2	66.3	-66.2	-81.1	-15.19	A′
6	30.0	60.0	-60.0	90.0	42.0	73.3	-42.3	99.1	-14.73	B
7	-30.0	-60.0	60.0	90.0	-37.6	-66.4	67.5	80.6	-14.58	A
8	-90.0	60.0	-60.0	-30.0	-95.8	47.9	-71.4	-35.9	-14.04	B′
9	150.0	60.0	-60.0	-90.0	-96.0	45.0	-71.4	-36.9	-14.03	B′
10	90.0	60.0	-60.0	30.0	76.8	57.7	-78.4	-32.7	-13.76	A′
					PHC3I					
1	90.0	-60.0	60.0	30.0	99.1	-59.0	74.1	36.1	-12.61	B
2	90.0	60.0	-60.0	-30.0	84.6	52.2	-74.4	-36.7	-12.10	A′
3	30.0	60.0	-60.0	-90.0	35.2	68.6	-66.8	-83.3	-12.03	A′
4	-90.0	-60.0	60.0	30.0	-81.3	-48.2	75.5	37.8	-11.93	A
5	-90.0	60.0	-60.0	-30.0	-97.1	53.2	-75.9	-37.2	-11.68	B′
6	-150.0	60.0	-60.0	-90.0	-95.9	55.0	-74.3	-38.2	-11.56	B′
7	90.0	-60.0	60.0	-30.0	106.0	-55.1	82.4	34.1	-11.28	B
8	30.0	60.0	-60.0	90.0	39.6	74.3	-45.0	97.3	-11.05	B
9	150.0	-60.0	60.0	90.0	95.0	-60.2	74.8	45.6	-10.99	B
10	-30.0	-60.0	60.0	90.0	-33.1	-69.9	68.0	80.0	-10.88	A

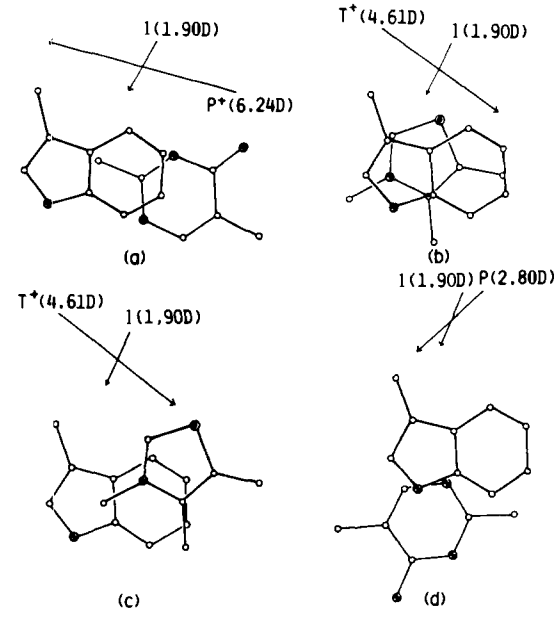

Figure 7. Stacking modes of indole-thiamin aromatic rings viewed perpendicular to the indole ring. The vectors of permanent dipole moments are represented by arrows above the respective figures. The unit of dipole moment is debye (D). The letters, a-d, correspond to the interaction modes listed in Table V.

the indole ring can stack with the neutral pyrimidine ring of thiamin as well as with the cationic one. In order to elucidate energetically stable stacking modes of the indole-pyrimidine or indole-thiazolium ring pair and also to investigate what extent the protonated pyrimidine ring affects on interaction with indole ring, we carried out the conformational analyses of the model compounds, TC3I, PC3I, and PHC3I, by using the empirical PPF method; the propylene group connecting with aromatic rings provides appropriate length for alteration of ring orientations.[30]

As an initially acceptable value for each torsion angle (Figure 2), the most reasonable one was selected on the basis of rotational barriers of sp^3–sp^3 and sp^3–sp^2 covalent bonds: ω_1 and $\omega_4 = \pm 30°$, $\pm 90°$, and 150°, ω_2 and $\omega_3 = \pm 60°$ and 180°. Thus, 324 different conformers were calculated and refined for TC3I, PC3I, and PHC3I, respectively.

Energy calculations for these three molecules indicated that the two conformers, i.e., the open and folded forms, are energetically stable. The initial conformations with $\omega_2 = \omega_3 = 180°$ as their starting angles are converged into the open forms regardless of their remaining torsion angles, and there is little difference among their energy values (-6 to -7 kcal/mol for TC3I, -7 to -8 kcal/mol for PC3I, and -5 to -6 kcal/mol for PHC3I, respectively). The open forms seem to be energetically metastable, even though there is no steric hindrance about the rotations of ω_1 and ω_4 at the state of trans zigzag orientation of ω_2 and ω_3.

On the other hand, energetically the most stable conformer is a folded one, in which indole and thiazolium, indole and pyrimidine, or indole and pyrimidinium rings are directly stacked parallel to each other. Ten stable conformers for each molecule are listed in Table VI, with their starting angles, final energies, and the stacking types. As was expected, the stable folded conformations mainly depend on the combination of ω_2 and ω_3 torsion angles: the (ω_2,ω_3) starting set of either (60°,-60°) or (-60°,60°) produces the energetically stable stacked conformers.

The folded conformers listed in Table VI could be further divided into four types, designated by letters A, A′, B, and B′. Figure 8 illustrates these four types of the most stable conformers for the respective molecules, as the projection onto the indole ring. Conformers A and B or A′ and B′ represent as having same mutual ring orientation but different stacking modes, i.e., face-to-face and back-to-face stackings. These could be related to each other

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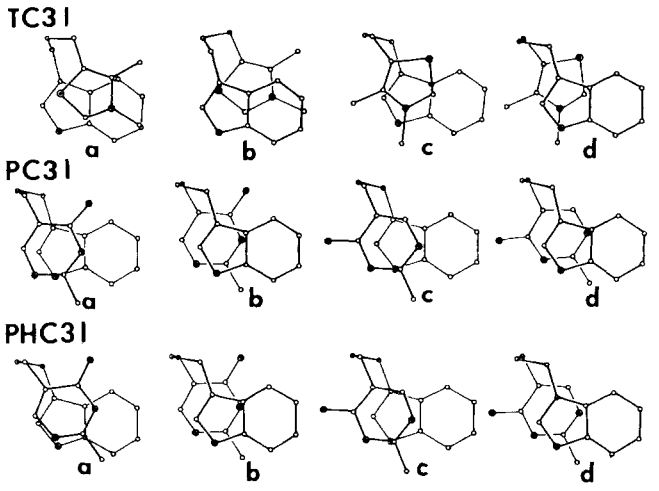

Figure 8. Calculated models of TC3I, PC3I, and PHC3I having the energetically stable conformation (a) form A, (b) form A′, (c) Form B, and (d) form B′.

by near 180° rotation about the ω_1 torsion angle. The CNDO/2 calculations showed that the B and B′ stacking forms of TC3I or PC3I were eventually stabilized by strongly coupled dipole–dipole interaction between indole and thiazolium or pyrimidine rings (nearly antiparallel alignment of each permanent dipole moment), while the A and A′ forms are significantly stabilized by the HOMO(indole)–LUMO(thiazolium or pyrimidine) interaction. Such HOMO–LUMO interaction may also exist in the case of the B and B′ stacking forms. In the case of PC3I, four types of stacking modes are mainly stabilized by the electrostatic interaction (−3 to −4 kcal/mol). On the other hand, neither electrostatic nor dipole–dipole interaction could be observed in the stacking pairs of PHC3I molecule.

The present energy calculations clearly show that the molecular energies accompanying the stacking interactions between the indole and neutral pyrimidine rings are in the range of −14 to −16 kcal/mol, which are nearly equal to those of indole–thiazolium ring stackings in TC3I. On the other hand, the protonation of pyrimidine makes the stacking interaction with the indole ring energetically less stable (−11 to −13 kcal/mol).

It is worthwhile to note that the conformer A is particularly favorable for TC3I. This implies that there is a site specificity in the stacking interaction between the indole and thiazolium rings. This is contrary to the stacking specificity between the indole and pyrimidine rings in PC3I, in which the conformers A, A′, and B are all energetically stable; that is, the indole ring can interact with both sides of the pyrimidine ring of thiamin with nearly the same stacking force.

Summary of the Results and Biological Implications. In order to elucidate the precise interaction mode between the thiamin coenzyme and the tryptophanyl residue in apoenzyme at the atomic level, we synthesized the suitable model compounds and studied the ring–ring interaction modes by spectroscopic and X-ray crystallographic methods, together with empirical energy calculations. The results clearly showed that the indole ring can stack with both pyrimidine and thiazolium rings of thiamin with nearly the same π–π stacking energies. These stacking interactions might be characterized by parallel alignment of both aromatic rings with the spacing of 3.4–3.6 Å, which are the suitable modes for the π–π charge transfer from the occupied molecular orbital of the indole ring to the unoccupied one of respective acceptor rings at their energy transition states. The ring orientation of the respective stacked pairs was subject to the HOMO–LUMO interaction, in addition to the electrostatic and/or dipole–dipole interaction.

It is important to note that the interaction forces between the indole ring and these acceptor rings are significantly strengthened, if both of the pyrimidine and thiazolium rings are linked by a methylene group such as thiamin.

The catalytic mechanism of the thiamin coenzyme has been proposed by Breslow;[31] the reaction proceeds through a formation of the thiamin C2 adduct of substrate, the so-called "active aldehyde". The thiazolium ring of the thiamin coenzyme plays a roll in the formation of this aldehyde, but the biological significance of the pyrimidine moiety is not yet clear. The X-ray results of the PTI crystal suppose that a pyrimidine moiety could play an important role at binding of the thiamin to apoenzyme by the prominent π–π stacking interaction with aromatic amino acid residue as tryptophan. This result also suggests that the most probable conformation of thiamin at the binding state with apoenzyme is probably the characteristic F form.

On the other hand, Jordan[32] showed by semiempirical energy calculations of thiamin and its pyrophosphate that the energetically most favorable conformation is apparently interconvertible at room temperature. Therefore, it seems reasonable to expect that the conformation of the thiamin coenzyme readily transfers from the F form to another one depending upon the environment of enzyme and/or substrate. Consequently, the intercalating equilibrium states of the tryptophan residue between the pyrimidine and thiazolium rings in thiamin[10] are conceivable because of having the nearly same stacking energies, and the π–π charge-transfer complex formation between the indole and thiazolium rings accelerates the formation of the ylide form of thiamin. This implies that, in addition to the binding of the thiamin coenzyme to apoenzyme, the trytophan residue plays an important role in accelerating the catalytic reaction of the coenzyme.[31]

Supplementary Material Available: Tables of observed and calculated structure factors, anisotropic thermal parameters of non-hydrogen atoms, atomic coordinates and isotropic thermal parameters of hydrogen atoms, and least-squares planes, dihedral angles, torsion angles, and hydrogen bond distances (47 pages). Ordering information is given on any current masthead page.

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