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Conformational Analysis of the D Ring of Lysergic Acid Amides and its Bioactive Conformation*

Jurkica Kidrič, Darko Kocjan and Dušan Hadži

Boris Kidrič Institute of Chemistry and Lek — Pharmaceutical and Chemical Works, Ljubljana, Yugoslavia

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^{1}H, ^{13}C nuclear magnetic resonance data on simple lysergic acid amides and ergopeptines indicate considerable flexibility of the D ring. The actual conformation depends upon the existence (or absence) of intramolecular hydrogen bonding between N6 and the central amide N20—H group. The results are in agreement with molecular mechanics calculations. The proposal for the bioactive conformation is based on the comparison of key geometric parameters of the possible ergolene conformers with ones derived for the conformationally restricted dopamine congeners.

INTRODUCTION

Ergoline derivatives are endowed with high biological activity and preparations from Claviceps sclerotia have been used since 1582 in the therapy of various diseases. The large diversity of pharmacological effects of ergoline derivatives results from interactions with dopaminergic, serotonergic and adrenergic receptors[1]. The requirement of selectivity towards a single type of receptor has led to numerous modifications of natural ergot alkaloids and the synthesis of partial ergoline structures[2]. Concomitant to the search for better drugs was the quest for the pharmacophore of ergoline nucleus which should be common with the physiological ligands to all the three groups of receptors[3], i. e. dopamine, serotonine and adrenaline as well as their synthetic congeners. The derivation of the pharmacophore may be subdivided into (i) the aromatic and (ii) the ethylamine fragments, both embedded in the ergoline nucleus. We shall be dealing only with (ii) in this paper whereas we are dealing with (i) elsewhere[4]. In particular, we shall presently be concerned with the question which is the conformation of the D ring of lysergic amides type ergolines that is most likely to be interacting with the dopamine receptor. This facet of the more general and, indeed, very complex problem of the ergoline pharmacophore was chosen because of the possibility of matching ergoline structures with the conformationally restricted congeners of dopamine[5]. The consideration of possible stereoisomers of ergolines in relation to their activity will be restricted to the conformation of the D ring including the C8 substituent because only molecules having the trans (5R, 10R) con-

* Dedicated to Professor Mihailo Lj. Mihailović on the occasion of his 60th birthday.

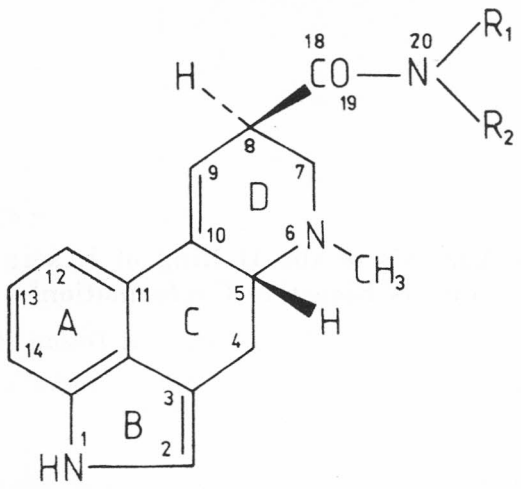

Figure 1. Molecular structure of ergolene derivatives; R_1 = alkyl or peptide, R_2 = = alkyl or H.

figurated C—D ring junction are biologically active[6]. The discussion will not include the medicinally very important 8-aminoergoline derivatives because of lack of appropriate conformational data.

The D ring of 9,10-unsaturated ergolines (ergolenes for short) may assume the D_1 and D_2 half chair conformations (Figures 1, 2) whereas the saturated D-ring may be in the chair or in either of the two boat conformations. Molecular mechanics calculations show the potential energy of the D_1 conformation to be lower than one of the D_2 conformation. The difference calculated by the QCFF/Pi method is 6.5 kcal/mole[7]. The energy of the chair conformation of ergoline is much lower than either of the two boat conformations. The energy of the ergolenes depends also on the orientation of the N_6-substituent and the calculations demonstrate that the equatorial methyl group is always energetically favoured[7]. A further conformational variable is the torsion angle O19-C18-C8-C9 which defines the orientation of the C8 substituent and thus the normal (8R or β) and the iso (or-inine, 8S or α) series.

Molecular mechanics calculations on ergotamine and ergotaminine indicate that for both epimers the D_1 ring conformation has the lower energy[8]. However, these calculations do not include the effect of the intermolecular hydrogen bond N2O—H . . . N6 which may exist in aprotic media with neutral ergolenes possessing a central NH group. Such hydrogen bonding will stabilize the D_2 conformation of β-ergolenes and the D_1 conformation of their α-epimers (Figure 2). The calculations may be compared with experimental results. X-ray structure determinations of neutral ergot alkaloids show the D ring to be in the hydrogen bonded D_2 conformation whereas the protonated alkaloids assume the D_1 conformation. The hydrogenoted ergot alkaloids are in the chair conformation exclusively.[9,10] Lysergic acid diethylamide is in the D_1 conformation[11].

The calculated energy difference between both half-chair conformations of 9, 10 ergolene and with N6—CH_3 equatorial is 25 kJ/mol, and for N6—CH_3 axial it is only 7.7 kJ/mol[7]. Since the calculations refer to the free molecules,

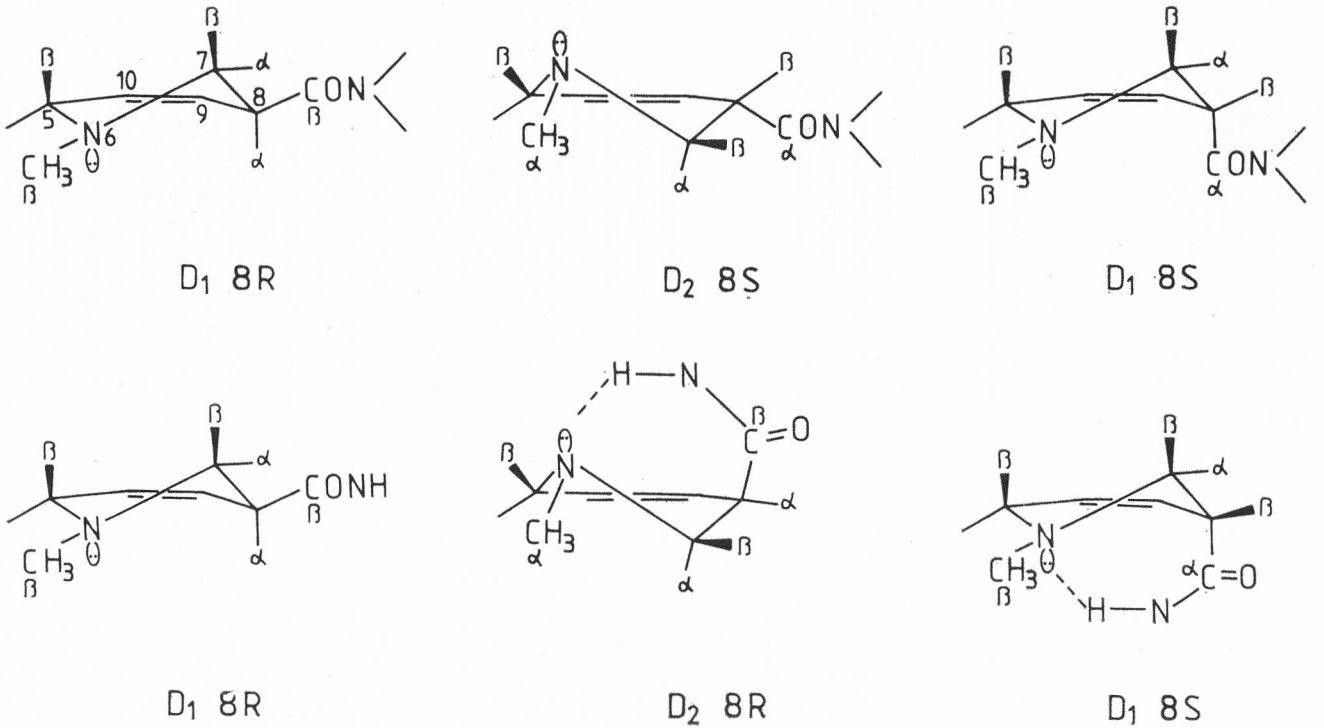

Figure 2. Conformations of D ring (R, S refers to configuration at C8). First row: lysergic dialkylamides, second row: lysergic monoalkylamides and ergopeptines.

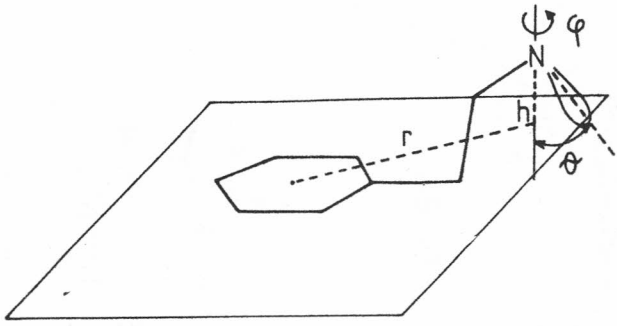

Figure 3. Geometrical parameters defining the spatial relations between the aromatic moiety and the N6-lone pair of some dopaminergic agonist skeletons with N6-methyl equatorial.

it may be expected that solvent effects and the existence or absence of intramolecular hydrogen bonds will influence the actual population of the various D-ring conformations in solution. The dominant conformation in solution may also be different from ones observed in the crystalline state. It therefore appeared necessary to study the conformation of the neutral and protonated ergolines by NMR methods.

Analysis of the ^1H NMR Spectra

NMR conformational analysis of several hydrogenated ergot alkaloids in $CDCl_3$ has shown them to exist in the chair conformation very similar to one observed in the crystals[11]. For neutral ergotamine in $CDCl_3$ the D_2 conformation has been determined whereas ergotaminine is in the D_1 conformation[8]. The conformation of dialkylamides of lysergic acid (DAM) and their epimers in $CDCl_3$ and C_6D_6 has been investigated by Baily et al.[12] using ^1H NMR. On the ground of long range coupling constants the conformation of lysergic acid dimethyl amide in $CDCl_3$ was unambigously assigned as D_1. For its epimer the D_2 form was proposed, but it could not be unambigously established.

We have investigated the ^1H and ^{13}C NMR spectra of both epimers of DAM in $CDCl_3$ and DMSO-d_6 [13]. The main progress in solving the problem of the D-ring form of DAM and of the orientation of C8-carboxamide group was the rationalization of the chemical shift differences between both epimers of DAM (epimeric shifts) by theoretical calculations of conformation dependent contributions of neighbour groups. In particular, the δ (H5, C4) are influenced by the magnetic anisotropy of the N6 lone pair and δ (C9) by the linear electric field of the C8 carbonyl group. Thus it was shown that the ring D of iso-DAM is indeed in the D_2 conformation whereas it is in the D_1 conformation with DAM. The carboxamide group is pseudoequatorial in both epimers, (Figure 2, first row). In contrast to lysergic acid dialkylamides, the formation of the N20—H . . . N6 hydrogen bond is possible with the neutral lysergic acid peptides, usually called ergopeptines. This complicates the conformational analysis based on ^{13}C spectra. No simple correlation between ^{13}C chemical shifts and the D-ring conformation is possible because of the difficulties in separating the through space effects from the through bond

effects. Therefore we have based the conformational analysis of ergopeptines in solution on the positions of the N20—H resonance signals which are indicative of intramolecular hydrogen bonding to N6, as well as on the values of the vicinal coupling constants J (7α, 8), J (7β, 8) and J (8, 9). As an example we consider ergosine (ESN), its epimer ergosinine (ESNN) and their methanesulphonates (ESNMS, ESNNMS) in $CDCl_3$ and DMSO-d_6. The downfield position of the N20—H resonance signal of ESN oi $CDCl_3$ (9.36 ppm/TMS) relative to dihydroergotamine (6.4 ppm)[14] and to dihydrolysergamides (5—6 ppm,[15] indicates intramolecular hydrogen bonding. The coupling constants of ESN in $CDCl_3$ J (7α, 8) = 3.7 Hz, J (7β, 8) = 4.1 Hz and J (8, 9) = 6.0 Hz are compatible with the dihedral angles characterizing the conformation D_2 with H8 in α-pseudoequatorial and the C8-substituent in β-pseudoaxial position. The coupling constants in DMSO-d_6 are 5.0 Hz, 12.5 Hz and 2 Hz, and they are compatible with dihedral angles connected with the D_1 conformation with H8 in α-pseudoaxial and the C8-substituent in the β-pseudoequatorial position. Obviously, the interconversion between the D_2 and D_1 conformation occours with change of solvent. DMSO is a strong proton acceptor and disrupts the intramolecular hydrogen bond that stabilizes the D_2 conformation. Without hydrogen bonding this conformation has the higher potential energy[8].

With N6 protonated the N20—H . . . N6 bonding is not possible and one expects the more stable D_1 form to predominate. This is supported by the X-ray structure of protonated bromocriptine[10], by the results of Pierri et al. for protonated ergotamine[8], and by the value of J (8, 9) of ESNMS in DMSO, which is 2 Hz.

Now we consider the conformation in the iso-series. The positions of the N20—H signal of ESNN (9.86 ppm, 16) and ergotamine (9.83 ppm, 8) in $CDCl_3$ indicate the existence of the intramolecular hydrogen bond. The vicinal coupling constants J (7α, 8) and J (8, 9) of ESNN in $CDCl_3$ which are 3 Hz and 5.0 Hz are in agreement with the D_1 half chair form with H8 in the β-pseudoequatorial and the C8-substituent in the α-pseudoaxial orientation. However, this form does not appear to be particularly stabilized by intramolecular hydrogen bonding since both the ^1H shifts and the coupling constants indicate that this conformation persists in DMSO solution[16] as well as with the protonated form.

Proposal for the Bioactive Conformation

The conformational analysis demonstrates that the D ring of 9,10-unsaturated ergolines with a central NH group may assume either the D_1 or D_2 conformation depending on the medium and the state of protonation of N6. Water as the biological medium may be assumed to have similar effects as DMSO on intermolecular hydrogen bonding, hence the D_1 conformation is likely to dominate in water solution both with neutral and protonated molecules. However, this does not necessarily mean that the ergolines are interacting in this form with the receptor. Part of the free energy of interaction with the latter may be used for ring inversion or, simply, the lower populated D_2 state might be picked out by the receptors. Such situations are often met with flexible ligands to receptors and present a challenge to drug designers

since it may be expected that a rigid ligand with the best steric fit to the receptor should have the highest affinity in a series of ligands with equal electronic components of the pharmacophore.

Endeavours to derive the steric requirements of dopamine receptors has led to the synthesis and biological testing of numerous hydroaromatic systems containing the hydroxyphenylethylamine pharmacophore (for references see 5). This series permits the derivation of the key geometric parameters relating the aromatic ring to the basic nitrogen atom and the orientation of the N6-lone pair[5]. The key parameters are the distance R between the center of the aromatic nucleus and the nitrogen, the height h of the latter above the aromatic plane and the angles ϑ and γ defining the orientation of the N6-lone pair (or the N^+—H bond in protonated ligands) Fig. 3. It should be emphasized that in case of small values of the angle ϑ variations in γ are of little importance for the actual location of the N6-lone pair (or proton) relative to the putative accepting site of the receptor. The skeletons of apomorphine and octahydrobenzo(f)quinoline may be taken as representatives of the rigid dopamine agonists and in Table I their parameters are given together with ones of ergolines. We have taken the pyrrole ring B of the latter to be equivalent to the catechole fragment of apomorphine following the arguments of Nichols[17] and substantiated by our work[4]. The superposition of apomorphine and ergolene skeletons is shown in Figure 4. The data in Table I demonstrate that parameters defining the lone pair direction in the D_1 conformation are closer to ones of dopamine congeners than to the corresponding parameters of the D_2 conformation. Actually, the most relevant difference lies in the values of the dihedral ongle ϑ. Thus D_1 conformation rather than D_2 appears to be the bioactive one. Pierri et al.[8] arrived at the opposite conclusion, i. e. that the D_2 conformation is bioactive. This was proposed for ergotamine in particular, but should be applicable to all lysergamides with an NH group. Their main argument was that the receptor binding site has hydrophobic character and hence the hydrogen bonded D_2 conformation, more stable in the hydrophobic solvent $CDCl_3$, should also be more appropriate for the interaction with the receptor.

The weak point of this hypothesis is the assumption that ergotamine acts on the receptor in the neutral form. It is generally accepted that the side chain nitrogen atom of biogenic amines or its cyclic analogues are the primary point of attachment to the receptor. However, the question whether the amines interact in the neutral or protonated form is open. The main argument in favour of the protonated from is the fact that with most, if not all, ligands the protonated form is the predominant one at physiological pH 7.4. Even for butaclamol, a dopamine receptor ligand with antagonistic properties, the claim for its low pK_A with the subsequent proposal (18) about its binding in the neutral form has to be corrected in view of new experimental data[19]. However, even assuming the ligand to be somehow deprotonated before actual binding to the receptor the intramolecular hydrogen bonded form may be expected to have a reduced affinity because of competition with the nitrogen lone pair binding to the receptor site.

The final argument in favour of the D_1 conformation being the bioactive one can be drawn from the overal shape of the molecule. The D_2 conformation

TABLE I

Geometrical Parameters Defining the Spatial Relations Between the Aromatic Moiety and the N6-Lone Pair of some Dopaminergic Agonist Skeletons with N6-Methyl Equatorial

		r	h	Θ	φ
apomorphine		5.0 Å	0.9 Å	21°	154°
octahydrobenzo [f] quinoline		5.2	0.3	17	131
9,10-ergolene	D_1	4.9	0.4	10	137
9,10-ergolene	D_2	4.9	0.4	160	—168
ergoline	chair	5.1	0.7	16	—167

with the peptide moiety bending over into the N6 region is bulky (see Figure 6 of ref. 8) whereas the D_1 conformation imparts a more planar form to the molecule which is also more flexible owing to the absence of the intramolecular hydrogen bond, leaving free access to the N6 reigon. The geometrical key parameters of the D_1 conformation are also very close to ones of the dihydroergolines (Table I) and this induced Weber[9] also to consider D_1 as the more likely bioactive conformation.

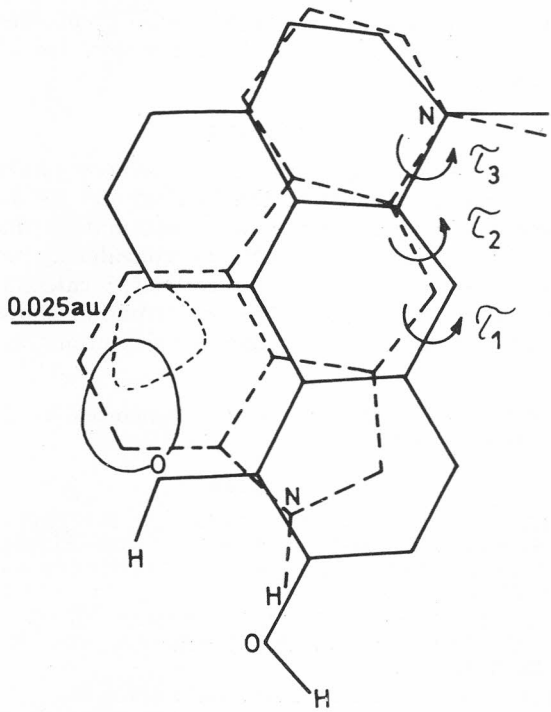

Figure 4. Superposition of ergolene (dashed) and apomorphine (full line) skeletons optimized for the positions of the molecular electrostatic potential minimum (indicated by the 0.025 a. u. contour) relative to the aliphatic ring nitrogen (ref. 4). The ring conformations are given by the dihedral angles $\tau_1 = 202°$ (216°), $\tau_2 = 177°$ (179°), $\tau_3 = 56°$ (54°) (values for apomorphine in parentheses).

So far we have been mainly concerned with the conformation of the D ring in the series of normal ergolines. The differences in biological activity between this and the iso series are difficult to rationalize. The extremely high activity of lysergamide is contrasted by the inactivity of its epimer isolysergamide[20]. However, either both epimers of some other ergolines with small C-8 substituents, e. g. CH_2OH and $C{\equiv}N$[21] are active in reducing prolactine secretion which is one of the characteristic dopamine agonists activities or even is the α-epimer the more active one (e. g. lisuride). For the ergopeptine series the belief was held up that the iso-peptines are devoid of any activity[22]. Recent pharmacological investigations, however, negate this belief although there are differences in pharmacokinetic characteristics of both series.[23,24] Binding assays using enriched receptor material have also shown that iso-peptines have affinities comparable to the normal peptines.[25]

In view of these facts the approach to the question of the bioactive D ring conformation of iso-ergolenes is rather speculative. Moreover, the pertinent experimental data are less complete than for the β-series. Confronting the inactivity of iso-DAM with the evidence that it is in the D_2 conformation one is tempted to exclude this conformation as a candidate for the bioactive conformation. Out of the two possible D_1 conformations, i. e. with and without intramolecular hydrogen bonding, the latter is more likely by arguments advanced for the series of β-epimers. The absence of the intramolecular hydrogen bonding also allows for better adaptability of the 8-substituent to the requirements of the receptor and, in particular, for its turning away from the N6 binding region.

CONCLUSION

The quest for the steric component of the ergolene pharmacophore arises from the conformational flexibility of the D ring and its 8-substituents. We hope to have presented arguments for the conformation that should satisfy the dopamine receptor steric requirements by considering the key geometric parameters derived from conformationally restricted catechole type agonists. These parameters are consistent with the conformation shown by NMR conformational analysis and molecular mechanics calculations to be energetically favourable.

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SAŽETAK

Konformaciona analiza i bioaktivna konformacija D-prstena amida lizergove kiseline

Jurkica Kidrič, Darko Kocjan i Dušan Hadži

^{1}H i ^{13}C NMR-podaci o jednostavnim amidima lizergove kiseline i ergopeptinima ukazuju na značajnu fleksibilnost D prstena. Konformacija zavisi od prisustva ili odsustva intramolekularne vodonične veze između N6 i N20—H centralne amidne grupe. Rezultati su u saglasnosti sa računima molekulske mehanike. Izbor bioaktivne konformacije osnovan je na upoređenju ključnih geometrijskih parametara mogućih konformera ergolena i konformaciono ograničenih srodnika dopamina.