ASSIGNMENT OF NITROGEN STEREOCHEMISTRY OF AGROCLAVINE AND ELYMOCLAVINE 6-*N*-OXIDES*

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Both possible 6-*N*-oxides of agroclavine (*I*) and elymoclavine (*II*) were prepared by hydrogen peroxide oxidation. Their ^{1}H and ^{13}C NMR spectra were assigned and the conformation of the D ring (half-chair) was determined. Absolute configuration at 6-N was established by NMR and molecular modelling.

Clavines (class of ergot alkaloid) are important precursors for a large group of semisynthetic ergot-based drugs. 6-*N*-Oxides of ergot alkaloids are better water soluble than the parent compounds and therefore they have better pharmacokinetical properties. They can be converted by the liver enzymatic systems to the parent alkaloids. Ergot alkaloid binding ability to the neurotransmitter receptors largely depends on the D ring conformation[1].

6-*N*-Oxides of ergot alkaloids can be prepared using different oxidants[2] (H_2O_2, 3-chloroperbenzoic acid). Until now they have not been found in nature although they can be often found as by-products in some reactions involving ergot alkaloids[3]. We have observed formation of the *N*-oxides of, e.g., agroclavine during *N*-1-ribosylation catalyzed by tin(IV) chloride in presence of air. It is expected that the *N*-oxides may be produced from ergot alkaloids also by peroxidases. To our knowledge no *N*-oxide of 8,9-ergolene was prepared yet in its pure form suitable for spectral characterization and conformational studies.

EXPERIMENTAL

Preparation: Agroclavine-6-*N*-oxides (*IIIa, IIIb*) and elymoclavine-6-*N*-oxides (*IVa*, *IVb*) were prepared by treatment of *I* (238 mg, 1 mmol) or *II* (254 mg, 1 mmol) with H_2O_2 (1 ml, 30%) in 2-propanol (25 ml) at 60 °C/2 h. Excess of H_2O_2 was destroyed by fine MnO_2 (catalytic amount). The mixture was evaporated in vacuo (30 °C). Reaction products were separated by flash chromatography (silica

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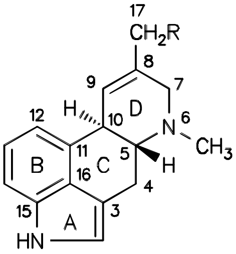
gel; CH_2Cl_2–CH_3OH 9 : 1) yielding 101 mg (37%) of a mixture *IIIa* and *IIIb* and 97 mg (30%) of a mixture *IVa* and *IVb*. The final purification was accomplished by preparative HPLC (column: 250 × 8 mm, Separon SGX C18, 7 mm, Tessek, Czech Republic; mobile phase CH_3OH–H_2O–NH_4OH 40 : 60 : 0.036 for *IIIa*, *IIIb*, and 20 : 80 : 0.036 for *IVa*, *IVb*, respectively, flow rate 3 ml/min, sample amount per run 10 mg). Retention times were 21.67 min for *IIIa*, 29.57 min for *IIIb*, 27.90 min for *IVa*, and 35.14 min for *IVb*, respectively.

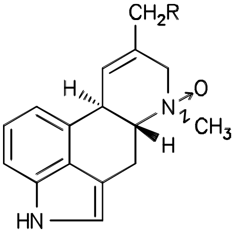
Compounds *IIIa*, *IIIb*, *IVa*, *IVb* were obtained as amorphous solids. Their IR spectra were not informative. Mass spectra contained very weak molecular ions and after ejection of oxygen[4] closely resembled those of parent alkaloids.

NMR spectroscopy: The NMR spectra (399.95 and 100.58 MHz) were measured on a Varian VXR-400 spectrometer in CD_3OD at 25 °C. H, C, APT, COSY, delayed COSY, HETCOR and NOESY experiments were performed using manufacturer's software. Residual solvent signals (δ_H 3.33, δ_C 49.3) were used as internal references.



I, R = H

II, R = OH



IIIa, R = H (*6S*)

IIIb, R = H (*6R*)

IVa, R = OH (*6S*)

IVb, R = OH (*6R*)

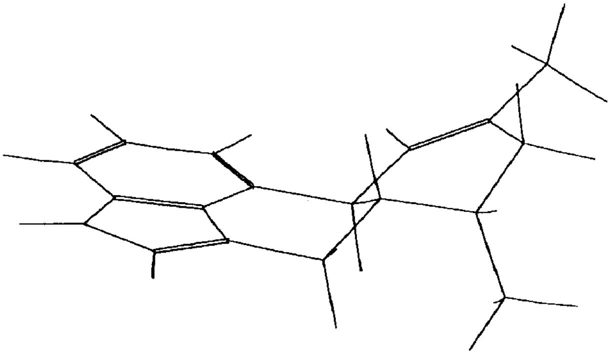


FIG. 1
Molecular modelling of structure *IIIb* based on the NMR data; PM3 MO calculations were performed by MOPAC6 program

Molecular modelling: PM3 MO calculations were performed by MOPAC6 program using standard parametrization. The geometries were fully optimized with respect of all degrees of freedom. Calculated structures were used for NOE predictions (Fig. 1).

RESULTS AND DISCUSSION

In both cases two reaction products from each (*I*) and (*II*) were formed in 3 : 2 (a : b) ratio (determined by HPLC from the reaction mixture). Comparison of the NMR data (Table I and Table II) of the products with that of parent alkaloids indicate the formation of 6-*N*-oxides (signals of N-Me, both H-7's, C-7, H-5 are shifted downfield). Formation of 6-*N*-oxides creates a new chiral centre on 6-N. Two *N*-methyl orientations are possible as well as two conformations of the D ring (half-chair and boat). Although molecular modelling studies have been performed to establish conformation of the D ring and also the optimal conformation for the interaction with the catecholamine receptors[5] they were limited only to 9,10-ergolenes and they were also impaired by flexibility of prochiral 6-N atom. One of the possibility of studying the real compounds with the

TABLE I
^{1}H NMR data of compounds *I*–*IV*

Proton	*I*	*IIIa*	*IIIb*	*II*	*IVa*	*IVb*	Typical multiplicity and *J* (Hz) for *Ia*
2	6.906	6.953	6.958	6.947	6.984	7.020	d, 1.5
4ax	2.717	3.244	2.834	2.796	3.278	2.906	dd, 4.2, 14.5
4eq	3.327	3.395	3.887	3.382	3.435	3.914	ddd, 1.5, 12.0, 14.5
5	2.468	3.370	3.423	2.632	3.510	3.517	ddd, 4.2, 12.0, 9.5
7a	2.931	3.667	~3.90	3.125	3.854	4.025	qddd, 0.8, 0.8, 2.5, 15.7
7b	3.238	4.070	~3.90	3.486	4.223	4.110	qddd, 1.3, 2.3, 3.6, 15.7
9	6.215	6.374	6.260	6.511	6.631	6.537	qddd, 0.5, 0.6, 2.5, 3.6
10	3.677	4.246	3.685	3.775	4.297	3.840	qdddd, 0.6, 0.8, 1.2, 2.0, 9.5
12	6.906	7.022	6.934	6.953	7.037	6.964	ddd, 7.3, 1.2, 0.8
13	7.064	7.120	7.097	7.080	7.118	7.105	dd, 7.3, 8.3
14	7.139	7.194	7.193	7.159	7.203	7.206	ddd, 8.3, 1.2, 0.8
17[a]	1.798	1.836	1.850	4.102	3.369	4.115	mt
17[a]				4.146	4.150	4.175	mt
N-Me	2.468	3.277	3.096	2.571	3.360	3.143	s

[a] In compounds *IVa*, *IVb* split of the methylene signal observed.

free electron pair blocked is preparation of 6-*N*-oxides making thus the conformation of the D ring rigid. This allowed us to establish the conformation of the D ring.

The *trans*-junction of C/D rings following from the *J*(5,10) values (about 10 Hz) restricts the possible conformations of the D ring to one half-chair with C-5 above the plane defined by C-10, C-7 and the double bond and to one boat form. NOE exhibited by both H-7 protons to C-8 methyl (H-17, compounds *IIIa*, *IIIb*) as well as their long-range couplings to H-9 are consistent with the half-chair conformation only. In the boat conformation, only one H-7 should exhibit NOE to the olefinic methyl and also only one H-7 is capable of coupling to H-9. Downfield shifts at H-4_{ax} and H-10 found in *IIIa* and *IVa* with respect to those in *IIIb* and *IVb* (Table I) indicate an axial N–O bond in the former compounds. On the other hand, H-4_{eq} resonates in the lower field in *IIIb* and *IVb* then in *IIIa* and *IVa*. Owing to the different orientation towards the N–O bond, the chemical shift difference between the H-4 protons is larger in *IIIb* and *IVb* (equatorial N–O); for the H-7 protons, this difference is larger with *IIIa* and *IVa* (axial N–O). NOE between the *N*-methyl and H-4_{eq} and both H-7 protons observed in *IIIa* and *IVa* agree

TABLE II
^{13}C NMR data of compounds *I*–*IV*

Carbon	*I*	*IIIa*	*IIIb*	*II*	*IVa*	*IVb*
2	119.72	120.69	120.78	119.88	120.78	120.89
3	112.06	110.20	110.55	111.81	110.10	110.49
4	27.71	22.88	23.05	27.66	23.03	23.17
5	65.72	74.57	77.43	65.94	74.88	77.91
7	61.57	73.35	75.38	57.95	70.85	72.67
8	135.58	128.23	130.79	132.19	132.46	135.03
9	121.24	121.69	121.23	122.12	122.46	121.96
10	42.10	38.65	41.83	41.64	38.72	41.84
11	133.00	131.13	130.67	135.61	130.66	130.29
12	113.31	114.19	114.15	113.38	114.19	114.14
13	123.66	124.17	124.11	123.68	124.17	124.11
14	110.07	110.65	111.08	110.25	110.76	111.19
15	132.81	135.62	135.89	136.91	135.65	135.92
16	127.82	127.14	127.48	127.79	127.15	127.50
17	21.18	21.01	20.75	65.44	65.42	65.55
N-Me	41.25	58.50	49.83	41.14	58.37	49.78

with the equatorial orientation of this group. With *IIIb* and *IVb* (axial *N*-methyl), the NOE was observed between this group and H-4_{ax}, one H-7 and H-10. Thus, the absolute configuration at 6-N is *S* for *IIIa* and *IVa* and *R* for *IIIb* and *IVb*.

The comparison of corresponding carbon chemical shifts in the pairs of isomers *IIIa–IIIb* and *IVa–IVb* (Table II) reveals further diagnostic rules: C-5, C-7, C-8, and C-10 resonate 2–3 ppm upfield in compounds having an axial N–O bond; the equatorial *N*-methyl carbon resonates around 58 ppm whereas the axial one resonated 8 ppm upfield. The later mentioned signal provides the most reliable criterion for the determination of the *N*-methyl orientation in the compounds studied.

As the major reaction products *IIIa* and *IVa* were identified, (6-N is (*S*), 6-*N*-methyl is equatorial) in both cases. The difference between calculated heats of formation for *IIIa* and *IIIb* is not important, there is no thermodynamic reason for such discrimination.

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