Conformational Analysis of the Ergot Alkaloids Ergotamine and Ergotaminine

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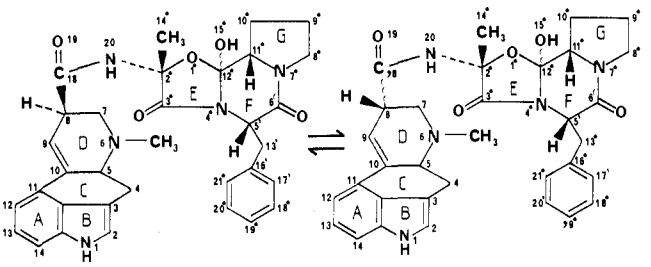
Conformational analyses by ^1H NMR and potential-energy calculations are reported for the ergot alkaloids ergotamine and ergotaminine, both as free bases and as the protonated species. In the neutral forms in $CDCl_3$, two strong intramolecular hydrogen bonds fix the molecules in folded conformations, but the protonated species adopt a more extended conformation, with a single intramolecular hydrogen bond. Of the 24 alternative conformations available to ergotamine, the most likely biologically active species in environments with low dielectric constants, e.g., the presumed ergotamine binding site, is the folded, hydrogen-bonded conformation observed for the neutral molecule in $CDCl_3$ solution.

Ergotamine (1) is an ergot alkaloid which can be an



1 2

effective drug for the treatment of migraine if it is introduced into the blood early in a migraine attack.[1] Problems can arise in ergotamine therapy of migraine because (1) the drug has a relatively slow rate of absorption from the gastrointestinal tract and (2) the drug has a tendency to epimerize to the pharmacologically inactive alkaloid ergotaminine (2).

Several studies have been conducted on the mechanism of absorption of 1 and the manner in which this is enhanced by caffeine.[2-5] The present paper is a study of the alternative conformations accessible to 1 and 2. This information was sought as a basis for the design of prodrugs of 1 that would be more stable with respect to epimerization or of analogues that would be likely to possess similar biological activity. Molecular conformations were investigated using ^1H NMR and molecular orbital and classical potential-energy calculations.

Experimental Section

^1H NMR spectra were recorded on a Bruker HX270 spectrometer at the National NMR Centre, Canberra. They were measured at 25 °C using dilute solutions (0.02 M) in $CDCl_3$, with tetramethylsilane as internal standard. Trifluoroacetic acid was added to the solutions for measurements on the protonated forms.

Calculations were done on the neutral and protonated forms of 1 and 2. At physiological pH, assuming pK_a values of 6.25 (1) and 6.72 (2),[3] the neutral froms would predominate. The molecular geometries were estimated using crystal data for LSD[6,7] and *aci-p*-iodobenzoylaminocyclol,[8] since crystal structures for 1 and 2 were not available.

The calculations were performed with a Cyber 72 computer using the program COMOL.[9] The program performs classical conformational calculations by pairwise summation of Van der Waal's interactions between nonbonded atoms, together with electrostatic and torsional potentials. The parameterization, which was developed by Giglio on the basis of a series of hydrocarbon and amide structures,[10] has been used to study a number of systems of biological interest.[11-13]

The atomic charges, used to calculate the electrostatic potentials, were obtained from a MINDO/3 molecular orbital calculation[14] on the above geometry. The calculations were carried out at fixed values of all bond lengths and bond angles. Preliminary calculations indicated that relaxation of this condition did not affect the qualitative nature of the potential-energy surfaces.

Four torsional angles are required to describe the conformations of ergotamine or ergotaminine, and these are defined in Figure 1. Initially, these variables were considered two at at time and approximate potential-energy surfaces were computed for each compound using torsional intervals of 12°. The calculations for each pair were then repeated for alternative values of the other two torsion angles. With both pairs of torsional variables near their minimum-energy values, very little interaction was found between the amide pair and the benzyl pair.

A further conformational variable is provided by the D ring, which may assume either a flap up (I) or flap down (II) conformation. In addition, the methyl group on N_6 may adopt positions α or β with respect to the proton on C_5 (defined as β). Calculations were performed for each combination of these alternative D-ring conformations, i.e., flap up or flap down, methyl α or methyl β. Conformational energy maps were prepared using a modification of the contouring program KONTOR.[15]

Ergotamine (1) was prepared from ergotamine tartrate (Boehringer Ingelheim) and recrystallized from acetone–water (90:10). Compound 2 was prepared from 1 by refluxing with MeOH and glacial HOAc (0.20%) under a N_2 atmosphere for 1 h.

Results and Discussion

^1H NMR. The chemical shifts and coupling constants for the free bases 1 and 2 in deuteriochloroform are presented in Tables I and II, along with the splitting pattern for each hydrogen. The assignments were made by consideration of chemical shifts and coupling constants, supported by spin-decoupling experiments, with reference to similar analyses of the spectra of lysergic acid di-

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Table I. ^{1}H Chemical Shifts and Coupling Constants in Ergotamine (1)

H resonances	chem shift, δ	J, Hz											
		H2	4α	4β	5β	7α	7β	8α	9	5′	13′α	13′β	11′
1	8.137	<1.5											
2	6.907		1.8	<0.5									
4α	2.790	1.8		14.2	11.9								
4β	3.322	<0.5	14.2		5.0								
5β	3.733		11.9	5.0				1.5	2.0				
6-CH_3	2.606												
7α	2.958						11.9	3.9					
7β	2.777					11.9		3.4					
8α	3.176				1.5	3.9	3.4		5.5				
9	6.342				2.0			5.5					
20-NH	9.039												
12-14	7-7.5												
2′CH_3	1.506												
5′	4.687										6.6	5.5	
8′ & 11′	~3.6												
9′ & 10′	~2.1												
13′α	3.455									6.6		14.0	
13′β	3.263									5.5	14.0		
15′OH	6.970												1.7

Table II. ^{1}H Chemical Shifts and Coupling Constants in Ergotaminine (2)

H resonances	chem shift, δ	J, Hz											
		H1	2	4α	4β	5β	7α	7β	8β	9	5′	13α	13β	11′
1	8.000	*	1.5											
2	6.906	1.5	*	1.5	1.6									
4α	3.590		1.5	*	15.3	4.9								
4β	2.616		1.6	15.3	*	10.4								
5β	3.234			4.9	10.4	*			1.3	2.0				
6-CH_3	2.611													
7α	3.131						*	11.7	1.7	1.3				
7β	2.758						11.7	*	3.6					
8β	3.074					1.3	1.7	3.6	*	6.3				
9	6.524					2.0	1.3		6.3	*				
20-NH	9.833													
12-14														
2′CH_3	1.493													
5′	4.609										*	6.6	5.4	
8′ & 11′														
9′ & 10′														
13′α	3.398										6.6	*	13.9	
13′β	3.218										5.4	13.9	*	
15′OH	6.941													2.0

methylamide and its C_8 epimer by Bailey and Grey.[16]

The NMR spectrum of 1 gives a specially clear picture of the stereochemistry of the unsaturated D ring. Thus, the signal for H_5, clearly that of an axial hydrogen, shows the expected vicinal coupling to $H_{4\alpha}$ (11.9 Hz) and $H_{4\beta}$ (5.0 Hz), as well as smaller allylic and homoallylic couplings to H_9 and H_8, respectively.[16] A decision between the two half-chair conformations I and II can be made by studying the couplings between H_8 and the pair of hydrogens at C_7.

The coupling constants for I and II may be predicted by using Karplus' rule and taking reasonable dihedral angles about the C_7–C_8 bond.[17] Thus, for the flap-up conformation (I), the expected dihedral angles and associated coupling constants are $H_{7\alpha}$–C_7–C_8–$H_8 \simeq 60°$, $J_{7\alpha,8} \simeq 3$ Hz, and $H_{7\beta}$–C_7–C_8–$H_8 \simeq 180°$, $J_{7\beta,8} \simeq 10$ Hz, while for the flap-down conformation (II), the corresponding numbers are $H_{7\alpha}$–C_7–C_8–$H_8 \simeq -60°$, $J_{7\alpha,8} \simeq 3$ Hz, and $H_{7\beta}$–C_7–C_8–$H_8 \simeq 60°$, $J_{7\beta,8} \simeq 3$ Hz. The two C_7 hydrogen signals can be identified as a pair with 11.9 Hz mutual coupling, but we cannot tell which is which. However, the

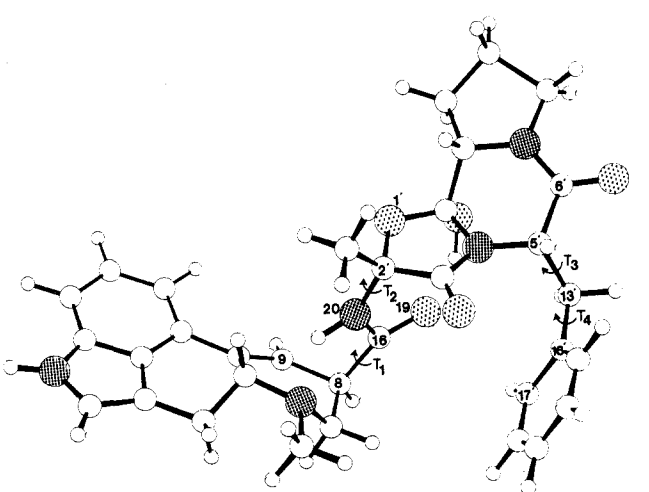

Figure 1. Conformational variables for ergotamine (1); the torsion angles are defined by clockwise rotations around the appropriate bonds. The torsion angles as illustrated here are: τ_1 (O_{19}–C_{18}–C_8–C_9) = −90°; τ_2 (O_1–C_2–N_{20}–C_{18}) = 45°; τ_3 (C_{16}–C_{13}–C_5–$C_{6'}$) = 160°; τ_4 (C_{17}–C_{16}–C_{13}–$C_{5'}$) = 120°. The corresponding torsion angles in ergotaminine (2) are defined by the same sets of atoms. Light and dark shadings represent oxygen and nitrogen atoms, respectively.

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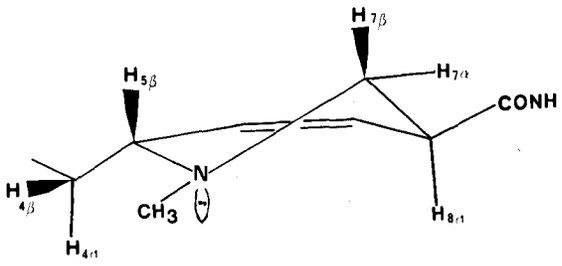
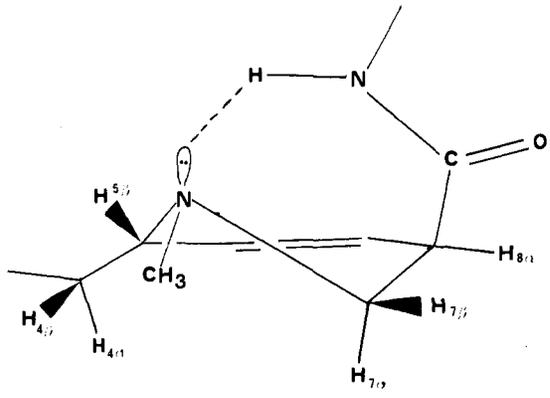


I

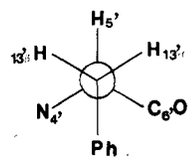


II

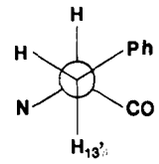
couplings to H_8 are 3.9 and 3.4 Hz, thus clearly identifying the structure of 1 in the free base form in $CDCl_3$ solution as the flap-down conformation, II.

In this conformation, the C_8 carboxamide substituent is pseudoaxial, which seems a priori to be of higher energy than the pseudoequatorial disposition of I. The energy balance is affected, however, by hydrogen bonding between the carboxamide NH and the nitrogen in ring D (as shown in II). It seems reasonable to allot an equatorial position to the *N*-Me, leaving an axial lone pair suitably placed for hydrogen bonding. Such a hydrogen bond was first proposed by Stoll[18] in 1954 as a result of pK_a studies with secondary and tertiary amides of lysergic acid. It is also found in the crystal structure of bromocriptine,[19] an alkaloid closely related to ergotamine. We would expect the formation of this hydrogen bond to affect the chemical shift of the carboxamide hydrogen (H_{20}), and at 9.8 ppm this does seem to be strongly deshielded. A reasonable model is dihydroergotamine, in which the trans C/D ring junction locks ring D in a chair configuration with the carboxamide group equatorial and quite unable to form an intramolecular hydrogen bond, and the amide NH resonates at 6.4 ppm.[20,21] In 2-azadihydroergotamine, the amide chemical shift is 6.3 ppm, and the configuration again prevents its intramolecular hydrogen bonding.[20,21]

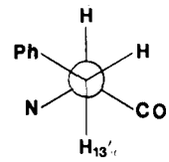
Turning to the peptide moiety (formally a pyruvylphenylalanylprolyldiketopiperazine), the NMR spectrum gives us information about the conformation of the benzyl group around $C_{5'}$–$C_{13'}$ and about the ortho amide OH ($H_{15'}$). If we adopt the usual formalism that only staggered rotamers (III) are observed for phenylalanine side chains we can calculate[22] their populations from the two vicinal



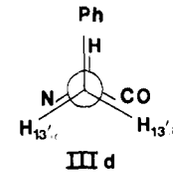
IIIa



IIIb



IIIc



IIId

couplings ($J_{5',13'}$), 5.5 and 6.6 Hz. These are for IIA–c in 1, 0.38:0.26:0.36. However, the crystal structures of 1, 2, and related alkaloids all adopt conformations IIIb or IIIc,[19] and calculations (see below) suggest that IIIc and the eclipsed intermediate region (IIId) are the more probable conformations in this series. The NMR data do not distinguish between these possibilities, since the predicted coupling constants for IIIa and IIId would be identical.

The ortho amide OH, $H_{15'}$, gives rise to a doublet with J = 1.7 Hz in the NMR spectrum, and its chemical shift (6.97 ppm) suggests that it is hydrogen bonded. The obvious hydrogen-bonding partner is the amide carbonyl at C_{18}, and with this restraint, exchange of the hydroxyl hydrogen might well be slow enough that coupling is observed. The coupling partner, along a W pathway,[17] is the $C_{11'}$ hydrogen. The connection between these two has been established by mutual decoupling experiments. When $OH_{15'}$ is irradiated, the signal for $H_{11'}$ appears at 3.56 ppm (in the difference spectrum) as a doublet of doublets, J = 7 and 9 Hz, which seem to be appropriate values for the couplings between $H_{11'}$ and two $C_{10'}$ hydrogens.[23] The geometrical requirements for this four-bond coupling require the OH to be oriented toward $C_{2'}$ and are consistent with the formation of a hydrogen bond to the carbonyl of the secondary amide group. The slow exchange of this hydroxyl hydrogen, as evidenced by the clear retention of a 1.7-Hz coupling, together with its relatively deshielded position in the NMR spectrum, allows us to propose the existence of such a hydrogen bond in $CDCl_3$ solution, as invariably observed in the crystal structures of 1, 2, and related alkaloids.[19,24]

The assignments for 2 were made in similar fashion. Again H_5 is axial and, compared to ergotamine, is shielded by 0.5 ppm; this suggests that in ergotaminine the nitrogen lone pair is trans diaxial with respect to H_5.[25] The bulky equatorial *N*-methyl thus becomes a β-substituent, and this means that the ring has the alternative half-chair conformation IV (cf. I). Support for this proposal comes from the H_7–H_8 vicinal couplings 1.7 and 3.6 Hz, which are consistent[17] with the expected dihedral angles for 7α (ca. −60°) and 7β (ca. 60°) in conformation IV.

Again we see the carboxamide group in an axial position, with its NH hydrogen bonded to the ring nitrogen, as evidenced by the extreme chemical shift (9.83 ppm) for the amide hydrogen. Judged from the chemical shifts, this hydrogen bond appears to be stronger in 2 than in 1. A

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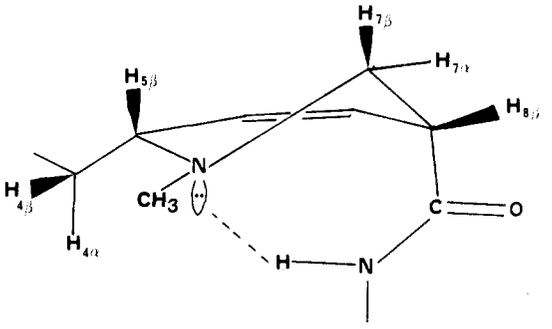

IV

final consequence of this stereochemistry is the four-bond W coupling of 1.3 Hz, between H_9 and $H_{7\alpha}$.

The peptide moiety of ergotaminine is almost exactly the same as that in ergotamine: the vicinal couplings to the benzyl CH_2 are almost unchanged, and the hydroxy is hydrogen bonded to the amide carbon. The long-range coupling to this OH, 2.0 Hz, is again believed to originate with $H_{11'}$.

The picture that emerges from this and other[19,26] studies is that the D ring of the lysergic acid derivatives is always in a half-chair conformation, and that an "inert" substituent at C_8 prefers the pseudoequatorial position. When the C_8 substituent can form a hydrogen bond with the ring nitrogen, however, it does so from the alternative half-chair conformation in which the small energetic cost of the pseudoaxial substituent is more than offset by the hydrogen bond.

Further evidence for this view is provided by our NMR studies on the cationic species, in which the formation of the N_{20}–H···N_6 hydrogen bond is precluded by protonation at N_6. Analyses of the spectra for protonated 1 and 2 were complicated by line broadening, but particularly significant differences were observed for the coupling constants involving H_8 in protonated 1. The observed couplings are $J_{7\alpha,8}$ = 4.0 Hz, $J_{7\beta,8}$ = 11.4 Hz, and $J_{8,9}$ < 1 Hz and are clearly consistent with the flap-up conformation I, with the substituent group in the pseudoequatorial position. This is also the conformation observed for the protonated forms of 1[19] and the related alkaloid bromocriptine[24] by X-ray crystallography. In 2 however, no collapse of the multiplet associated with H_9 is observed on protonation, indicating that 2 may remain in the flap-up conformation in both neutral and cationic forms. This could not be confirmed by reference to the H_7 couplings because of excessive line broadening in the spectrum but is in agreement with the results of potential-energy calculations (see below). No crystal structures for protonated 2 derivatives are available.

Potential Energy Calculations. The conformational variables describing the molecular conformations of 1 and 2 may be divided into three groups: (1) the amide torsion angles τ_1 and τ_2; (2) the benzyl torsion angles τ_3 and τ_4; and (3) the conformation of the D ring, which may be either flap up or flap down, with the *N*-methyl group either α or β relative to H_5 (defined as β) and N_6 either neutral or positively charged. From the calculated relative energies it appears that each group of conformational variables is relatively unaffected by changes to the other two. The three groups will therefore be considered independently. In each case, nonbonded interactions play a major role, with torsional and electrostatic terms contributing very little to the overall potential-energy maps.

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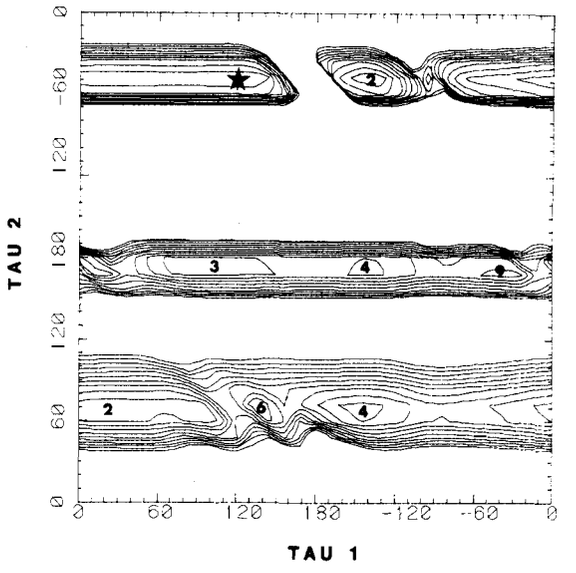

Figure 2. Contour map showing the relative energies of conformations defined by rotations τ_1 and τ_2 in neutral 1. The contour interval is 5 kcal/mol and the first 10 contour lines are shown. τ_3 is set at 120°, and the D ring conformation is flap down, methyl α.

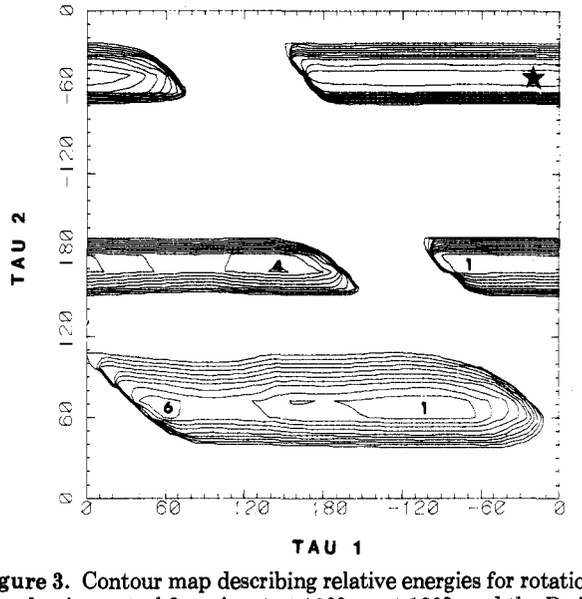

Figure 3. Contour map describing relative energies for rotations τ_1 and τ_2 in neutral 2. τ_3 is set at 160°, τ_4 at 120°, and the D ring conformation is flap up, methyl β. The first ten 5 kcal/mol contour intervals are shown.

Amide Torsion Angles. The potential-energy surfaces produced by stepwise rotation of the torsion angles τ_1 and τ_2 in 1 and 2 are shown in Figures 2 and 3, respectively. In both cases, there is a broad low-energy region corresponding to extended conformations of the LSD–amide bond (τ_1 = 0 to 120° in 1, 0 to −120° in 2) and a secondary minimum in which the amide group is folded back over the ring (τ_1 = −120° in 1, 120° in 2) to allow formation of the N_{20}–H···N_6 hydrogen bond observed in solution.

For τ_2 there are three low-energy regions, corresponding to torsion angles of 60°, 180°, and −60°. Two of these correspond to extended conformations, while the other (τ_2 = 60°) brings the amide oxygen back over the cyclol ring system, thus enabling the formation of the O_{15}–H···O_{19} bond observed both X-ray crystallographically[19,24] and in $CDCl_3$ solution.

There are thus six alternative conformations for the amide linkage, of which one is consistent with our analysis of the NMR data for both 1 and 2. In 1 this corresponds

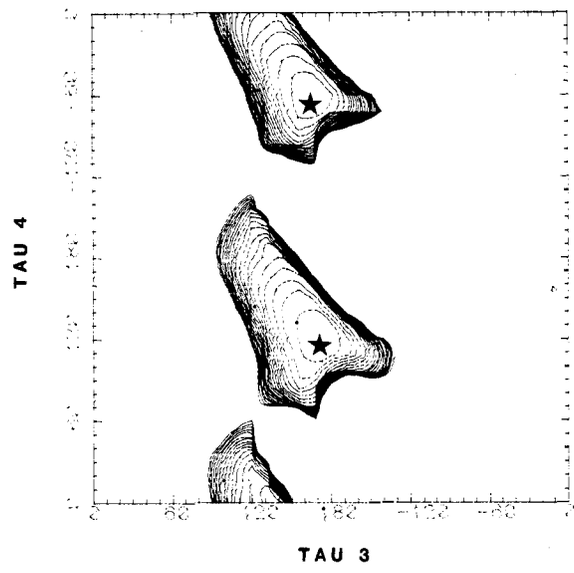

Figure 4. Contour map obtained for rotations τ_3 and τ_4 in neutral 1. The D ring conformation is flap down, methyl α, and τ_1 and τ_2 are set to −90° and 45°, respectively. The first twenty 5 kcal/mol contour lines are shown.

to the conformation $\tau_1 = -120°$, $\tau_2 = 60°$, while in **2** it is defined by $\tau_1 = 120°$, $\tau_2 = 60°$. In both cases, however, the conformation in $CDCl_3$ is relatively high in energy. This is probably because our empirical potential-energy calculations do not account for stabilization due to hydrogen-bond formation; indeed, they may introduce a repulsive term into the potential function in situations where hydrogen bonding can occur. The hydrogen-bonded conformations of 1 and **2** will therefore be more stable than the calculated potential-energy surfaces imply. Nevertheless, it is clear that all six alternative conformations of the amide linkage must be taken into account in assessing the likely biologically active conformation.

Benzyl Torsion Angles. The potential-energy map obtained for rotations of the benzyl group in 1 is given in Figure 4; the corresponding map for **2** is almost identical. There is a single minimum-energy region for τ_3, centered on 168°. This corresponds to a point intermediate between conformers IIIc and IIId discussed above and may be compared to the observed value of 165° in the crystal structure of bromocriptine. Published sketches of crystal structures for other ergopeptines indicate that the benzyl group generally adopts this minimum-energy conformation,[19] although a conformer nearer IIIb ($\tau_3 \simeq 60°$) is observed in **2**. The NMR data, discussed above, are also consistent with a slightly broader energy minimum, lying between $\tau_3 = 60°$ and $\tau_3 = 180°$. Due to the symmetry of the phenyl ring, there are two equivalent conformations for τ_4 (120°, −60°).

D-Ring Conformation. The alternative D-ring conformations for the neutral forms of 1 and **2** are illustrated in Figure 5. The relative energies of the D-ring conformations of the protonated species are almost identical. In each case, values of τ_1–τ_4 are optimized for the ring conformation concerned. It is clear that both flap-up and flap-down conformations are accessible to both 1 and **2** in either neutral or cationic forms. The *N*-methyl group is confined to the β face in all but the flap-down conformation of 1, where the *N*-methyl group is fixed in the α configuration.

These observations are entirely consistent with available crystallographic and solution data.

Thus, for the neutral species, flap-up and flap-down conformations are observed for analogues of both 1 (up, lysergamide;[16] down, bromocriptine,[19] ergotamine) and **2** (up, ergotaminine;[19] down isolysergamide[16]).

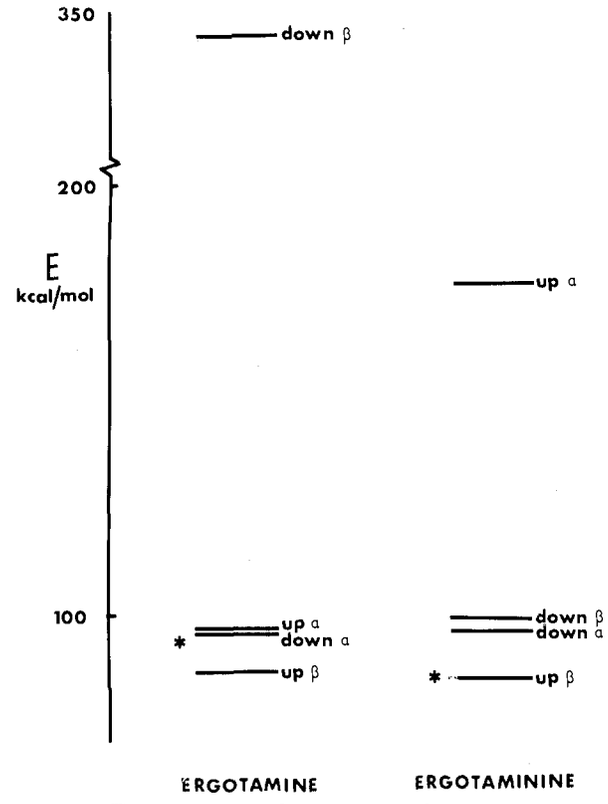

Figure 5. Energies of possible D ring conformations in neutral 1 and **2**. Values of τ_1, τ_2, τ_3, and τ_4 are optimized for each ring conformation. Conformations observed in solution are asterisked.

Conclusions

The alternative conformations available to 1, **2**, and related compounds can be summarized as follows: (1) τ_1 may be either extended or folded back to allow formation of the N_{20}–H···N_6 bond. The latter situation appears to apply whenever a hydrogen bond can be formed. In general, neutral molecules will adopt the folded H-bonded conformation while protonated species are in the extended forms. (2) τ_2 can adopt any of three conformations, but the folded conformation allowing formation of a $O_{15'}$–H···O_{19} is invariably observed in crystal structures and solution. (3) The benzyl group is restricted to a single relatively broad conformational region with the phenyl ring in an extended configuration relative to the cyclol ring. (4) The D ring may be either flap up or flap down. When the N_{20}–H···N_6 bond can be formed, the ring conformation is that which places the amide group pseudoaxial. In the absence of the hydrogen bond, the pseudoequatorial configuration is usually preferred, although the limited NMR data for protonated **2** suggests that it may be pseudoaxial despite its inability to form the hydrogen bond.

Biologically Active Conformations. The present calculations suggest that at least 24 conformations are accessible to 1 under physiological conditions. These comprise two alternative τ_1 regions, three τ_2 values, two alternative ring conformations, and two alternative ionization states. Considering all of the available data, however, two of these conformations emerge as the most probable in environments with low dielectric constants, such as would be expected for the ergotamine binding site. They are (1) the folded, hydrogen-bonded, flap-down conformation observed for neutral ergotamine in $CDCl_3$ solution (Figure 6a) and (2) the extended, flap-up conformation observed for protonated ergotamine in the

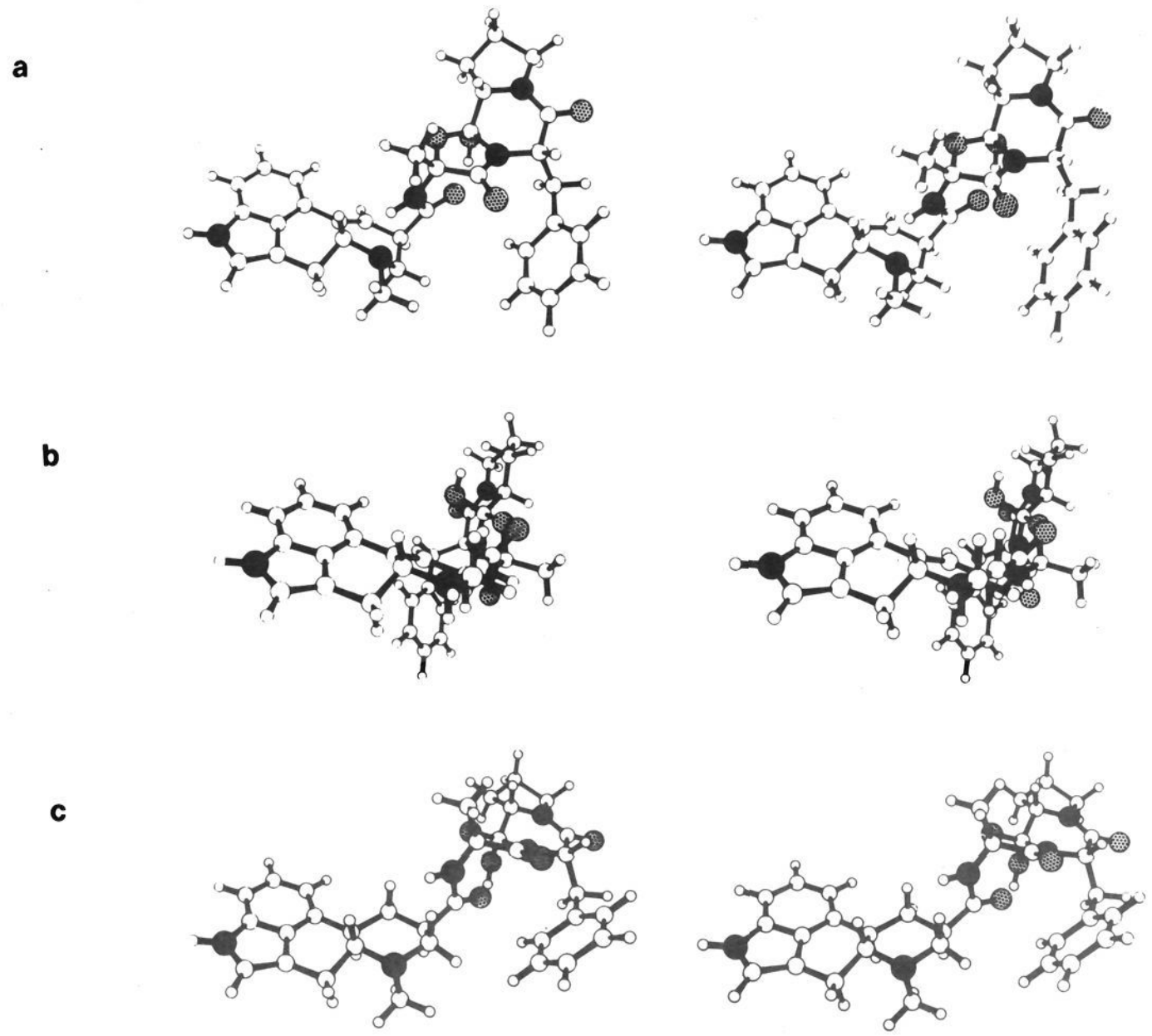

Figure 6. Stereoscopic comparisons of alternative conformations of **1** with dihydroergotamine: (a) folded, hydrogen-bonded, flap down conformation observed for neutral **1** in solution; (b) extended, flap up conformation observed for protonated **1** in solution; (c) dihydroergotamine in the chair conformation with τ_1 and τ_2 aligned to match neutral **1**. Light and dark shadings represent oxygen and nitrogen atoms, respectively.

crystal[19] or $CDCl_3$ solution (Figure 6b). In differentiating between these two possibilities, Weber[19] has invoked the ergotamine-like activity of dihydroergotamine as support for the latter conformation as the biologically active form. This is consistent with the fact that dihydroergotamine is sterically constrained to a chair conformation with C_7 above the plane of the indole ring and thus apparently similar to the flap-up conformation of **1**. However, comparison of molecular models for dihydroergotamine and the alternative conformations of **1** indicates that a much better overall fit can be achieved between one of the other low-energy conformations of dihydroergotamine (Figure 6c) and the hydrogen-bonded, flap-down conformation of **1** than between the crystal structure of dihydroergotamine[19] and the extended, flap-up conformation of **1**. The hydrogen-bonded structure, which is that favored by the neutral species in $CDCl_3$, thus appears to be the more likely biologically active conformation of ergotamine.

Possible Prodrugs and Analogues of 1. The favored conformations of **1** and **2** in $CDCl_3$ appear to be stabilized by two intramolecular hydrogen bonds. Consequently, it is likely that the relative thermodynamic stabilities of analogues of **1** and **2** that could not form intramolecular hydrogen bonds (either because N_6 was quarternized or because the protons on N_{20} and $O_{15'}$ were replaced by acyl, alkyl, or aryl groups) would be quite different. If such analogues were rapidly transformed to **1** or **2** within the body they would be potentially useful prodrugs.

An alternative approach would be the design of rigid analogues of **1** that mimic the proposed biologically active conformation. This could be achieved, for example, by the formation of a covalent bridge between N_{20} and N_6.

Future work will be concerned with the design, synthesis, and evaluation of prodrugs and analogues of **1**.

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