STRUCTURAL STUDY OF DOPAMINE AGONIST LISURIDE+

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One monoclinic and three orthorhombic structures of natural ergot alkaloid lisuride were solved by X-ray diffraction techniques. The conformation analysis has revealed that side chain orientations of lisuride differ significantly from those observed in related terguride (*trans*-dihydrolisuride). The conformation found in individual lisuride molecules corresponds to another minimum of total energy calculated by *ab initio* quantum-mechanical calculations for a simplified model of 3-(cyclohex-2-en-1-yl)-1,1-diethylurea.

Keywords: Indole alkaloids; Ergot alkaloids; Lisuride; Crystal forms; X-Ray diffraction; Crystal structure; Conformation analysis; Polymorphism; *Ab initio* calculations.

Together with pergolide, cabergoline and bromokryptine, lisuride ranks among dopamine receptor agonists used for the treatment of Parkinson's disease. It was found that dopamine D-2 receptor agonists may protect against glutamate-induced neurotoxicity[2–5], can improve motor fluctuations and reduce dyskinesia in Parkinsonian patients, provide a levodopa sparing effect and a better control of the side effects by the careful titration of the dosage in comparison with the levodopa monotherapy[6]. Hence, the therapeutic approach to Parkinson's disease has recently begun to change

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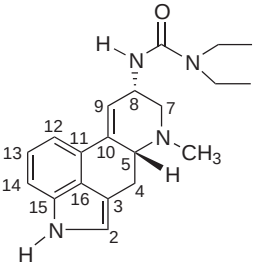
and the role of dopamine agonists becomes more prominent especially in early treatment[6]. This promoted also a new development in the field of dosage forms[7,8] and interest in the structure–activity relationship of D-2 receptor agonists.

Recently we reported the structures of lisuride maleate[9]. Here we report the structure determinations of four crystalline forms of lisuride base (**1**–**4**).



1, anhydrate
2, ethanol solvate
3, methanol solvate
4, monohydrate

EXPERIMENTAL

Preparation of Crystals

Lisuride anhydrate (**1**): Lisuride (100 mg, 99.5% HPLC, IVAX Pharmaceuticals s.r.o., Czech Republic) was dissolved in acetonitrile (2 ml) and the solution was allowed to stand overnight. The formed needle crystals were separated and dried in air. Lisuride anhydrate exhibits a sharp endothermic peak at the DSC curve at 189.5 °C (melting) and an additional weak endothermic effect at 206 °C (DSC, Mettler, Toledo, 50–300 °C, 10°/min).

Lisuride ethanol solvate (**2**): Lisuride (500 mg, 99.5% HPLC, IVAX Pharmaceuticals s.r.o., Czech Republic) was dissolved in ethanol (5 ml) and water (0.5 ml) was added. The solution was allowed to stand overnight. The formed crystals were mounted directly on the holder of the diffractometer and immediately cooled. Lisuride ethanol solvate exhibits a broad endothermic effect at roughly 102 °C at the DSC curve (desolvatation), a very weak endothermic effect at 160 °C, and other effects are essentially the same as with **1**.

Lisuride methanol solvate (**3**): Lisuride (400 mg, 99.5% HPLC, IVAX Pharmaceuticals s.r.o., Czech Republic) was dissolved in methanol (6 ml) and the solution was allowed to stand overnight. The formed crystals were mounted directly on the holder of the diffractometer and immediately cooled. Lisuride methanol solvate exhibits a broad endothermic effect at roughly 95 °C at the DSC curve (desolvatation) and other effects are essentially the same as with **1**.

Lisuride monohydrate (**4**): Lisuride (400 mg, 99.5% HPLC, IVAX Pharmaceuticals s.r.o., Czech Republic) was dissolved in *tert*-butyl methyl ether (100 ml; 100 μl of water) and the solution was allowed to stand overnight. The formed plates of crystals were separated and dried on air. Lisuride monohydrate exhibits a broad endothermic effect at roughly 108 °C at the DSC curve (dehydratation), a very weak endothermic effect at 160 °C, and other effects are essentially the same as with **1**. The effect at 206 °C is much more pronounced compared with all other forms.

X-Ray Structure Analysis

All data collection and refinement parameters are listed in Table I. International Tables for X-Ray Crystallography[10], and programs SDP[11], CRYSTALS[12] and PARST[13] were used. CCDC 186804–186807 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Ab initio Quantum-Mechanical Calculations

Dependence of the total energy on the torsion angle C9–C8–N18–C19 was studied using an *ab initio* quantum-mechanical method (base RHF/6-31G(d,p) as implemented in Gaussian 98[14]. 3-(Cyclohex-2-en-1-yl)-1,1-diethylurea served as a reasonable model of rigid ergolene ring system. The torsion angles were rotated using 10° steps, while the geometry of the molecule was optimized and the total energy calculated.

RESULTS AND DISCUSSION

Crystallization of lisuride from various solvents provided four crystalline forms with distinct powder patterns. Depending on the solvent, lisuride crystallizes as an anhydrous form (**1**), as ethanol (**2**) or methanol (**3**) solvates and monohydrate (**4**). Forms **1**–**3** are orthorhombic, form **4** crystallizes in the monoclinic symmetry. Rapid crystallization from diluted solutions seems to contribute to preferred crystallization of plate crystals of lisuride hydrate **4**, while the slow crystallization from supersaturated solutions gives compact crystals of **1**. These two forms frequently coexist.

The basic structure of lisuride is derived from the tetracyclic ring system designated as ergolene. The indole moiety (formed by A and B rings) is nearly planar. C and D rings are puckered (C ring – envelope E_3 conformation, D ring – halfchair $^6H_1$ conformation). However, the ergolene rings C and D do not adopt "ideal" conformations (Table II). The largest discrepancy of ring C was found for the hydrated form **4**, the conformation of which lies between E_3 and $^2H_3$. Ring D of both independent molecules of anhydrous form **1** deviates slightly towards ^6E from the ideal $^6H_1$ conformation. In comparison, the only structure of lisuride maleate[9] (**5**) adopts almost a perfect envelope and a halfchair for the rings C and D. A subtle decrease in the puckering amplitude (Q) can be found.

The asymmetric unit of **1** comprises two symmetry-independent lisuride molecules. Surprisingly, we have discovered no links between them. Thus, the hydrogen bond network is built of infinite chains oriented along a screw axis in X-direction for the one molecule and by a simple translation

TABLE I
X-Ray crystallographic and measurement data

Parameter	**1**	**2**	**3**	**4**
Formula	$C_{20}H_{26}N_4O$	$C_{20}H_{26}N_4O \cdot C_2H_5OH$	$C_{20}H_{26}N_4O \cdot CH_3OH$	$C_{20}H_{26}N_4O \cdot H_2O$
M_w	338.5	384.5	370.5	356.5
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
a, Å	12.1596(9)	10.2352(2)	10.3024(1)	7.4814(2)
b, Å	12.2906(6)	13.2834(2)	13.3666(2)	7.9931(3)
c, Å	25.267(1)	15.5874(3)	14.7946(2)	16.0839(6)
β, °	–	–	–	97.119(2)
Z; *V*, Å^3	8; 3776.1(4)	4; 2119.24(7)	4; 2037.34(5)	2; 954.39(6)
ρ_{calc}, g cm^{-3}	1.19	1.21	1.21	1.24
μ, mm^{-1}	0.60	0.08	0.08	0.08
F(000)	1459.71	832.14	800.13	384.06
Crystal dimensions, mm	0.28 × 0.32 × 0.84	0.14 × 0.35 × 0.56	0.11 × 0.28 × 0.46	0.5 × 0.5 × 0.075
Diffractometer	Enraf-Nonius CAD4	Nonius Kappa CCD	Nonius Kappa CCD	Nonius Kappa CCD
Radiation used λ, Å	1.54184	0.71073	0.71073	0.71073
Scan technique	ω/2θ-scan	ω-scan	ω-scan	ω-scan
Temperature, K	293	150	150	150
No. and θ range (°) of reflections for lattice parameter refinement	20, 38–40	21 506, 1–27	25 433, 1–27	28 928, 1–27
Range of *h*, *k* and *l*	–14→14, 0→14, 0→30	–13→13, –17→17, –20→20	–13→13, –17→17, –19→19	–9→9, –10→10, –20→20
Total number of reflections measured	7364	32 666	44 904	13 607
2θ range, °	4–136	2–55	2–55	2–54
No. of observed unique reflections	6101	2770	2666	2259
Criterion for observed reflections	$I > 1.96I_o$	$I > 1.96I_o$	$I > 1.96I_o$	$I > 1.96I_o$
Function minimised	$\sum w(\lvert F_o\rvert - \lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - \lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - \lvert F_c\rvert)^2$	$\sum w(\lvert F_o\rvert - \lvert F_c\rvert)^2$
Weighting scheme	Chebychev polynomial[16]	Chebychev polynomial[16]	Chebychev polynomial[16]	Chebychev polynomial[16]
Parameters refined	451	378	363	347
Value of *R*, *wR*, and *S*	0.0798, 0.0720, 0.9887	0.0385, 0.0459, 1.0148	0.0475, 0.0369, 1.0568	0.0324, 0.0383, 1.0379
Ratio of the maximum least-squares shift to e.s.d. in the last cycle	0.0004	0.0004	0.001	0.0002
Maximum and minimum heights in final Δρ map, e Å^{-3}	–1.01, 0.93	–0.39, 0.49	–0.56, 0.60	–0.36, 0.26

along *Y* for the other. The corresponding hydrogen bonds are N1–H···O20 ($x - 1/2, -y + 1, -z + 1$) and N51–H···O70 ($x, y + 1, z$).

The hydrogen bond network of **2** displays a strong interaction of ethanol with lisuride. The OH hydrogen atom in ethanol is utilized in O51–H···N6 bond, whereas a hydrogen atom attached to amide N18 is involved in the bond N18–H···O51. Infinite chains are formed through N1–H···O20 ($-x + 1/2, -y, z + 1/2$) contacts along a screw axis in *Z*-direction.

The molecular packing of **3** is roughly identical with that of **2**. Methanol is bound to lisuride over O51–H···N6 and N18–H···O51 bridges. The N1–H···O20 ($-x + 1/2, -y, z + 1/2$) linkage allows infinite chain growth.

The water molecule in **4** takes the site of the alcohol oxygen in preceding structures. However, the role of water is not fulfilled just by the presence of O51–H···N6 and N18–H···O51 bridges. Water molecules participate in the chain network described above, acting as bridge atoms for neighbouring molecules of lisuride forming thus a two-dimensional network. The hydrogen bonds are as follows N1–H ($-x, y + 1/2, -z$)···O50–H···O20 ($x + 1, y, z$).

Altogether, five symmetry-independent molecules can be found in the four structures of lisuride base described in this work and the sixth one was described in literature for lisuride maleate[9]. The effects of different crystal packings on the conformations of independent lisuride molecules appear to be generally very low. All the six independent molecules studied exhibit rather subtle conformation differences. The conformations of flexible chains are highly correlated in all structures. Slightly different conformation can be found with the second independent molecule in **1** only. Hence,

TABLE II
Ring puckering parameters of lisuride

Compound	Ring C				Ring D			
	φ, °	θ, °	*Q*, Å	conformation	Φ, °	θ, °	*Q*, Å	conformation
1	–63.0(4)	124.5(3)	0.410(2)	E_3	138.4(3)	127.8(2)	0.530(2)	${}^6H_1$–${}^6E$
	–62.6(4)	124.5(3)	0.440(2)	E_3	137.3(3)	127.7(2)	0.507(2)	${}^6H_1$–${}^6E$
2	–72.3(2)	127.1(2)	0.397(1)	E_3–${}^2H_3$	150.9(2)	128.4(1)	0.514(1)	${}^6H_1$
3	–68.7(2)	127.0(2)	0.387(1)	E_3–${}^2H_3$	151.0(2)	127.6(1)	0.527(1)	${}^6H_1$
4	–75.2(2)	125.4(2)	0.429(1)	E_3–${}^2H_3$	153.8(2)	125.7(1)	0.523(1)	${}^6H_1$
5	–65	126	0.36	E_3	151	130	0.48	${}^6H_1$

two molecules of **1** are given as an example of lisuride structure in Fig. 1. Whereas the first independent molecule exhibits the ordinary conformation with atoms N18, C19, O20, N21, C22 and C24 forming a plane (torsion angle C9–C8–N18–C19 72.7(3)°), the orientation of the plane is rotated in the second independent molecule (torsion angle C59–C58–N68–C69 162.2(2)°). Torsion angles of **1**–**5** are listed in Table III.

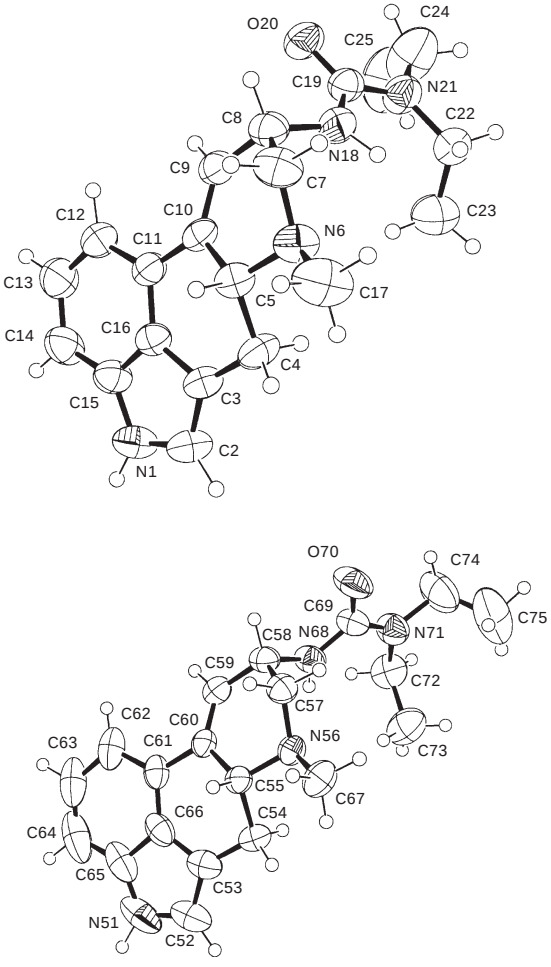


FIG. 1
The ORTEP drawing of two symmetry-independent molecules of lisuride anhydrate (**1**) with the crystallographic numbering

Surprisingly, except of this one independent molecule in **1**, which is related to the conformations in terguridies[15], all other independent molecules in **1**–**5** adopt a conformation, which was never found in any terguride molecule. Regardless, *ab initio* quantum-mechanical calculations of a simplified model of 3-(cyclohex-2-en-1-yl)-1,1-diethylurea (for lisuride) provided the very similar results reported for 3-(cyclohex-1-yl)-1,1-diethylurea (model for terguride)[15], ordinary conformation of all other independent molecules in **1**–**5** corresponds to other local minima on the total energy curve (Fig. 2).

TABLE III
Characteristic torsion angles of flexible chains

Compound	C9–C8–N18–C19 torsion angle, °	C19–N21–C22–C23 torsion angle, °	C19–N21–C24–C25 torsion angle, °
1	72.7(3)	82.4(3)	–90.4(5)
	162.2(2)	–81.0(4)	103.5(4)
2	88.5(1)	–97.1(1)	–81.7(2)
3	92.5(1)	–95.6(2)	–79.3(2)
4	109.3(1)	–91.0(2)	–88.5(1)
5	95.9	–119.0	–85.7

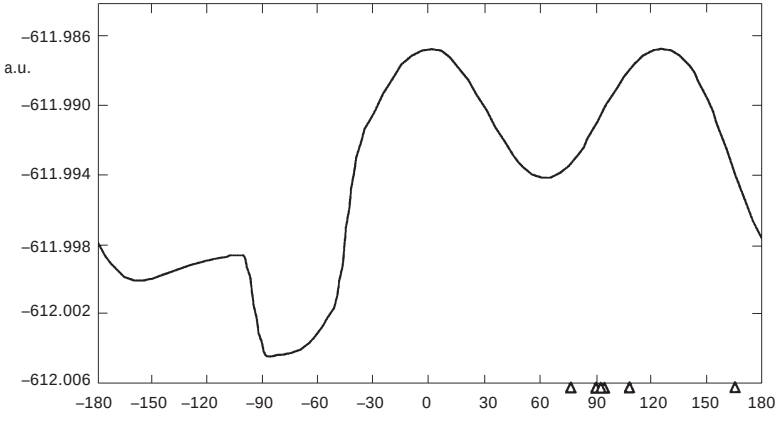


FIG. 2
Dependence of total energy (a.u.) on torsion angle C9–C8–N18–C19 calculated *ab initio* in base RHF/6-31G(d,p) for 3-(cyclohex-2-en-1-yl)-1,1-diethylurea. The corresponding experimental torsion angles are shown as (Δ) on the *x* axis

The *ab initio* method, however, has given no clue to the question "why the typical conformation of lisuride corresponds just to this local minimum".

Although it is not possible to classify overall lisuride conformations in the same manner as for terguride, we can summarize some features that might also contribute to the explanation of different pharmacological activity of related structures of lisuride and terguride. One criterion is conformation of ring D. A typical envelope or slightly distorted envelope was found in lisuride. On the other hand, no double bond is present in terguride, which results in regular chair conformation. Another key feature is the orientation of the side chain discussed above. Lisuride molecules strongly prefer the chain orientation corresponding to the sharp local minimum at the calculated total energy curve (Fig. 2). Although an analogous minimum lies also on the total energy curve calculated for terguride[15], such conformation has never been found in any of twelve independent terguride molecules found in various crystal structures. A very broad and shallow local minimum on the total energy curve seems to be the cause of the broad distribution of the chain alignments, allowing various types of packing and hydrogen bond networks found in terguride structures[15]. On the other hand, the V-shaped minimum is responsible for the generally smaller spread of side chain conformations in lisuride and lower flexibility. It also facilitates the uniform hydrogen bond network found in different crystal forms. With respect to the fact that conformations of molecules found in the solid state usually correspond to the dominant conformation in solution, the different biological activities of lisuride and terguride might be also affected by differences in populations of their conformers available for primary interactions with various receptors. This estimation also supports a relatively high value of rotation barrier (0.008 a.u. ≈5 kcal/mol, Fig. 2), which indicates that completely free rotation is in fact rather restricted.

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