Crystal and Molecular Structure of 6-Hydroxymelatonin, a Final Metabolite of Tryptophan

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As a series in the structural studies of tryptophan metabolites by an X-ray diffraction method, the crystal and molecular structure of 6-hydroxymelatonin, one of the final tryptophan metabolites, has been determined. The space group is *Pbca* with cell dimensions $a=9.309(1)$, $b=12.061(1)$ and $c=22.301(1)$ Å. The structure was refined to $R=0.053$ for 2108 ($|Fo|>0.0$) observed reflections. In contrast to a nearly planar *trans* conformation of melatonin, a precursor of 6-hydroxymelatonin, a folded conformation of the *N*-acetylaminoethyl side chain to the indole ring was observed, which is also energetically stable as shown by quantum chemical modified neglect of diatomic overlap calculations for melatonin and 6-hydroxymelatonin molecules. The oxygen atom of 6-hydroxy group participates in two intermolecular hydrogen bond formations as an electron donor and an acceptor. Three-dimensional molecular packing is essentially stabilized by these and NH (indole) ··· O (acetyl) hydrogen bonds.

Keywords 6-hydroxymelatonin; tryptophan metabolite; crystal structure; X-ray analysis; molecular conformation; indolealkylamine; conformational energy calculation

Indole derivatives, widely distributed in living cells as tryptophan metabolites, play respective biological functions. Because of their physiological and pharmacological importance, indolealkylamines such as serotonin and melatonin have been investigated in various fields. Conformational studies on these compounds have also been done to find a possible relationship with their biological activities.

As a series elucidating the conformational characteristics of tryptophan metabolites; indolealkylamines of melatonin,[1] tryptamine,[2] 5-methoxytryptamine[3] and serotonin[4] were previously subjected to X-ray single crystal analyses. Thus, *N*-acetylserotonin and 6-hydroxymelatonin molecules still remain to be crystallographically analyzed as indolealkylamines in tryptophan metabolic pathway (see Fig. 1). Recently we succeeded in obtaining single crystals of 6-hydroxymelatonin suitable for X-ray study. The present paper deals with its crystal and molecular structure and the conformational characteristics of melatonin and 6-hydroxymelatonin are considered by quantum chemical modified neglect of diatomic overlap (MNDO) calculations.

Experimental

X-Ray Study 6-Hydroxymelatonin (Sigma Chemical Co., U.S.A.) was dissolved in 60% aqueous ethanol solution. Transparent platelet crystals were obtained by slow evaporation of the solution kept at 10 °C. Oscillation and Weissenberg photographs indicated that they belong to orthorhombic; the space group was uniquely determined as *Pbca* from systematic absent spectra. The crystal data are given in Table I, where the cell constants were refined by least-squares method using 2θ angles of 25 reflections ($40°<2\theta<60°$), and the crystal density was measured by a flotation method in C_6H_6–CCl_4 mixture. A single crystal with dimensions of approx. $0.3\times0.3\times0.1$ mm^3 was used for the X-ray diffraction data collection. The intensities were measured with a Rigaku AFC-5 diffractometer using graphite-monochromated CuK_α radiation. A total of 2167 independent reflections within $2\theta=130°$ ($\sin\theta/\lambda<0.588$ Å^{-1}) was collected in the ω–2θ scan mode with a peak range of $(1.15+0.15\tan\theta)°$ and a scan speed of 3 ° min^{-1} in 2θ; the background was counted for 3 s at both sides of each reflection. The data were corrected for Lorentz and polarization factors, but not for absorption effect.

The structure was solved by the combination of a direct method (program MULTAN87[5]) and successive Fourier syntheses. The positional parameters obtained for nonhydrogen 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, where 2108 observed reflections with $|F_o|>0.0$ were used for the refinement. All the hydrogen atoms were revealed on a difference Fourier map, and were included in further refinement with isotropic thermal parameters.

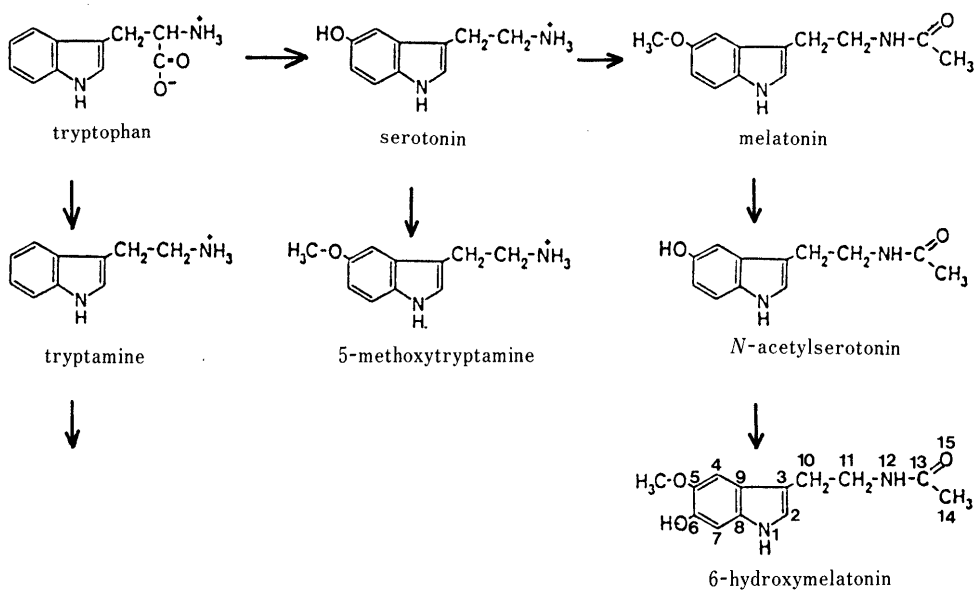

Fig. 1. Metabolic Pathway of Indolealkylamines from Tryptophan

Table I. Crystal Data of 6-Hydroxymelatonin

Chemical formula	$C_{13}H_{16}N_2O_3$
Molecular weight	248.28
Space group	*Pbca*
a (Å)	9.309 (1)
b (Å)	12.060 (1)
c (Å)	22.301 (1)
V (Å^3)	2503.7 (3)
Z	8
D (measd) (g·cm^{-3})	1.315 (2)
D (calcd) (g·cm^{-3})	1.317
μ (CuK_α) (cm^{-1})	7.40
F (0 0 0)	1056
T (K)	293

Table II. Final Atomic Coordinates of 6-Hydroxymelatonin with Their e.s.d.s in Parentheses

Atom	x	y	z	B_{eq}
N(1)	0.5722 (2)	−0.1510 (2)	0.1642 (1)	3.84 (8)
C(2)	0.4551 (2)	−0.1008 (2)	0.1378 (1)	3.58 (9)
C(3)	0.4989 (2)	−0.0186 (2)	0.09960 (9)	2.83 (8)
C(4)	0.7606 (2)	0.0482 (2)	0.07636 (9)	2.68 (8)
C(5)	0.9023 (2)	0.0289 (2)	0.09028 (9)	2.77 (8)
O(5)	1.0176 (2)	0.0847 (1)	0.06663 (8)	3.96 (7)
C(5)M	0.9858 (3)	0.1683 (2)	0.0232 (1)	4.6 (1)
C(6)	0.9399 (2)	−0.0554 (2)	0.13154 (9)	2.72 (8)
O(6)	1.0839 (1)	−0.0693 (1)	0.14359 (7)	3.42 (6)
C(7)	0.8376 (2)	−0.1197 (2)	0.1587 (1)	2.97 (8)
C(8)	0.6938 (2)	−0.0998 (2)	0.14390 (9)	2.86 (8)
C(9)	0.6529 (2)	−0.0166 (2)	0.10303 (9)	2.58 (8)
C(10)	0.4039 (2)	0.0578 (2)	0.06456 (9)	2.88 (8)
C(11)	0.3629 (2)	0.1616 (2)	0.10016 (9)	2.91 (8)
N(12)	0.2760 (2)	0.1297 (1)	0.15188 (8)	2.92 (7)
C(13)	0.2905 (2)	0.1714 (1)	0.20600 (9)	2.65 (8)
C(14)	0.2057 (3)	0.1161 (2)	0.2549 (1)	4.2 (1)
O(15)	0.3697 (2)	0.2521 (1)	0.21728 (7)	3.68 (6)

e.s.d.s: estimated standard deviations.

Refinement was carried out to minimize $\sum w(|F_O|-|F_C|)^2$, where $|F_O|$ and $|F_C|$ were observed and calculated structure amplitudes, respectively. The weight (w) used for the last refinement was as follows:

$$w=1.0/[\sigma(F_O)^2+0.07032|F_O|-0.00045|F_O|^2]$$

where $\sigma(F_O)^2$ is a standard deviation for observed intensity based on counting statistics. The final $R(=\sum(|F_O|-|F_C|)/\sum|F_O|)$, $Rw(=[\sum w(|F_O|-|F_C|)^2/\sum w|F_O|^2]^{1/2})$ and $S(=[\sum w(|F_O|-|F_C|)^2/(M-N)]^{1/2}$, where $M=2108$ (no. of reflections) and $N=228$ (no. of variables)) are 0.053, 0.066 and 0.942, respectively. During the last cycle of refinement, none of the positional parameters shifted by more than one fifth of the estimated standard deviations. The residual fluctuations on the difference Fourier map were in a range of −0.23 to 0.29 e·Å^{-3}. Final atomic coordinates and isotropic temperature factors, which were calculated using $B_{eq}=4/3\sum_i\sum_j \boldsymbol{a}_i\boldsymbol{a}_j B_{ij}$, for nonhydrogen atoms, are listed in Table II.[6] The UNICS programs[7] were used for all crystallographic computations and the atomic scattering factors were taken from published data.[8]

Quantum Chemical Energy Calculation Total energies for the various conformers of melatonin and 6-hydroxymelatonin molecules were calculated by a MNDO program.[9] The atomic coordinates used for the calculations were derived from the present X-ray results. The stabilities of the respective electronic energies were used to verify the convergence in the iteration calculations. Various conformers were built up by the rotations of the torsion angles χ(C2–C3–C10–C11) and ϕ(C3–C10–C11–N12) in 10° increments from 0° to 360°.

The numerical calculations were performed on a Micro VAX II computer at the Computation Center, Osaka University of Pharmaceutical Sciences.

Results and Discussion

Molecular Dimension and Conformation The bond lengths and angles for nonhydrogen atoms are listed in Table III. Torsion angles for the molecular conformation are listed in Table IV, where those of melatonin[10] are also given for the sake of comparison. A stereoscopic (ORTEP[11]) view of the 6-hydroxymelatonin molecule observed in the crystal structure is shown in Fig. 2.

Table III. Bond Lengths and Angles of 6-Hydroxymelatonin with Their e.s.d.s in Parentheses

Bond	Distance (Å)	Bond	Distance (Å)
Bond lengths			
N(1)–C(2)	1.379 (3)	C(6)–O(6)	1.378 (2)
N(1)–C(8)	1.367 (3)	C(6)–C(7)	1.369 (3)
C(2)–C(3)	1.369 (3)	C(7)–C(8)	1.400 (3)
C(3)–C(9)	1.436 (3)	C(8)–C(9)	1.408 (3)
C(3)–C(10)	1.497 (3)	C(10)–C(11)	1.531 (3)
C(4)–C(5)	1.375 (3)	C(11)–N(12)	1.460 (3)
C(4)–C(9)	1.403 (3)	N(12)–C(13)	1.314 (3)
C(5)–O(5)	1.372 (3)	C(13)–C(14)	1.501 (3)
C(5)–C(6)	1.415 (3)	C(13)–O(15)	1.247 (2)
O(5)–C(5)M	1.429 (3)		

Bond	Angle (°)	Bond	Angle (°)
Bond angles			
C(2)–N(1)–C(8)	108.3 (1)	C(6)–C(7)–C(8)	117.6 (1)
N(1)–C(2)–C(3)	110.41 (1)	N(1)–C(8)–C(7)	129.5 (1)
C(2)–C(3)–C(9)	106.1 (1)	N(1)–C(8)–C(9)	108.3 (1)
C(2)–C(3)–C(10)	126.5 (1)	C(7)–C(8)–C(9)	122.2 (1)
C(9)–C(3)–C(10)	127.3 (1)	C(3)–C(9)–C(4)	134.5 (1)
C(5)–C(4)–C(9)	119.7 (1)	C(3)–C(9)–C(8)	106.9 (1)
C(4)–C(5)–O(5)	125.5 (1)	C(4)–C(9)–C(8)	118.6 (1)
C(4)–C(5)–C(6)	120.4 (1)	C(3)–C(10)–C(11)	112.3 (1)
O(5)–C(5)–C(6)	114.1 (1)	C(10)–C(11)–N(12)	109.4 (1)
C(5)–O(5)–C(5)M	116.4 (1)	C(11)–N(12)–C(13)	124.6 (1)
C(5)–C(6)–O(6)	117.1 (1)	N(12)–C(13)–C(14)	116.3 (1)
C(5)–C(6)–C(7)	121.5 (1)	N(12)–C(13)–O(15)	123.0 (1)
O(6)–C(6)–C(7)	121.4 (1)	C(14)–C(13)–O(15)	120.7 (1)

Table IV. Selected Torsion Angles of 6-Hydroxymelatonin with Their e.s.d.s in Parentheses, along with Those of Melatonin

Bond sequence	6-Hydroxymelatonin	Melatonin
C(2)–C(3)–C(10)–C(11): χ	87.5 (2)°	5.5
C(9)–C(3)–C(10)–C(11)	−88.2 (2)	−174.9
C(3)–C(10)–C(11)–N(12): ϕ	−64.6 (2)	−171.4
C(10)–C(11)–N(12)–C(13)	137.7 (2)	170.9
C(11)–N(12)–C(13)–C(14)	−171.4 (2)	−176.3
C(11)–N(12)–C(13)–O(15)	8.4 (2)	4.0
C(4)–C(5)–O(5)–C(5)M	−1.1 (2)	−0.1
C(6)–C(5)–O(5)–C(5)M	178.0 (2)	179.2

Compared with the bonding parameters of melatonin, no noticeable discrepancy was observed; they are in agreement within the estimated standard deviations. The bond length of C(6)–O(6), 1.378 (2) Å, is essentially the same as that of C(5)–O(5) in the serotonin molecule.[4] The bond lengths and angles of *N*-acetyl group, including C(11)–N(12) bond, show dimensions typical for peptides. The planarities of the indole ring and peptide group were examined in terms of the equations of the best-fit, leasts-squares planes. The indole ring is almost planar with a fluctuation of −0.009 (2) Å (C(9)) to 0.015 (3) Å (C(2)), and the atoms directly attached to the ring lay approximately on this plane. It is interesting to note that irrespective of the atomic repulsion

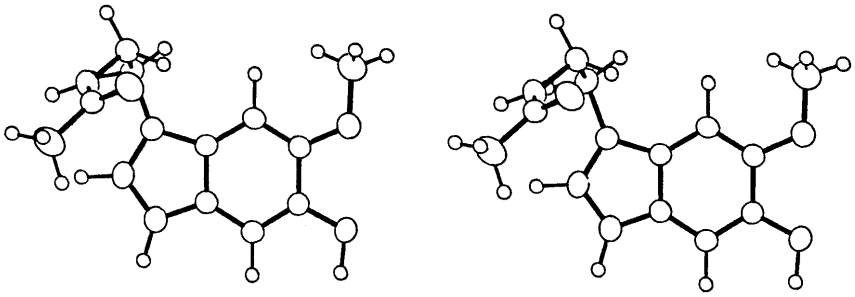

Fig. 2. Stereoscopic View of Molecular Conformation of 6-Hydromelatonin

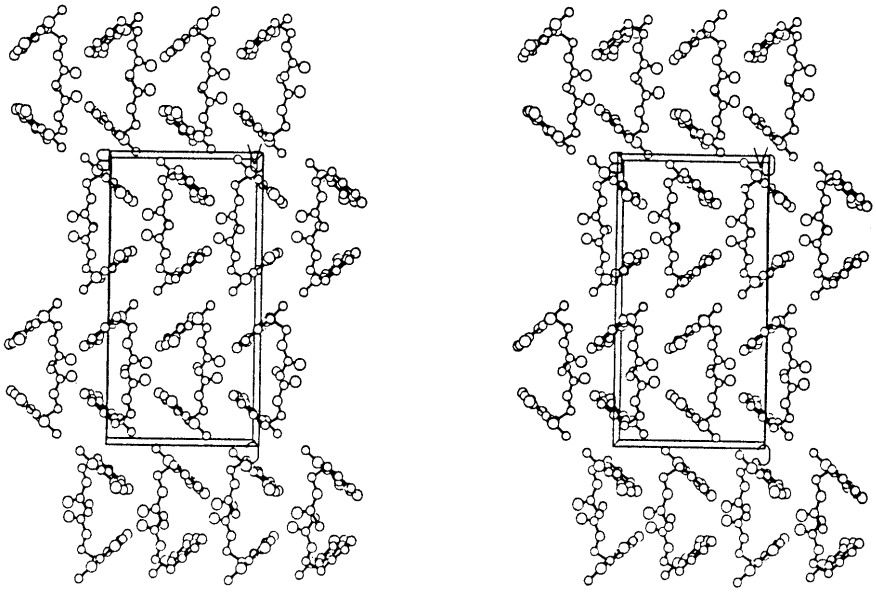

Fig. 3. Stereoscopic View of Crystal Packing Viewed along the *a*-Axis

between the C(4) and C(5)M atoms, the methoxy group essentially lays on the indole ring: the torsion angle of C(4)–C(5)–O(5)–C(5)M is -1.1 (2)°. Since this *cis* orientation of methoxy group to the indole ring has always been observed for melatonin,[1,10,12] 5-methoxytryptamine[3,13] and 5-methoxyindole-3-acetic acid,[3,14] it appears to be a conformational characteristic of methoxyindole compounds. The peptide linkage also forms a plane with *trans* conformation, and its dihedral angle with the indole ring is 108.9 (2)°.

The 6-hydroxymelatonin, as a whole, takes a folded conformation where the torsion angles χ and ϕ are in the *+syn.clinal* and *−syn.clinal*, respectively. As is obvious from Table IV, this is in contrast with the melatonin conformation; this molecule takes an extended planar conformation with $\chi = 5.5°$ and $\phi = -171.4°$.

Crystal Packing and Hydrogen Bond Stereoscopic crystal packing, viewed down the *a*-axis, is shown in Fig. 3. Two kinds of double layers consisting of the hydrophobic indole rings and the hydrophilic *N*-acetylamino ethyl side chains alternatively run parallel to the *b*-axis. The indole ring layers are stabilized by usual van der Waals contacts, and no noticeable stacking interactions were observed. On the other hand, *N*-acetylaminoethyl side chain layers are stably held by three-dimensional hydrogen bond formations. Hydrogen bond parameters and short contacts are summarized in Table V; the hydrogen bonding mode is shown in Fig. 4. The polar atoms of 6-hydroxymelatonin all participated in the hydrogen bond formations. The indole

TABLE V. Hydrogen Bonds and Short Contacts

Donor (D) at x, y, z	Acceptor at symmetry (A) operation		Distance D...A (Å)	Distance H...A (Å)	Angle D–H...A (°)
Hydrogen bonds					
N(1)	O(15)	$-x+1, y-0.5, -z+0.5$	2.939 (2)	1.86 (3)	178 (2)
O(6)	O(15)	$-x+1.5, y-0.5, z$	2.743 (2)	1.66 (3)	180 (3)
N(12)	O(6)	$x-1, y, z$	2.998 (2)	1.92 (3)	175 (3)

Atom at x, y, z	Atom at symmetry operation		Distance (Å)
Short contacts less than 3.5 Å			
C(2)	O(6)	$x-1, y, z$	3.479 (3)
C(11)	O(5)	$x-1, y, z$	3.428 (3)
N(12)	O(5)	$x-1, y, z$	3.114 (2)
C(8)	C(14)	$x+0.5, y, -z+0.5$	3.448 (3)
C(11)	O(6)	$-x+1.5, y+0.5, z$	3.423 (3)
O(15)	C(6)	$-x+1.5, y+0.5, z$	3.492 (3)
O(15)	C(7)	$-x+1.5, y+0.5, z$	3.394 (3)

N(1)–H is hydrogen-bonded to the neighboring O(15) atom. The hydroxyl O(6) atom participated in two hydrogen bond formations as an electron donor and an acceptor atom: O(6)–H···O(15) and N(12)–H···O(6). Consequently these hydrogen bonds stabilize three-dimensional molecular packing of 6-hydroxymelatonin.

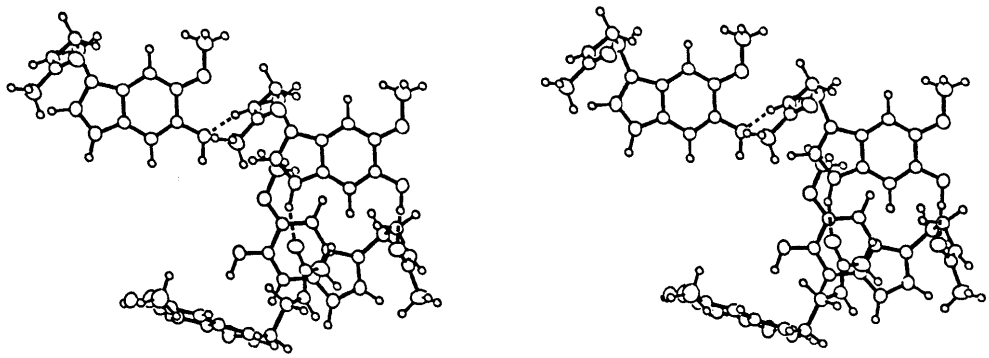

Fig. 4. Stereoscopic View of Intermolecular Hydrogen Bonding Mode
The dotted lines represent the hydrogen bonds.

TABLE VI. Torsion Angles of Indolealkylamines in Tryptophan Metabolites

Compound	ϕ	χ	Notation	Reference
N-Unsubstituted				
Tryptamine	−63.5	90.1	Conformer I	2*a*
Tryptamine thymin-1-ylacetic acid	−75.6	−4.1		2*b*
Tryptamine lumiflavin-10-acetic acid	−65.9	114.4	Conformer I	2*f*
Tyrptamine hydrochloride	−60.5	110.8	Conformer I	2*e*
Tryptamine picrate	−61.9	100.7	Conformer I	2*h*
Tryptamine phenylacetic acid	−59.7	107.2	Conformer I	2*c*
Tryptamine adenin-9-ylacetic acid	−65.8	108.4	Conformer I	2*d*
Tryptamine m7GMP	−170.3	−5.0	Conformer II	2*g*
Tryptamine m3CMP	171.0	−112.0		Unpublished
Serotonin picrate	−66.6	115.3	Conformer I	4*b*
Serotonin hydrogen oxalate	179.7	12.6	Conformer II	4*c*
Serotonin creatine sulfate	172.6	9.1	Conformer II	4*a*
5-MT	−54.7	116.3	Conformer I	13
5-MT indole-3-acetic acid	−70.2	−11.9		3
5-MT 5-methoxyindole-3-acetic acid	−64.8	105.6	Conformer I	3
N-Monosubstituted				
Melatonin	−171.4	5.5	Conformer II	10
6-Hydroxymelatonin	−64.6	87.5	Conformer I	This work

5-MT: 5-methoxytryptamine.

Conformational Analyses of Melatonin and 6-Hydroxymelatonin In this section, we consider the conformational characteristics of indolealkylamines, especially *N*-monosubstituted one. Table VI shows torsion angles of indolealkylamines hitherto subjected to X-ray crystal analyses. The majority of indolealkylamines could be grouped into two kinds of conformations, *i.e.*, conformer I ($\chi = 90$—$110°$, $\phi = -60$—$-80°$) and conformer II ($\chi = -10$—$10°$, $\phi = 170$—$190°$ ($= -170°$)). This table suggests the preference of *N*-unsubstituted indolealkylamines for conformer I rather than conformer II, which has also been verified by the conformation analysis of tryptamine[2a] and serotonin[15] by quantum chemical calculations. On the other hand, it could be guessed that conformer II becomes energetically favorable for *N*-monosubstituted indolealkylamines (melatonin and 6-hydroxymelatonin), because of the steric hindrance between the indole ring and the *N*-substituted group. Since X-ray results for these compounds are limited to only two examples, the conformational analyses of melatonin and 6-hydroxymelatonin were carried out by the quantum chemical MNDO method to investigate the energetic stabilities of conformers I and II.

The energy contour (χ, ϕ) map for melatonin is shown in Fig. 5; essentially the same contour map was also obtained for 6-hydroxymelatonin.[16] This figure is more or less similar to those calculated for tryptamine and serotonin. There are two global energy minima corresponding to

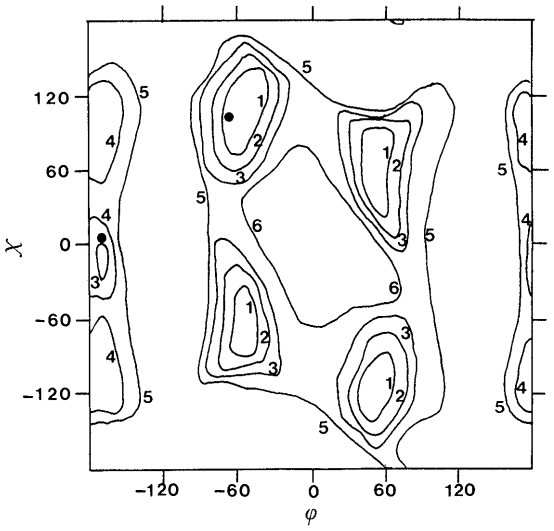

Fig. 5. A Contour Energy (χ, ϕ) Map of Melatonin Molecule
The number in the map represents the energy difference (in kcal/mol) from the most stable conformation. The dotted circles correspond to the conformations of melatonin and 6-hydroxymelatonin observed in their crystal structures.

conformer I and III ($\chi = ca.\ 90°$, $\phi = ca.\ 60°$). Contrary to our prediction, conformer II is not an energetically stable form for the molecule itself, but belongs to the secondary stable (or metastable) region. Thus it could be interpreted that the extended conformation of melatonin results from the steric requirements imposed for the molecule accompanying the crystal packing.

It is interesting to note that the majority of indolealkylamines fall in conformer I, and conformer III has not yet been observed irrespective of its energetic stability. Although it may not be a definitive reason for this fact, the following is imaginable. It could be thought that the molecule actually takes a conformation in which, in addition to the energetic stability itself, each polar atom more easily forms a hydrogen bond with a neighboring polar atom. In the case of indolealkylamines, conformer I would provide a space wide enough to form the hydrogen bond in which the amino NH group participates, while such a situation could not be formed in conformer III as a result of the steric hindrance with the bulky indole ring.

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16) The torsion angle of C(10)–C(11)–N(12)–C(13) ($=\psi$) appears to produce little effect on the (χ, ϕ) contour energy map, because the map calculated with $\psi = 180°$ was almost the same with $\psi = 138°$.