saline at pH 7. Animal experiments were carried out on groups of five adult female mice (35 g), injected first with ^{238}Pu citrate (about 1.5 μCi/kg, iv), followed 1 h later by a single 20 to 30 μmol/kg of body weight (ip) dose of test compound. Radioactivity measurements (whole body counts) were made at injection and 24 h later. One group of mice received compound 8, another compound 10, and a control group isotonic saline. The counts showed 27, 51, and 94% retention of plutonium, respectively. Continued administration of ten daily injections of compound 8 for 14 days produced no grossly observable signs of toxicity.

These initial animal experiments indicate that the 4-carboxylate tetramer 8 is even more effective in promoting plutonium excretion than the corresponding 5-sulfonate derivative[17] (35% retention), which was previously tested and reported as the most effective compound to date.[6] There is also a strong correlation of the Pu removal capability and the number of substituted DHB groups in the molecule: the monomeric catechol carboxylate is ineffective as a Pu removal agent, a dimer has not been tested, and the trimer removes 49% and the tetramer removes 73%. These single-dose results are consistent with the hypothesis that a chelate able to provide an eight-coordinate metal ion environment will be most effective as a Pu(IV) removal agent.

It is also pertinent that the 4-CO_2^- substituent not only increases the solubility of these compounds but is potentially a ligating group as well, which is not true of the 5-SO_3^- groups of the previous compounds. Finally, a 0.2 mM solution of trimeric 10 removes Fe(III) from iron-saturated human transferrin with an apparent first-order rate constant of 2.1×10^{-3} min^{-1}, which is essentially the same rate as with enterobactin.[18] This shows that these carboxylate-substituted compounds are both kinetically and thermodynamically capable of removing iron from this iron-transport protein.

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Comparison of Solution Conformational Preferences for the Hallucinogens Bufotenin and Psilocin Using 360-MHz Proton NMR Spectroscopy

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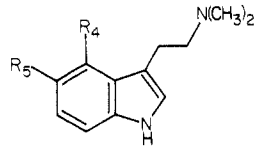
The 360-MHz ^1H NMR spectra of bufotenin and psilocin were obtained, both as the free bases in $CDCl_3$ and as protonated salts in D_2O. Coupling constants for the side-chain methylenes were derived using the LAOCN3 program. These time-averaged coupling constants indicate that the trans and gauche rotamers of both compounds have about equal energy in D_2O. There is a slight excess of the trans rotamer of bufotenin in $CDCl_3$. For psilocin, in contrast, the gauche form is highly favored in $CDCl_3$. The magnitude of this stabilization was estimated at about 1 kcal/mol using rotamer populations and free energy of transfer from published partitioning studies. It is suggested that this could result from a very weak hydrogen bond. On the other hand, the difference in partitioning between bufotenin and psilocin, which seems to be a major determinant of biological activity, is largely due to a difference in the basicity of the two compounds. The pK_a values for the amino group of psilocin and bufotenin were determined to be 8.47 and 9.67, respectively.

Bufotenin (I) and psilocin (II) are isomeric compounds



I, $R_4 = H$; $R_5 = OH$
II, $R_4 = OH$; $R_5 = H$

which are classified as hallucinogens. This classification seems unequivocal for psilocin.[1] Whether or not bufotenin is hallucinogenic is still a source of some dispute.[2] In any case, in man an oral dose of 100 mg of bufotenin was without effect, whereas 4–8 mg orally of psilocin elicits quite pronounced hallucinogenic intoxication.[3] The in vivo distribution of these two isomeric compounds also differs markedly. Although psilocin penetrates the CNS readily, Vogel[4] has reported that only small concentrations of bufotenin are detectable in the brain following intravenous administration. However, at the receptor bufotenin may actually possess quite high intrinsic activity. This has been pointed out by Vogel and Evans[5] and is supported by studies by Glennon and Gessner,[6] Lovell and Freedman,[7] Fillion et al.,[8] and others. In vitro serotonin receptor binding assays demonstrate that bufotenin possesses high affinity for the 5-HT receptor and potent serotonin-like activity.

Gessner et al.[9] suggest that the low potency for bufotenin following peripheral administration is due to low lipid solubility and consequent inability to penetrate into the central nervous system. This concept is supported by

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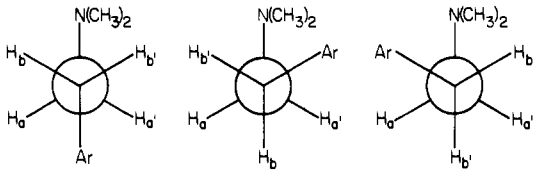
Table I. NMR Data and Calculated Conformational Preferences for Psilocin and Bufotenin in $CDCl_3$ and D_2O[a]



solvent	compd	J_{ab}	$J_{ab'}$	N	L	calcd ΣE[b]	conformer preference
$CDCl_3$	bufotenin (360 MHz)	10.8	5.7	16.5	−5.2	13.2	trans
D_2O	bufotenin (360 MHz)	7.3	7.3	14.6	0	13.3	no preference
$CDCl_3$	psilocin (360 MHz)	2.7	7.4	10.2	4.7	15.2	gauche
D_2O	psilocin (360 MHz)	8.0	6.4	14.4	−1.6	13.8	slightly trans

[a] H_a (or $H_{a'}$) and H_b (or $H_{b'}$) refer to the side chain α and β hydrogens, respectively. [b] The theoretical Huggins electronegativity for psilocin and bufotenin is 13.9.

Gessner and co-workers in a prodrug approach using 5-acetoxy-*N*,*N*-dimethyltryptamine.[9,10] This compound penetrates the CNS, is hydrolyzed to bufotenin, and elicits behavioral effects.

As noted above, psilocin is centrally active when administered systemically in relatively low doses. Several years ago Snyder and Richelson[11] suggested that psilocin could mimick the C ring of LSD by forming an intramolecular hydrogen bond between the amino and the 4-hydroxy. While this idea received little support as a mechanistic explanation, it does seem possible that such an interaction, if it occurs, might alter the lipid solubility characteristics of psilocin relative to bufotenin. Such intramolecular hydrogen bonding is not possible in bufotenin. It should be noted that X-ray crystallographic study of crystals of the free base of psilocin have not given geometries indicative of an intramolecular hydrogen bond.[12]

We therefore decided to study the solution conformational properties of bufotenin and psilocin both under protonated aqueous and nonprotonated "lipid" environments to determine whether intramolecular hydrogen bonding could be detected. The availability of a high-resolution 360-MHz spectrometer made this approach appealing (see Experimental Section).

Results

The conformer populations of four spin systems, such as bufotenin and psilocin, can be estimated using a numerical approach,[13,14] which gives the approximate percentages of trans and gauche rotamers (if the assumptions commonly used are correct), or using the N and L spectral parameter method,[15] which indicates simply whether the trans or gauche conformers predominate. The results of the N and L method generally agreed with one set of percentages obtained using the numerical approach and are reported in Table I. For bufotenin in both D_2O and $CDCl_3$ the quantitative approach indicated either no preference or a slight excess for the trans rotamer. For psilocin in D_2O there appears to be a slight preference for the trans conformer. However, for psilocin in $CDCl_3$ the gauche conformers appear highly favored with <20% of the conformational population in the trans rotameric form.

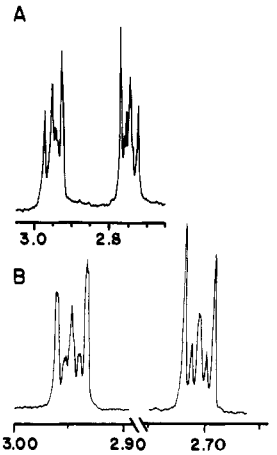


Figure 1. (A) The 360-MHz spectrum for the α (H_a and $H_{a'}$) and β (H_b and $H_{b'}$) protons of bufotenin free base in $CDCl_3$. (B) The 360-MHz spectrum for the α and β protons of psilocin free base in $CDCl_3$. The chemical shifts are measured relative to an internal Me_4Si standard.

Bufotenin. Figure 1A shows the 360-MHz spectrum for the methylenes of bufotenin (free base) in $CDCl_3$, at 2.75 and 2.95 ppm. Vicinal coupling constants derived from LAOCN3 were J_{ab} = 10.8 Hz and $J_{ab'}$ = 5.7 Hz. Bufotenin in D_2O (monooxalate, pD 2.95) showed triplets at 3.16 and 3.44 ppm. The derived coupling constants are $J_{ab} = J_{ab'}$ = 7.3 Hz.

Psilocin. Figure 1B shows the 360-MHz spectrum for the methylenes of psilocin (free base) in $CDCl_3$ at 2.71 and 2.95 ppm. The derived vicinal coupling constants were J_{ab} = 2.7 Hz and $J_{ab'}$ = 7.4 Hz. In D_2O (monooxalate, pD 2.95) the side-chain methylene protons were observed as triplets at 3.28 and 3.51 ppm. Derived coupling constants were J_{ab} = 8.0 Hz and $J_{ab'}$ = 6.4 Hz.

Calculation of Spectral Parameters.[15] The procedure is as follows: (1) let $N = J_{ab} + J_{ab'}$ and $L = J_{ab} - J_{ab'}$; (2) since J_{ab} and $J_{ab'}$ cannot be differentiated except by stereospecific labeling experiments, L can have either a positive or negative value. However, the sign of L can be tentatively established by calculating the sum of the electronegativity ($\sum E$) of the substituents on Cα and Cβ. Using the equation $1/2N + 1/6L = 17.97 - 0.80 \sum E$, the obtained $\sum E$ is compared to the calculated $\sum E$ from the Huggins electronegativity scale.[16] If L is positive, the gauche conformers predominate. If L = 0, all three conformers are of equal energy.

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Table II. Partition Coefficients, Measured pK_a Values, and Calculated Free Energy of Transfer for Psilocin and Bufotenin

compd	pK_a	P_{CHCl_3}[a]	P_{CHCl_3} (true)[b]	ΔG_{Tr}, kcal/mol	P_{oct}[c]	log P_{oct}[d]
bufotenin	9.67 (N)[e] 10.88 (OH)	0.06	11.2	−1.5	0.16	1.19
psilocin	8.47 (N) 11.33 (OH)	3.30	42.1	−2.3	0.68	1.45

[a] From ref 6 and 9. [b] From ref 6 and 9 but corrected for ionization. [c] Experimental determination, octanol/water, uncorrected for ionization. [d] Corrected for ionization. [e] Reference 22 reports $pK_{a_1} = 9.8$ and $pK_{a_2} = 11.2$.

Application of the above methods to the data for bufotenin in $CDCl_3$ gives $N = 16.48$, $L = \pm 5.16$, and the theoretical $\sum E$ (Huggins) = 13.69. If $L = +5.16$ the calculated $\sum E = 10.86$, while if $L = -5.16$ the calculated $\sum E$ 13.01. Hence, the negative value for L is preferred, indicating a preference for the trans conformer (see Table I). Bufotenin in D_2O when similarly analyzed gives $L = 0$, indicating similar energy for the conformers and no solution conformational preference. A similar calculation for psilocin in D_2O gave $L = -1.57$, indicating a slight preference for the trans conformer.

In contrast to the above three cases, the spectrum of psilocin in $CDCl_3$ was slightly asymmetric. Attempts were therefore made to match the spectrum using two coupling constants (J_{ab} and $J_{ab'}$) and four coupling constants (J_{ab}, $J_{ab'}$, $J_{a'b}$, and $J_{a'b'}$). While the best simulated spectrum was obtained with four coupling constants, the error values were the same as for the fit obtained using two coupling constants; RMS = 0.11, error = 0.04 (Hz). The coupling constants were $J_{ab} = 7.4$ and $J_{ab'} = 2.7$. As seen in Table I, these results indicate that as the free base in $CDCl_3$ the gauche conformation is highly favored for psilocin. The nature of this stabilization was of some interest; it was speculated that it might result from an intramolecular hydrogen bond. Using molecular geometries obtained from crystallographic coordinates,[12] it was found that hydrogen bonding is likely in one gauche side-chain conformer of psilocin. The amino to oxygen distance is 2.2 Å, with other bond angles and distances nearly ideal. This is a conformation where the plane defined by atoms C(3)–C(α)–C(β) is nearly perpendicular to the plane of the indole ring. However, infrared spectroscopy dilution studies failed to support the existence of such a bond. This might not be surprising if the interaction was very weak. Depending on the exact rotamer populations one uses, the NMR data for the two compounds in $CDCl_3$ indicate that the strength of this interaction is between 0.5 and 1.5 kcal/mol.

It was reasoned that the magnitude of this stabilization could also be approximated from the difference in free energy of transfer[17] between bufotenin and psilocin. Given in Table II are the reported[6,9] apparent partition coefficients for bufotenin and psilocin in the system $CHCl_3/H_2O$, pH 7.4. Listed also in this table are the pK_a values determined in this study and the true partition coefficients, corrected for ionization using these values. Free energy of transfer listed in Table II was estimated from the equation $\Delta G_{T_Y} = -RT \ln P$, where P is the true $CHCl_3/H_2O$ partition coefficient.[17] It was assumed that the phosphate salts of the amines have negligible solubility in $CHCl_3$. However, in view of the fact that many amine salts have appreciable chloroform solubility, this assumption is not necessarily valid, and we caution that the free-energy values are approximations. Nevertheless, the difference in free energy of transfer does correspond to the estimation of energy differences obtained from the NMR data.

Also reported in Table II are the octanol/water partition coefficients determined in this study. The apparent partition coefficients (P), uncorrected for ionization, indicate that psilocin partitions into lipid about 4 times better than does bufotenin. The difference in pK_a values is largely responsible for this. However, even after correction for ionization, as reflected by the octanol/water log P values, psilocin has higher lipid solubility. This again may indicate additional stabilization factors for psilocin, since psilocin and bufotenin are isomeric. Additivity principles would lead one to believe that the uncharged species should partition identically for the two compounds.

It has previously been suggested that a minimum octanol/water log P value of about 1.4 is required to obtain in vivo hallucinogenic action in humans.[18] The value for bufotenin is well below this, while psilocin is in a range where activity would be expected.

In conclusion, the difference in in vivo partitioning and the consequent effect on biological activity seems largely attributable to the difference in pK_a between bufotenin and psilocin. Psilocin is less basic by more than an order of magnitude in K_a. Thus, there is a significant increase in the concentration of the uncharged species available at physiological pH for partitioning into the CNS. No evidence was obtained for the presence of an intramolecular hydrogen bond in psilocin. However, the NMR data do indicate stabilization of the gauche conformer(s) for psilocin in $CDCl_3$. The strength of this interaction can be estimated from the rotamer populations to be on the order of 0.5–1.5 kcal/mol. This is supported by the difference in free energy of transfer for $CHCl_3/H_2O$ partitioning. This probably reflects an intramolecular interaction which can partially compensate for the loss of solvation energy when psilocin partitions from an aqueous into a "lipid" environment. Although the most likely candidate for this interaction is an intramolecular hydrogen bond, if it does occur it is certainly weak. Structural constraints preclude any similar possibility for bufotenin.

Experimental Section

Preliminary nuclear magnetic resonance (NMR) spectra were measured on either a Varian EM 360 or FT-80 spectrometer. Samples were then submitted to the Purdue University Regional Biochemical Magnetic Resonance Laboratory for analysis on a Nicolet 360-MHz superconducting Fourier transform NMR spectrometer. The following solvents were used for the NMR studies: chloroform-d_1 (99.8 atom % D) and deuterium oxide (99.7 atom % D). Tetramethylsilane was used as the internal standard in $CDCl_3$. In D_2O experiments, the chemical shifts are reported relative to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP).

Concentrations of solutions were as follows: 10–30 mg/0.5 mL for the EM-360, 5–10 mg/0.5 mL for FT-80, and 2–3 mg/0.5 mL for the Nicolet 360-MHz spectrometer. All solutions were filtered through a fritted glass filter and immediately placed in 5-mm sample tubes. Tubes were flushed with nitrogen to prevent oxidation.

The AA'BB' spectra of the side-chain methylenes of bufotenin and psilocin were analyzed using the LAOCN3 program.[19]

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pK_a Determinations. The pK_a determinations were made at 30 °C as described by Albert and Serjeant.[20] The monooxalate salts were titrated using standardized carbonate-free KOH and a Radiometer Copenhagen Autoburette ABU12 and TTA60 titration assembly. The pH was measured using a Radiometer PHM standard pH meter. During the titration the solution was blanketed with N_2. The pK values for the amino and phenol were resolved using the Fortran program described in ref 20. Values reported for psilocin are the mean of four determinations and those for bufotenin the mean of duplicate determinations.

Octanol/Water Partition Coefficients. Partition coefficients were determined by standard methods[21] using 0.1 M phosphate buffer and 1-octanol, pH 7.40. Concentrations in the aqueous phases were determined using ultraviolet spectroscopy, and solutions were kept blanketed with N_2 to prevent oxidation. Apparent partition coefficients (P), uncorrected for ionization, are reported in Table II. Log P values reported in the table are for the un-ionized species.

Conversion of Bufotenin Monooxalate to the Free Base. Bufotenin monooxalate monohydrate (Sigma Chemical Co), 50 mg, was dissolved in distilled water (25 mL). Sodium bicarbonate was added in excess, and the free base was extracted with several 10-mL portions of $CHCl_3$. The combined organic extract was washed with saturated NaCl solution and dried (Na_2SO_4). Filtration and evaporation of the solvent gave the free base as a thick oil.

Psilocin was obtained as the free base from the National Institute on Drug Abuse. For solutions in D_2O, 1 equiv of reagent grade oxalic acid was added to the psilocin free base.

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Studies on Position 1 of Angiotensin II: Effects on Affinity and Duration of Action from Alkyl Amide Substitution[1]

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The synthesis and the biological activities of [asparagine]angiotensin II analogues with alkyl-substituted amide groups are reported. This study was performed in order to elucidate further the importance and the influence of the side chain in position 1 of angiotensin II. The two synthesized analogues [1-(N^4,N^4-dipropyl)asparagine]- and [1-(N^4,N^4-diisopropyl)asparagine]angiotensin II were compared to [1-asparagine]angiotensin II (hypertensin, Ciba) and to [1-(N^4,N^4-dimethyl)asparagine]angiotensin II in vitro and in vivo. All compounds had full intrinsic activity, but their potency decreased with increasing alkyl size of the substituted carboxamide group. Despite their reduced potency, the alkylated analogues showed enhanced duration of action on rabbit aorta strips. The relative potencies of the series hypertensin, dimethyl, dipropyl, and diisopropyl analogues on rabbit aorta strips were 100, 46, 16, and 9%, respectively. In the rat blood pressure assay they were 100, 30, 9, and 7%, respectively.

The side chain of the aspartic acid residue in position 1 of angiotension II can be replaced by a free carboxamide group or other amino acids, such as sarcosine, either without a significant loss or an increase of activity.[2] However, its modification to a dimethyl amide, as in [1-(N^4,N^4-dimethyl)asparagine]angiotensin II, reduces the relative potency of the analogue, without altering the intrinsic activity.[3] In order to study further the influence of the carboxamide side chain on receptor binding and stimulation, we have synthesized analogues which have bulky alkylated amide groups. The analogue [1-(N^4,N^4-diisopropyl)asparagine]angiotensin II was synthesized according to the general solid-phase procedure,[4] using chloromethylated polystyrene resin, esterified with Boc-Phe by the *Loffet* esterification.[5] The synthesis of N^2-(*tert*-butyloxycarbonyl)-N^4,N_4-dipropylasparagine and N^2-(*tert*-butoxycarbonyl)-N^4,N^4-diisopropylasparagine was effected by coupling N^2-(benzyloxycarbonyl)aspartic acid α-benzyl ester with dipropyl- or diisopropylamine, a method we have described earlier.[6] The benzyl ester was cleaved with catalytic hydrogenation, and the free amino group was acylated with S-(butyloxycarbonyl)-4,6-dimethyl-2-mercaptopyrimidine.[7] The octapeptide analogues were liberated from the resin and from the side-chain protecting groups in liquid HF.[8] The resulting analogues were purified by gel filtration and partition chromatography, and their purity was assessed by TLC (thin-layer chromatography).

The compounds were tested on rabbit aorta strips (in vitro) and in the rat blood-pressure test (in vivo), and their biological activities were compared to those of hypertensin and to the aforementioned dimethyl analogue. The results of both biological assays were very similar and are present in Table I.

(1) All optically active amino acids are to the L configuration. Abbreviations used follow the recommendations of the IUPAC–IUB as found in *Biochemistry*, 14, 449 (1975) and *Biochem. J.*, **126**, 773 (1972).
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