

CRYSTAL FORMS OF SEMISYNTHETIC ERGOT ALKALOID TERGURIDE⁺

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Two new structures of semisynthetic ergot alkaloid terguride created by unusual number of symmetry-independent molecules were determined by X-ray diffraction methods at 150 K. Form A (monoclinic, $P2_12_12_1$, $Z = 12$) contains three symmetry-independent terguride molecules and two molecules of water in the asymmetric part of the unit cell. The form C_A (monoclinic, $P2_1$, $Z = 8$) is an anhydrate remarkable by the presence of four symmetry-independent molecules in the crystal structure. Conformations of twelve symmetry-independent molecules that were found in four already described terguride structures are compared with torsion angles obtained by *ab initio* quantum-mechanical calculations for the simplified model of *N*-cyclohexyl-*N'*-diethylurea.

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

The semisynthetic ergot alkaloid terguride, 1,1-diethyl-3-(6-methyl-ergolin-8-yl)urea, is a dopamine agonist with inhibiting effect on prolactin secretion and anti-Parkinsonian efficiency². The molecule of terguride pos-

+ In this 23rd paper on structure and polymorphism of ergot alkaloids we report an unprecedented case of a small molecule of terguride, which occurs under very similar conditions in four different crystalline forms created by twelve symmetry-independent molecules. For preceding paper of the series, see ref.¹

sesses three chiral centres. Only the (5*R*,8*S*,10*R*)-enantiomer (Fig. 1) is pharmaceutically used as a free base (Teluron®) or as its maleate (Mysalfon®). The existence of some terguride solvate was noted already by Zikán and Semonský³, but the true nature of several other crystal forms remained puzzling until current crystal structure determinations. Depending on the water content in the solvent, terguride can crystallize as anhydrous or in the form of various hydrates and solvates under very similar conditions. Originally these forms were denoted by letters **A**, **B**, **C**, and **M**, which we use also here. Recently we reported the structures of terguride–water⁴ (1 : 1) (form **B**), terguride–methanol⁵ (1 : 1) (form **M**), and terguride maleate⁶. A structural and synthetic study of the (5*R*,8*S*,10*S*)-enantiomer is also available⁷.

Here we report low-temperature structure determinations of the anhydrous form (denominated as **C_A**), and terguride–water (1 : 0.66) (form **A**), a comparison of all symmetry-independent terguride molecules, and the *ab initio* quantum-mechanical calculations of the relationship between the total energy and selected torsion angles in the terguride molecule.

EXPERIMENTAL

Preparation of Crystals

Form **A**. Terguride (200 mg, dried, 99.5% HPLC, IVAX CR, Czech Republic) was dissolved in acetone (6 ml), a small amount of water was added (10 µl) and the solution was allowed to stand overnight. The plate crystals were separated and dried in air.

Form **C_A**. Terguride (200 mg, dried, 99.5% HPLC, IVAX CR, Czech Republic) was dissolved in acetone (6 ml), several grains of molecular sieve was added (3 Å) and the solution was allowed to stand overnight. Prismatic needle crystals were immediately mounted on a holder of the Nonius Kappa CCD area detector and cooled to 150 K.

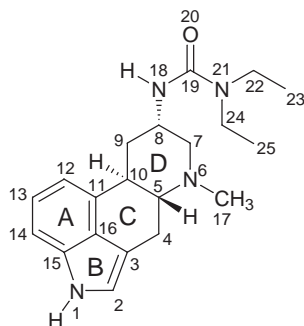


FIG. 1

Terguride molecule with atom numbering and ring designation

X-Ray Structure Analysis

All data collection and refinement parameters are listed in Table I. Both structures were solved by SHELXS86 (ref.⁸) by direct methods (Table II). Programs DENZO (ref.⁹), CRYSTALS (ref.¹⁰) and PARST (ref.¹¹) were used for other processing. All non-hydrogen atoms were refined anisotropically by full-matrix least squares. Carbon and nitrogen hydrogens were placed in the positions found from geometry. The water hydrogens for form **A** were placed in the positions found from a difference Fourier map. The C22–C23 ethyl group of molecule 2 in **C_A** was found to be disordered in two positions (refined with an occupancy factor fixed to 0.5). CCDC 170641 and 170642 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

The optimum geometry of flexible torsion angles was studied. An *ab initio* quantum-mechanical method (base 6-31G** as implemented in Gaussian 98, ref.¹²) was used for the total energy–torsion angle dependence calculation. The whole rigid ergot alkaloid ring system was replaced by cyclohexane to simplify the calculation. It means that *N*-cyclohexyl-*N*-diethylurea molecule was used as a model to study the side chains conformations. The examined torsion angles were changed in 10° steps. The geometry of the model molecule was optimised for each angle and the total energy calculation was made.

RESULTS AND DISCUSSION

The existence of various crystalline forms, which may affect the stability, pharmacokinetic profile and bioavailability of various dosage forms, is well known in the solid-state chemistry of drugs¹³. Recent research in the field of ergot alkaloids showed that more than 50% of them crystallize as various solvates, some of them occur as polymorphic forms¹⁴ (the use of the term “solid-state polymorphism of drugs” is not uniform: some authors include both polymorphs and solvates). However, the existence of several crystalline forms of terguride crystallizing under very similar conditions and containing such number of independent molecules seems to be very unusual.

Terguride monohydrate⁴ (historically form **B**) is the most stable form of terguride, which easily crystallizes from various aqueous solvents. Terguride methanol solvate⁵ (denoted as **M**), crystallizes from dry methanol, but can be easily converted to **B** even by air humidity. The third form, terguride–water (1 : 0.66) (form **A**), crystallizes from acetone containing water (typically 0.1–0.5%). At higher contents of water, both **A** and **B** crystallize simultaneously.

When the water/terguride ratio further decreases, the fourth form of terguride results (anhydrous **C_A**) or can coexist together with the form **A**.

TABLE I
X-Ray crystallographic and measurement data

Parameter	C_A	A
Formula	$C_{20}H_{28}N_4O$	$C_{20}H_{28}N_4O \cdot 2/3H_2O$
M_w	340.46	352.48
Space group	$P2_1$ (No.4)	$P2_12_12_1$ (No. 19)
a , Å	8.1640(3)	7.9340(2)
b , Å	23.3159(6)	12.8148(3)
c , Å	19.9975(8)	56.467(1)
β , °	95.870(2)	–
Z	8	12
V , Å ³	3 786.3	5 741.2
ρ_{calc} , g cm ⁻³	1.19	1.12
μ (MoK α), mm ⁻¹	0.08	0.08
$F(000)$	1 472.19	2 288.35
Crystal dimension, mm	$0.5 \times 0.5 \times 0.175$	$0.5 \times 0.5 \times 0.12$
Diffractometer used	Nonius Kappa CCD area detector	
Radiation, Å	λ (MoK α) = 0.71073	
Scan technique	ψ and ω scans to fill the Ewald sphere	
Temperature, K	150(2)	
No. and ϕ range of reflections for lattice parameter refinement, °	46 813; 1.02–24.11	29 299; 2.91–33.14
Range of h , k , and l	$-9 \rightarrow 9$, $-26 \rightarrow 26$, $-22 \rightarrow 22$	$-9 \rightarrow 9$, $-15 \rightarrow 15$, $-61 \rightarrow 61$
Total number of reflections measured; θ range, °	23 828; 0–24	33 637; 0–33
No. of unique reflections observed	4 954	3 318
Criterion for reflections observed	$I \geq 1.96\sigma(I)$	
Function minimised	$\Sigma w(F_o - F_c)^2$	
Weighting scheme	Chebyshev polynomial with 3 parameters	
Parameters refined	910	694
Value of R , wR , and S	0.049, 0.050, 1.0691	0.050, 0.053, 1.0445
Ratio of the maximum least-squares shift to e.s.d. in the last cycle	0.019	0.143
Maximum and minimum heights in final $\Delta\rho$ map, e Å ⁻³	-0.44, 1.25	-0.31, 0.61

Single crystals of C_A can be easily crystallized from anhydrous acetone, particularly if several grains of molecular sieve are added to absorb possible traces of water. The crucial problem is the stability of single crystals of C_A . Form C_A can absorb water from air depending on its humidity conditions yielding form C. This transformation ($C_A \rightarrow C$) is reversible and does not influence significantly the crystal parameters of form C_A . However, this transformation is accompanied by almost immediate transition of single crystal to a typical filamentous structure of polycrystalline materials unsuitable for X-ray crystal structure determination. This process is associated with swelling or shrinkage of the unit cell as confirmed by X-ray powder patterns.

TABLE II

Hydrogen bond system in C_A (the atoms are numbered as in Fig. 1 + $x00$ with x corresponding to the number of symmetry-independent molecule) and in A

Donor	Acceptor	Donor-acceptor distance, Å	Acceptor symmetry operation
Terguride form C_A			
N101	O320	2.776(4)	$-x, +y - 1/2, -z$
N201	O420	2.764(4)	$-x, +y - 1/2, -z + 1$
N301	O120	2.813(4)	$-x + 2, +y + 1/2, -z + 1$
N401	O220	2.859(4)	$-x + 1, +y + 1/2, -z$
Terguride form A			
O1	N106	2.788(4)	x, y, z
O2	N306	2.754(6)	x, y, z
N118	O1	3.114(5)	x, y, z
N318	O2	3.206(7)	x, y, z
O2	O320	2.893(7)	$x + 1, +y, +z$
O1	O120	2.694(4)	$x + 1, +y, +z$
N101	O320	2.835(5)	$x + 1, +y + 1, +z$
N201	O1	2.960(5)	$-x + 1, +y - 1/2, z + 1/2 + 1$
N301	O220	2.822(5)	$x + 1/2, -y + 1/2, -z + 2$

Form **A** contains three symmetry-independent terguride molecules and two molecules of water in the asymmetric part of the unit cell, hence the resulting stoichiometry is terguride–water (1 : 0.66). Terguride molecules are linked through water molecules forming a three-dimensional hydrogen bond network. The first and second water molecules are involved in 4 and 3 H-bonds, respectively. In addition, two other H-bonds are formed between the terguride N–H groups and O atoms (Table II).

Whereas the stoichiometry of **A** was known from the preliminary crystal structure determination¹⁵, attempts to examine the structure of **C_A** took roughly ten years and were successfully accomplished using a CCD area detector and the low-temperature measurement technique. The crystal structure determination finally proved that **C_A** is really the anhydrous form of terguride. The form **C_A** is remarkable by the presence of four symmetry-independent molecules in the structure; one of them has a disordered side chain (denoted as v1 and v2). All N–H groups and O atoms in molecules are involved in a hydrogen bond network holding the structure together (Table II).

Twelve symmetry-independent molecules can be accommodated in four already described terguride structures. Such an enormous number of independent molecules formed by a relatively small and simple compound prompted us to investigate in detail their individual conformations and perform some quantum-mechanical calculations. The basic structure of terguride is derived from the tetracyclic ring system of ergoline (Fig. 1). The indole moiety (formed by A and B rings) is nearly planar. C and D rings are puckered (C ring, ¹E conformation with C5 atom above the plane; D ring, ¹C₄ conformation with N6 atom above and C9 atom below the plane) for all 12 independent terguride molecules studied so far.

As expected, the overlap of all 12 terguride molecules (Fig. 2) shows that the torsion angles of the urea side chain are flexible, whereas the geometry of the ergoline skeleton is relatively fixed. The shapes of separated molecules are shown in Fig. 3. The side chain flexibility is documented by torsion angle values (Table III). Figure 4 shows the dependence of the model molecule total energy on the three flexible torsion angles. The C9–C8–N18–C19 torsion angle does never had in reality the value found for the theoretical global energetic minimum (about -70°) – such effect could be explained by the cyclohexane approximation used. The relatively high energy for C19–N21–C24–C25 torsion angle value -133.7° in **B** molecule 2 is probably caused by crystal packing forces.

A detailed classification of possible side chain conformations is given in Table IV. Four types of conformations are possible depending on the side chain orientation. Apparently, any reasonable explanation is missing for

the occurrence of individual stereoisomers in a particular structure. It is interesting to note that the conformation with both ethyl groups oriented towards the ergoline moiety does not occur in any molecule. Among other structures, the skewed conformations slightly predominate. Thus the ability of terguride to form various hydrogen bond networks, the flexibility of the diethylamino group and the nearly identical total energies of various molecules seem to be the factors contributing to the diversity of terguride structures.

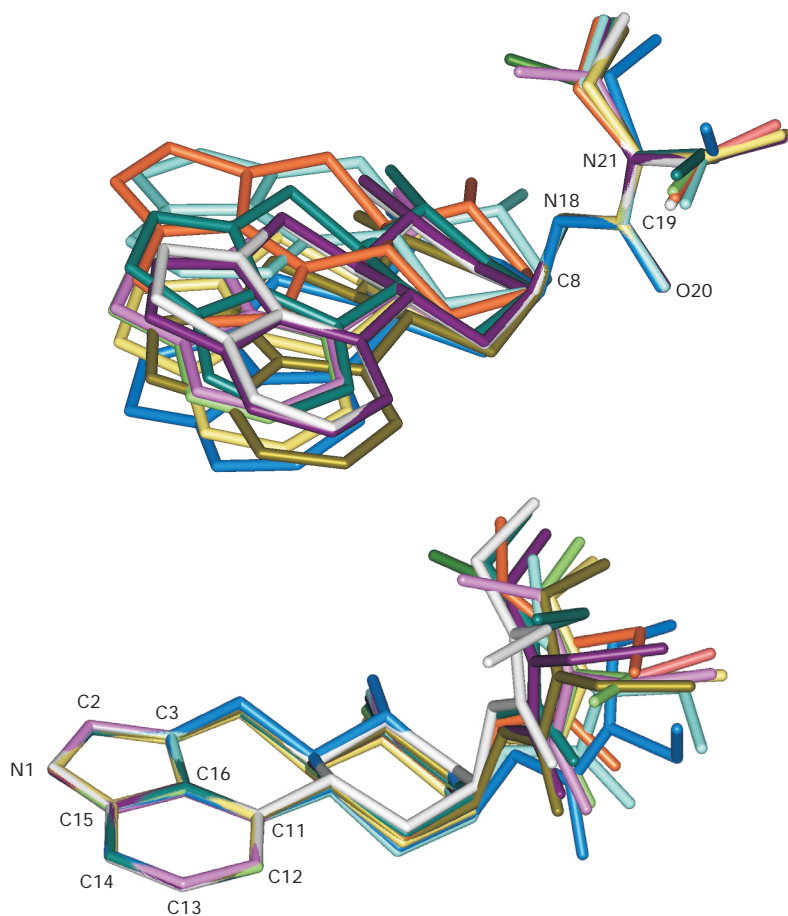


FIG. 2

The shape differences of 12 overlapped terguride molecules. The rigid parts of the molecules were fitted by least squares. Atoms C8, C19, N18, N21 and O20 were chosen as a rigid fragment (a); rings A and B (atoms N1, C2, C3, C11, C12, C13, C14, C15, C16) were chosen as rigid fragment (b)

TABLE III
Comparison of flexible side-chain torsion angles of different independent tergride molecules

Molecule No.	Molecule identification	Torsion angle, °		
		C9-C8-N18-C19	C19-N21-C22-C23	C19-N21-C24-C25
1	M , molecule v1 ^a	-155.0	-93.2	99.6
2	M , molecule v2 ^a	-155.0	-93.2	-85.0
3	B , molecule 1	-154.2	-91.4	91.4
4	B , molecule 2	-149.2	-77.9	-133.7
5	C_A , molecule 1	-134.0	-95.4	-82.1
6	C_A , molecule 2 v1 ^a	-174.2	64.7	82.3
7	C_A , molecule 2 v2 ^a	-174.2	-82.6	82.3
8	C_A , molecule 3	-118.4	-97.2	-77.5
9	C_A , molecule 4	-173.2	-94.1	-82.6
10	A , molecule 1	-166.7	-77.8	88.2
11	A , molecule 2	-162.2	-90.3	-98.9
12	A , molecule 3	-154.9	83.8	85.5

^a v1, v2 are disordered forms.

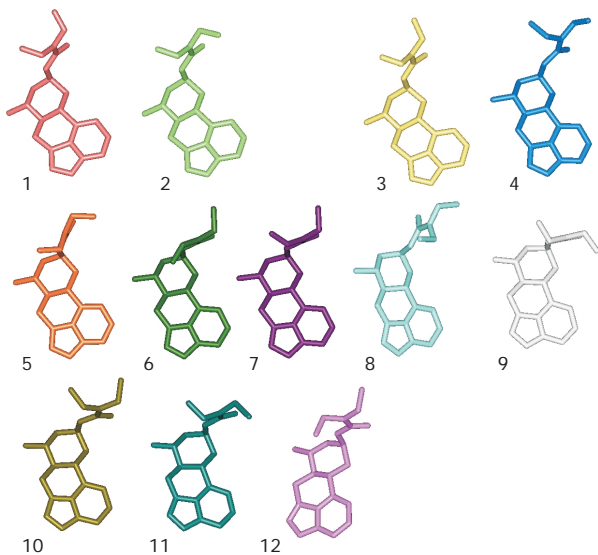


FIG. 3

Conformation of individual tergride molecules. The molecule numbering corresponds to that used in Table III. Colour coding corresponds to Fig. 2

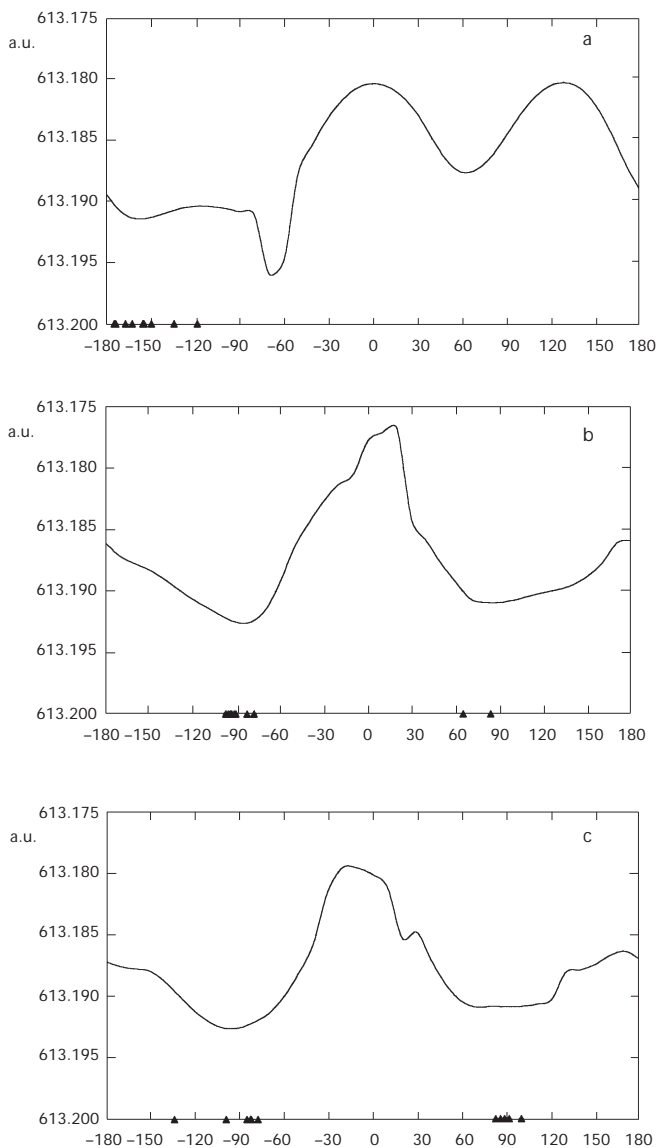


FIG. 4
Dependence of total energy (a.u.) on selected torsion angles (C9-C8-N18-C19 (a), C19-N21-C22-C23 (b) and C19-N21-C24-C25 (c)) as calculated *ab initio* in base 6-31G** for *N*-cyclohexyl-*N*-diethylurea. The experimental corresponding torsion angles found in 12 symmetry-independent molecules of terguride are shown as ▲ on the x axis

TABLE IV
Classification of ethyl side-chain conformations

Ethyl direction ^a	C19–N21–C22–C23 torsion angle range, °	C19–N21–C24–C25 torsion angle range, °	Molecule No. ^b	Population of the particular form ^c				Total occurrence
				M	B	C _A	A	
A	–	–	none	0	0	0	0	0
B	(–93.2, –77.8)	(88.2, 99.6)	1, 3, 7, 10	0.5	1	0.5	1	3
C	(–97.2, –77.9)	(–133.7, –77.5)	2, 4, 5, 8, 9, 11	0.5	1	2	1	5.5
D	(64.7, 83.8)	(82.3, 85.5)	6, 12	0	0	0.5	1	1.5

^a A, both ethyl groups are directed towards the ergoline moiety; B, both ethyl groups are directed outwards the ergoline moiety; C, skewed orientation 1; D, skewed orientation 2.
^b See Table III. ^c Non-integer population of a given conformation reflects the fractional occupation.

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