MOLECULAR STRUCTURE OF *cis*- AND *trans*-TERGURIDES*

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Four isomers of terguride, a semisynthetic ergot alkaloid derivative, have been prepared by catalytic hydrogenation of (5*R*,8*S*)- and (5*S*,8*S*)-lisuride [1,1-diethyl-3-(6-methyl-8-ergolenyl)urea]. Relative stereochemistry of the isomers is based on NMR and CD spectra. Absolute configuration of all the series has been confirmed by the X-ray crystal structure determination of (5*R*,8*S*,10*S*)-terguride 2-bromobenzoate [1,1-diethyl-3-(6-methyl-8-isoergolenyl)urea, *cis*-dihydrolisuride].

Key words: Tergurides; Ergot alkaloids; Crystal structure determination; NMR spectroscopy; Absolute configuration.

Terguride, a semisynthetic ergot alkaloid derivative, possesses three chiral centers at C5, C8 and C10 atoms. Among eight possible isomers, the only (5*R*,8*S*,10*R*)-one[2] has been described as an active substance "terguride" in the manufacture of drug for the treatment of Parkinsonism and for the inhibition of prolactin secretion (Mysalfon®). Until now, all terguride structures[2–5] were reported as the (5*R*,8*S*,10*R*)-isomers, assuming that the absolute configuration of the parent skeleton present in the starting natural compounds was not changed during the chemical modifications[6]. In order to study the conformational flexibility/rigidity of ergoline skeleton, four terguride isomers have been prepared. The (5*R*,8*S*,10*R*)-configuration is simply referred as *trans*-D-terguride

* This paper is the 20th continuation-in-part of our systematic study of the molecular structure and polymorphism of ergot alkaloids[1].

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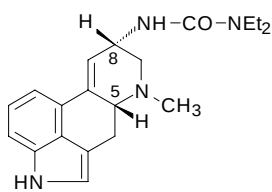
and the (5*R*,8*S*,10*S*)- as *cis*-D-terguride in this work, according to the relative positions of H5 and H10 hydrogen atoms (Fig. 1).

EXPERIMENTAL

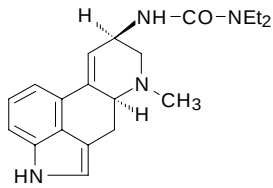
General Synthetic Route to Tergurides

Either (5*R*,8*S*)- or (5*S*,8*R*)-lisuride (20 g, Galena Co., Czech Republic) was dissolved in methanol (400 ml) and hydrogenated over 5% Pd/C catalyst (8 g) in a 600 ml stainless-steel autoclave (65 °C, 6 MPa). The reaction mixture was filtered and evaporated to dryness. Hydrogenation of (5*R*,8*S*)-lisuride afforded predominantly (5*R*,8*S*,10*R*)-terguride with (5*R*,8*S*,10*S*)-terguride as the minor product. Similarly, (5*S*,8*R*,10*R*)-terguride and (5*S*,8*R*,10*S*)-terguride were obtained in the same way from (5*S*,8*R*)-lisuride. The crude tergurides were purified by column chromatography on a silica gel (350 g, Merck Kieselgel 60, 0.063–0.100 mm) in dichloromethane–methanol, with step gradient [1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, and 7.0% of methanol (v/v), fractions 1 200 ml]. Fractions containing pure isomers were pooled, evaporated and crystallized from methanol or acetone.

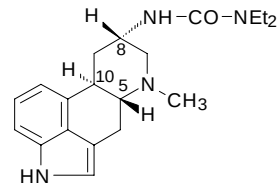
Compounds were characterized by their ^{1}H and ^{13}C NMR spectra (Varian VXR-400, $CDCl_3$), 2D NMR experiments (COSY, delay-COSY, NOESY, ROESY, HETCOR, HOM2 J) (Tables I and II), and CD spectra (Jobin–Yvon 5, 190–350 nm, methanol) (Table III, Fig. 2). Following compounds were used as models for CD spectra comparison: ergometrine, ergometrine maleate, dihydroergotamine mesylate, α-bromokryptine, α-bromokryptinine, α-bromokryptine mesylate, α-ergokryptine, α-ergokryptinine, ergotamine, ergotamine tartrate, pergolide, pergolide mesylate, and metergoline (all from Galena Co.,



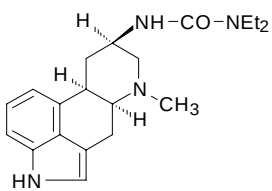
(5*R*,8*S*)-lisuride



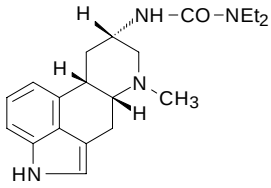
(5*S*,8*R*)-lisuride



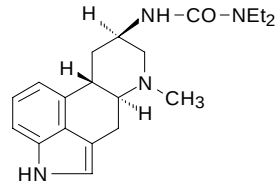
(5*R*,8*S*,10*R*)-terguride



(5*S*,8*R*,10*R*)-terguride



(5*R*,8*S*,10*S*)-terguride



(5*S*,8*R*,10*S*)-terguride

FIG. 1
Stereochemistry of tergurides

Czech Republic). EI-MS spectra (Finnigan MAT 90, ionizing energy 70 eV, emission current 1 mA, accelerating voltage 5 kV, direct inlet).

Preparation of (5*R*,8*S*,10*S*)-Terguride 2-Bromobenzoate

(5*R*,8*S*,10*S*)-Terguride (100 mg) was dissolved in ethanol (3 ml) and mixed with 2-bromobenzoic acid (59 mg in 3 ml ethanol, molar ratio 1 : 1). Subsequently, water (0.5 ml) was added and the solution was allowed slowly to evaporate.

Crystal Structure Determination

(5*R*,8*S*,10*S*)-Terguride 2-bromobenzoate, $C_{20}H_{28}N_4O \cdot C_7H_6BrO_2$ (M_r = 541.49), space group $P2_1$, a = 8.650(2), b = 14.018(3), c = 11.687(3) Å, = 111.15(2)°, V = 1 321.7(6) Å^3, Z = 2, D_{calc} = 1.361 g cm^{-3}, μ(MoKα) = 1.57 mm^{-1}, F(000) = 564.

TABLE I
^{13}C and ^{1}H NMR spectra (400 and 100 MHz, $CDCl_3$, 25 °C) of (5*R**,8*S**,10*S**)-tergurides (data taken from spectra of (5*R*,8*S*,10*S*)-terguride)

Atom	δ(C), ppm	Multiplicity	δ(H), ppm	*n* H	Multiplicity	*J*, Hz
2	118.27	d	6.886	1	ddd	2.0, 1.0, 1.0
3	110.74	s	–	–		
4	14.90	t	2.937	1	ddd	15.5, 4.1, 1.0
			3.060	1	m	
5	59.92	d	3.354	1	ddd	10.6, 4.1, 4.0
7	54.79	t	2.480	2	m	
8	47.01	d	4.211	1	m	
9	35.65	t	2.484	1	m	
			4.197	1	m	
10	38.25	d	2.116	1	m	
11	134.34	s	–	–		
12	115.18	d	6.849	1	dd	6.7, 1.0
13	122.86	d	7.100	1	dd	8.2, 6.7
14	108.41	d	7.147	1	dd	8.2, 1.0
15	132.92	s	–	–		
16	126.21	s	–	–		
N–CH_3	42.68	q	2.491	3	s	
N–C=O	156.54	s	–	–		
CH_3	13.63	q	1.048	6	t	6.2
CH_2	42.68	t	3.123	4	q	6.2
N–H	–	–	8.678	1	d	2.0

The structure was solved by the heavy-atom technique and refined by full-matrix least-squares based on *F* values. Anisotropic temperature factors were applied to bromine and oxygen atoms whereas the remaining atoms could be refined isotropically only. As the terminal methyl group C25 was disordered, two carbon positions, designated as C251 and C252 were refined. Sum of their occupancy factors [0.56(2) and 0.44(2)] was held to be 1, and for both U_{iso} = 0.1 was taken. Since the geometry of some molecular parts became unrealistic during refinement, the restraints procedure was applied to set of the distances: N21–C22, N21–C24, C22–C23, C24–C251 and C24–C252. The values 1.455(7) Å for N–C and 1.48(1) Å for the C–C bonds were taken for calculating of weights. Hydrogen atoms were included in ideal positions, with fixed U_{iso} values equal to U_{iso} or U_{eq} ones of the bond partners. The position of 2-bromobenzoate OH-hydrogen was not localized. Flack's enantiopole parameter[7] was calculated from the equation $|F(h,x)|^2 = (1 - x)\,|F(h)|^2 + x\,|F(-h)|^2$. Parameters used for the crystal structure analysis are listed in Table IV.

TABLE II
^{13}C and ^{1}H NMR spectra (400 and 100 MHz, $CDCl_3$, 25 °C) of (5*R**,8*S**,10*R**)-tergurides (data taken from spectra of (5*R*,8*S*,10*R*)-terguride)

Atom	δ(C), ppm	Multiplicity	δ(H), ppm	*n* H	Multiplicity	*J*, Hz
2	117.86	d	6.895	1	dd	1.8, 1.8
3	111.27	s	–			
4	26.95	t	2.698	1	ddd	14.6, 11.1, 1.8
			3.414	1	dd	14.6, 4.3
5	67.57	d	2.211	1	ddd	11.1, 9.7, 4.3
7	61.80	t	2.484	1	dd	11.7, 2.6
			2.890	1	ddd	11.7, 2.5, 2.5
8	45.03	d	4.315	1	m	
9	32.52	t	1.634	1	ddd	13.4, 12.8, 3.3
			2.813	1	dddd	13.4, 4.2, 2.6, 2.5
10	36.53	d	3.088	1	m	
11	133.04	s	–	–		
12	112.90	d	6.857	1	ddd	6.9, 1.3, 1.1
13	122.85	d	7.135	1	dd	8.2, 6.9
14	108.59	d	7.182	1	ddd	8.2, 1.1, 0.7
15	133.30	s	–	–		
16	126.21	s	–	–		
$N–CH_3$	43.30	q	2.417	3	s	
N–C=O	156.67	s	–	–		
CH_3	13.80	q	1.164	6	t	7.1
CH_2	41.09	t	3.266	2	dq	14.8, 7.1
			3.362	2	dq	14.8, 7.1
N–H	–	–	8.726	1	s	
N–H	–	–	5.645	1	d	8.2

RESULTS AND DISCUSSION

Relative Stereochemistry

All four compounds provided molecular ion-radical (*m/z* 340, $C_{20}H_{28}N_{40}$) and following ergine ions : *m/z* 154 ($C_{11}H_8N$, ABC-ring system), *m/z* 167 ($C_{12}H_9N$, increment of CH), and *m/z* 225 (whole ABCD-ring system). The urea part was characterized by ion *m/z* 100 [$^+CO–N(C_2H_5)_2$]. However, no differences in the mass spectra allowing the discrimination of individual stereoisomers or *cis*- and *trans*-tergurides were observed.

NMR spectra provide the relative stereochemistry at all chiral centers with (5*R**,8*S**,10*S**)-tergurides, the magnitude of *J*(5,10) = 4.0 Hz combined with a strong cross-peak between these protons in the NOESY spectrum indicate a *cis*-junction. The

TABLE III
CD spectra of tergurides and lisurides

trans-Tergurides			*cis*-Tergurides			Lisurides		
λ, nm	$\Delta\varepsilon_{max}$		λ, nm	$\Delta\varepsilon_{max}$		λ, nm	$\Delta\varepsilon_{max}$	
	5*R*,8*S*,10*R*	5*S*,8*R*,10*S*		5*R*,8*S*,10*S*	5*S*,8*R*,10*R*		5*R*,8*S*	5*S*,8*R*
296	0.9	–0.9				322	5.5	–5.5
286 sh	1.5	–1.5				295 sh	3.0	–3.0
270	2.7	–2.7	275	4.9	–4.9	264	3.4	–3.4
240	1.8	–1.8				250	–10	10

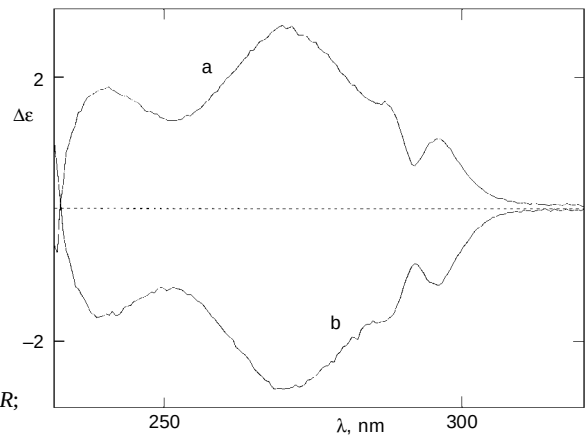


FIG. 2
CD spectra of *trans*-tergurides: a 5*R*,8*S*,10*R*; b 5*S*,8*R*,10*S*

linewidth of H-8 (21 Hz) implies its equatorial position. Several signals in both ^{1}H and ^{13}C NMR spectra are seriously broadened[13,14], indicating a possible exchange process involving conformational changes in C and D rings. In contrast, large vicinal constants J(5,10) = 9.7, J(4a,5) = 11.1, J(9a,10) = 12.8 Hz require nearly antiperiplanar arrangement of the protons in (5*R**,8*S**,10*R**)-terguride (*trans*-terguride). That dictates also a chair conformation of ring D. Small couplings of both H-7 protons to H-8 and also small J(8,9a) and J(8,9e) indicate that H-8 is equatorial (the urea moiety is, therefore, axial). Nuclear Overhauser effect (NOE) observed between H-9 and H-12 suggests a short distance between these protons. A measurable effect between H-5 and H-7a reflects their 1,3-diaxial orientation. Finally, the NOE between *N*-methyl and H-4e and H-7e is consistent with an equatorial orientation of the *N*-methyl group. However, enantiometric terguride pair gave identical NMR spectra and thus are undistinquishable by this technique.

Although the CD spectra are considered to be rather obsolete and scarcely used nowadays, nevertheless, it is the only technique suitable for a quick differentiation of

TABLE IV
Data collection and refinement parameters for (5*R*,8*S*,10*S*)-terguride 2-bromobenzoate

Crystal dimensions	0.3 × 0.3 × 0.2 mm
Diffractometer and radiation used	Enraf–Nonius CAD4, MoKα, λ = 0.71073 Å
Scan technique	ω/2θ
Temperature	293 K
Number and θ range of reflections for lattice parameter refinement	20; 18–19°
Range of h, k and l	–9→9, –15→15, –12→12
Standard reflections monitored in interval; intensity fluctuation	120 min; –2%
Total number of reflections (Friedel pairs not merged)	2 809
R_{int}	0.037
Criterion for observed reflections	$I \geq 1.96\sigma(I)$
Function minimized	$\sum w (\lvert F_o \rvert - \lvert F_c \rvert)^2$
Weighting scheme	$w = [\sigma^2(F_o)]^{-1}$
Parameters refined	166
Value of R, wR and S	0.105, 0.114 and 3.88
Ratio of max. least-squares shift to e.s.d. in the last cycle	0.08
Max. and min. heights in final Δρ map	1.77, –2.07 e Å^{-3}
Source of atomic scattering factors	International Tables for X-Ray Crystallography[8]
Programs used	CRYSTALS (ref.[9]), SDP (ref.[10]), SHELXS86 (ref.[11]), PARST (ref.[12])

enantiomers. In the ergot alkaloid series the first band in spectra corresponds to π–π^* ^1L_b and ^1L_a transitions in the UV region 270–300 nm (ref.[14]). For 9-ergolenes related to D-lysergic acid, a pronounced positive Cotton effect was typical [*e.g.*, ergometrine 322 (5.3), α-ergokryptine 318 (4.9), α-bromokryptine 315 (7.0), ergotamine 318 (5.0), (nm, $\Delta\varepsilon$)]. Epimerization at the C8 carbon (compounds belonging to D-isolysergic acid series) did not change the sign of the Cotton effect [*e.g.*, lisuride, Table III, α-bromokryptinine 322 (15), see also ref.[16]]. Ergot alkaloid salts exhibited in methanol spectra identical with those of free bases. Corresponding transitions in ergoline derivatives afforded only a weak Cotton effects. For the series of compounds used in this study derivatives corresponding to D-dihydrolysergic acid exhibited Cottot effect ($\Delta\varepsilon$) approximately –1 at 290 nm (dihydroergotamine, metergoline, pergolide), whereas in (5*R*,8*S*,10*R*)-terguride (corresponds to D-dihydroisolysergic acid) positive Cotton effect was found (Table III). Although the exact structural parameters are available from the crystal structure determinations of some similar compounds (terguride[2–5], metergoline[17], pergolide[18], ergotamine[19,20], dihydroergotamine[21], bromokryptine[22,23], lisuride[24], and ergometrine[25,26]), we did not found any simple parameter (like torsion angles about the C/D ring junction) which would enable the prediction of their CD spectra. Nevertheless, if the absolute configuration of the only one member of certain series of isomers is known, CD spectra then make possible fast identification of the remaining members.

Absolute Configuration of (5R,8S,10S)-Terguride

In order to obtain the absolute configuration, the crystal structure determination of (5*R*,8*S*,10*S*)-terguride with the heavy-atom-containing 2-bromobenzoate anion was undertaken. The final positional and thermal parameters of the non-H atoms of (5*R*,8*S*,10*S*)-terguride 2-bromobenzoate are deposited in Cambridge Crystallographic Database. Figure 3 shows *cis*-terguride molecule with the atom numbering. Higher

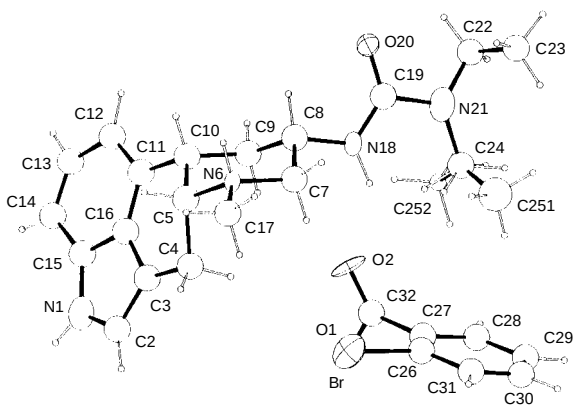


FIG. 3
Ortep drawing of (5*R*,8*S*,10*S*)-terguride 2-bromobenzoate

values of reliability factors are probably caused by a poor quality of the single crystal available (repeated attempts of preparation of better crystals were not successful). The correct assignment of the (5*R*,8*S*,10*S*)-stereoisomer was confirmed by Flack's enantiopole parameter[6] value of –0.02(3). The indole moiety, formed by A and B rings of ergoline skeleton, is almost planar, as usual. The χ^2 test value 1.06 for phenyl A ring and 1.76 for pyrrole B ring, respectively, was obtained. Dihedral angle of A and B ring planes is 1.4(6)°. Some conformation puckering was found for rings C (C5,C4,C3,C16,C11,C10) and D (N6,C5,C10,C9,C8,C7) (Table V). The C ring is enveloped (^1E), while the ring D has a chair conformation $^4C_1$. The system of hydrogen bond is formed by following contacts: N18–H…O2: 2.86(2) Å, 143(2)°, N1–H…O20(x, y, z – 1): 2.86(3) Å, 164(2)° and N6–H…O1(x – 1, y, z): 2.67(2) Å, 156(1)°.

Until now, four (5*R*,8*S*,10*R*)-terguride crystal structures have been described in literature (terguride hydrogen maleate monohydrate[2], terguride two-third hydrate[3], terguride monohydrate[4], and terguride methanol solvate[5]) including seven symmetrically-independent (5*R*,8*S*,10*R*)-terguride molecules altogether. This enables us to compare ring-puckering parameters[27] of individual terguride isomers (Table V). Significant difference was found for the ring D of (5*R*,8*S*,10*S*)-terguride (*cis*-terguride), where the inversion chair conformation ($^4C_1$) is caused by a different configuration at C10. On the other hand, this difference does not influence the conformation of *cis*-terguride ring C. The effect of protonation at N6 on the geometry of terguride molecules was not proved.

TABLE V

Comparison of ring-puckering parameters[26] of C and D rings in tergurides. Estimated standard deviations are approximately 0.005 Å and 0.5°, for $\phi < 10°$ are one order higher

Compound[a]	Ring C				Ring D			
	Q, Å	ϕ, °	θ, °	Conformation	Q, Å	ϕ, °	θ, °	Conformation
A, 1	0.456	–11.4	49.0	^1E	0.597	51.9	7.7	$^1C_4$
A, 2	0.429	–6.6	54.8	^1E	0.600	4.9	3.2	$^1C_4$
A, 3	0.437	–11.0	52.6	^1E	0.578	–93.2	2.3	$^1C_4$
B, 1	0.466	–7.1	51.0	^1E	0.580	17.6	1.3	$^1C_4$
B, 2	0.408	3.7	49.5	^1E	0.590	–59.4	10.0	$^1C_4$
M	0.475	9.7	52.3	^1E	0.566	–123.7	1.6	$^1C_4$
G	0.458	–9.9	51.4	^1E	0.588	–39.4	1.9	$^1C_4$
cis-T	0.426	–5.6	51.9	^1E	0.548	117.7	174.9	$^4C_1$

[a] A terguride two-thirds hydrate; B terguride monohydrate; M terguride hydrogen maleate monohydrate; G terguride methanol solvate; *cis*-T (5*R*,8*S*,10*S*)-terguride 2-bromobenzoate; numbers denote the independent molecules in the crystal structure.

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