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J. Nat. Prod., **1991**, 54 (2), 390-395• DOI:
10.1021/np50074a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 2, pp. 390-395, Mar-Apr 1991

DEGRADATION PRODUCTS OF ERGOT ALKALOIDS

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ABSTRACT.—Two new metabolites of *Claviceps purpurea* strain 129/35, (4a*S*),(10b*S*)-7-amino-3,4,4a,5,6,10b-hexahydro-2,4-dimethyl-6-oxobenzo[*f*]quinoline [**1**] and (4a*S*),(10b*S*)-7-amino-3,4,4a,5,6,10b-hexahydro-2-hydroxymethyl-4-methyl-6-oxobenzo[*f*]quinoline [**2**], were isolated from the culture broth. It is supposed that these metabolites are products of ergot alkaloid turnover.

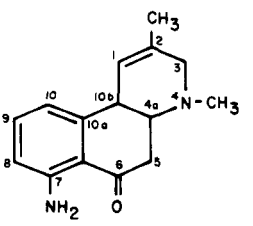
Ergot alkaloid degradation was described by Robbers (1). A high concentration of inorganic phosphate in saprophytic cultures of *Claviceps* sp. inhibits alkaloid biosynthesis. It was also shown that this effect (inhibition of alkaloid production) could be initiated at any time during the course of the fermentation. Another effect of inorganic phosphate is an increased activity of alkaloid-degradative enzymes. On the basis of these results Robbers supposed that alkaloid concentration is in a dynamic state, that there is alkaloid turnover, and that the alkaloids cannot be considered simply as end products of metabolism.

The analysis of kinetic parameters of a submerged fermentation of clavine alkaloids by *Claviceps purpurea* 129/35 showed that overall efficiency of the fermentation process was decreased due to a partial degradation of agroclavine and elymoclavine (2). As the first step in search for the alkaloid-degrading system, we aimed at finding the products of this degradation and determining their structure.

RESULTS AND DISCUSSION

Inspection of thin-layer chromatograms revealed the presence of orange-colored spots (van Urk reagent) accompanying in different concentrations both main products (agroclavine and elymoclavine). Production of these compounds was higher with high-producing strains and increased with fermentation time and upon phosphate addition. They were shown also to occur in *Claviceps fusiformis* and in *Claviceps* sp. SD 58. These facts indicated that these substances could be the degradation products.

The mass spectrum of compound **1** exhibits a molecular ion at *m/z* 242 ($C_{15}H_{18}N_2O$). Two hydrogen atoms are replaced by deuterium upon CD_3OD treatment in the ion source of the mass spectrometer. Fifteen carbon atoms observed in the ^{13}C-nmr spectrum consist of two methyls, two methylenes, six methines (two aliphatic and four aromatic/olefinic), and five quaternary carbon atoms (all sp^2 hybridized). That leaves two hydrogen atoms attached to heteroatom(s). COSY, delayed-COSY, and



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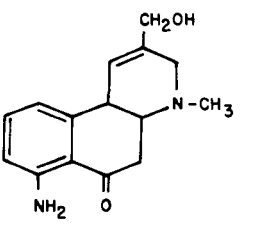

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homodecoupling experiments revealed the system $-CH_2CHCHCH=C(CH_3)CH_2-$, analogous to the B- and C-ring protons of agroclavine. The value of $J_{4a,10b} = 10$ Hz indicates a trans junction of these rings. The other signals present in the ^1H-nmr spectrum (Tables 1 and 2), an *N*-methyl group and an ABC system of three vicinal aromatic protons, complete the picture. Carbon atoms belonging to the identified protons were assigned by a (^1H, ^{13}C)-COSY experiment. The ^{13}C-nmr spectrum of our compound (Table 3) differs from that of agroclavine by the absence of the C-2 signal, by the presence of a carbonyl resonance, and by a downfield shift of the C-5 (corresponding to C-4 of agroclavine) and one sp^2-hybridized quaternary carbon. According to its ^{13}C chemical shift, the carbonyl group is conjugated with a double bond and participates in hydrogen bonding. At this stage, the elemental balance can be completed. The only oxygen atom in the molecule is part of a carbonyl; one nitrogen atom is *N*-methylated, so that the remaining nitrogen atom belongs to an NH_2 group. This group has to be close to the carbonyl to be capable of hydrogen bond formation. The carbon atom carrying it resonates undoubtedly at 151.36 ppm. Coupling between both C-5 protons and the carbonyl resonance, observed in a (^1H, ^{13}C)-COSY experiment optimized for the detection of geminal and vicinal coupling constants (3), requires a close proximity of C-5 and the carbonyl. Their vicinity readily explains both the downfield shift of C-5 with respect to agroclavine and the increase in the magnitude of the geminal coupling $J_{5d,5u}$ from 14.4 to 16.8 Hz (4). The final structure **1** can be derived by disrupting the indole ring of agroclavine, removing C-2 so that there is a C=O at C-3 and NH_2 at C-15.

Compound **2** has a molecular formula $C_{15}H_{18}N_2O_2$ (hrms *m/z* 258) and incorporates three deuterium atoms. Mass spectra of both compounds contain a common fragment *m/z* 159. The ^1H-nmr spectrum (Tables 1 and 2) contains an *N*-methyl group, three vicinal aromatic protons, and a system $-CH_2CHCHCH=C(CH_2OH)CH_2-$, pointing to elymoclavine. Again, the B/C ring junction is trans ($J_{4a,10b} = 10.0$ Hz).

TABLE 1. ^1H-nmr Chemical Shifts.

Proton	Compound		Proton	Compound	
	agroclavine[a]	elymoclavine[b]		**1**[a]	**2**[b]
H-2	6.897	6.922	—	—	—
H-4d[c]	3.320	3.357	H-5d	3.084	3.108
H-4u[d]	2.773	2.798	H-5u	2.546	2.577
H-5	2.513	2.622	H-4a	2.399	2.470
H-7d	3.242	3.444	H-3d	3.169	2.906
H-7u	3.920	3.026	H-3u	2.819	2.394
H-9	6.169	6.464	H-1	5.992	6.294
H-10	3.738	3.798	H-10b	3.407	3.471
H-12	6.976	6.907	H-10	6.702	6.696
H-13	7.154	7.121	H-9	7.240	7.248
H-14	7.012	7.191	H-8	6.527	6.547
Me	1.769	—	Me	1.788	—
CH_2d	—	4.141	CH_2d	—	4.191
CH_2u	—	4.106	CH_2u	—	4.154
N-Me	2.491	2.523	N-Me	2.354	2.392
NH	8.059	—	NH_2	6.523	6.524

[a]Solvent $CDCl_3$.
[b]Solvent $CDCl_3$-CD_3OD (4:1).
[c]d = downfield.
[d]u = upfield.

TABLE 2. ^{1}H-nmr Coupling Constants (Hz).

J	Compound		J	Compound	
	agroclavine[a,c]	elymoclavine[b]		1[a]	2[b]
NH,2	1.9	1.5	—	—	—
2,4d[d]	0.8	<1	—	—	—
2,4u[e]	1.8	1.7	—	—	—
4d,4u	−14.4	−14.3	5d,5u	−16.8	−16.6
4d,5	4.1	3.9	4a,5d	4.0	3.7
4u,5	11.6	11.6	4a,5u	12.7	12.7
5,10	9.3	9.6	4a,10b	10.0	10.0
7d,7u	−16.2	−16.2	3d,3u	−16.2	−16.2
7d,9	1.2	0.9	1,3d	1.1	0.9
7d,10	2.4	2.3	3d,10b	2.2	2.1
7d,Me	1.2	—	3d,Me	0.9	—
7d,17d	—	<1	3d,CH_2d	—	1.2
7d,17u	—	n.d.	3d,CH_2u	—	0.5
7u,9	2.3	2.4	1,3u	2.5	2.7
7u,10	4.0	n.d.	3u,10b	3.9	3.8
7u,Me	1.1	—	3u,Me	1.2	—
7u,17d	—	<1	3u,CH_2d	—	0.9
7u,17u	1.1	1.4	3u,CH_2u	—	1.0
9,10	2.3	n.d.	1,10b	1.0	n.d.
9,Me	2.1	—	1,Me	1.2	—
9,17d	—	<1	1,CH_2d	—	1.6
9,17u	—	<1	1,CH_2u	—	1.7
10,12	1.5	1.0	10,10b	1.2	1.3
10,14	<1	0.8	8,10b	0.8	1.0
10,Me	1.2	—	10b,Me	—	—
10,17d	—	1.5	10b,CH_2d	—	1.5
10,17u	—	1.6	10b,CH_2u	—	1.6
12,13	7.9	7.0	9,10	7.5	7.6
12,14	1.5	1.1	8,10	1.2	1.1
13,14	7.7	8.2	8,9	8.3	8.4
17d,17u	—	−13.1	CH_2d,CH_2u	—	−13.1

[a]Solvent $CDCl_3$.
[b]Solvent $CDCl_3$-CD_3OD (4:1).
[c]$J_{4d,9}$ = 0.8 Hz.
[d]d = downfield.
[e]u = upfield.

This compound is similar to **1**: it also contains a carbonyl and a downfield resonating sp^2-hybridized quaternary carbon, and its C-5 signal is shifted downfield with respect to elymoclavine. Thus, the structure **2** is obtained by repeating the deduction outlined above.

The discovery and structure determination of these two new compounds **1** and **2** shed some more light on the stability and metabolic fate of ergoline alkaloids. We suppose that these products are formed by the action of enzymes of the kynurenic pathway, i.e., by the action of tryptophan dioxygenase and formamidase, enzymes of "primary metabolism." There are many theories discussing features of primary and secondary metabolism (5,6) and various hypotheses about why secondary metabolites are biosynthesized (7). The possible mechanism of clavine alkaloid turnover is given in Figure 1. Oxidation and cleavage steps leading from **2** to anthranilic acid (dotted lines) are speculative only and will be the subject of our future study.

TABLE 3. ^{13}C-nmr Data of Agroclavine, Elymoclavine, and Compounds **1** and **2**.

Carbon	Compound		Carbon	Compound	
	agroclavine	elymoclavine		**1**	**2**
C-2	117.85	119.09	—	—	—
C-3	112.16	111.45	C-6	199.13	198.87
C-4	26.69	27.17	C-5	44.88	42.05
C-5	63.85	64.76	C-4a	61.01	61.32
C-7	60.63	57.20	C-3	59.37	55.66
C-8	132.25	131.74	C-2	133.84	137.64
C-9	119.41	132.12	C-1	118.63	120.05
C-10	40.95	40.98	C-10b	42.22	42.05
C-11	132.42	134.37	C-10a	145.58	144.77
C-12	112.62	112.83	C-10	112.12	112.06
C-13	122.84	129.56	C-9	134.61	134.67
C-14	108.51	109.60	C-8	115.28	115.52
C-15	133.54	151.44	C-7	151.36	151.44
C-16	126.33	126.88	C-6a	114.68	114.80
C-17	20.83	65.06		21.35	65.35
NMe	40.85	41.15	N-Me	40.72	40.81

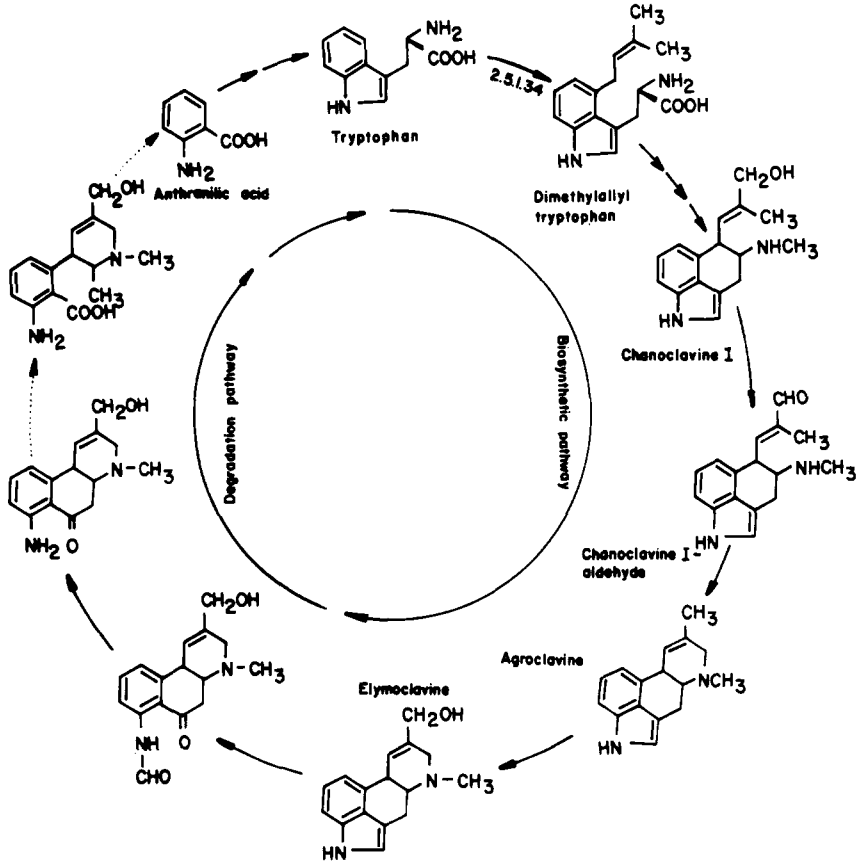

FIGURE 1. Possible mechanism of clavine alkaloid turnover.

EXPERIMENTAL

STRAIN.—The strain *C. purpurea* 129/35 originated from the Collection of Microorganisms of the Institute of Microbiology, Czechoslovak Academy of Sciences, Prague. For cultivation conditions see Pažoutová *et al.* (8).

ALKALOID SEPARATION.—A volume of 5 liters of the fermentation broth was extracted with 10 liters EtOAc, the extract was concentrated to 300 ml, and the precipitated elymoclavine was removed by filtration. The mother liquors were concentrated to a honey-like residue, dissolved in MeOH, and left to crystallize in the cold. Crystalline agroclavine was removed by filtration, and evaporation of the mother liquor yielded a total of 2.84 g residue containing a mixture of agroclavine, elymoclavine, and compounds **1** and **2**. The residue was dissolved in CH_2Cl_2 and chromatographed on a column of Si gel Merck 100 (30 g). The elution was performed by CH_2Cl_2 with an MeOH gradient (0–20%). It afforded 310 mg of the crude compound **1** and 120 mg of the crude compound **2**. Both substances were rechromatographed to yield 78 mg substance **1** (crystallization from Me_2CO) and 27 mg substance **2** (crystallization from Et_2O). Their purity was checked by gc and gc-ms. In both cases it was higher than 99.5%.

CHROMATOGRAPHY.—Tlc was performed on Si gel (Merck) in the system $CHCl_3$-MeOH (4:1), and the following R_f values were found: **1**, 0.81; **2**, 0.56; agroclavine, 0.62; elymoclavine, 0.31.

Gc was performed on an SP 7100 Spectra Physics instrument on a fused silica capillary column HP-1, (5 m × 0.53 mm, particle size 2.65 μm), carrier gas He, temperature 250°, flow rate 150 ml/min, injector temperature 300°. Retention times were: **1**, 1.78; **2**, 3.70; agroclavine, 2.13; elymoclavine, 4.12 min.

Gc-ms was carried out on a Finnigan MAT, MAT 90 instrument coupled directly with Varian 3400 gas chromatograph. Hewlett-Packard ultraperformance fused silica capillary column was used, cross-linked with 5% phenyl methyl silicon, i.d. 0.2 mm, length 50 m, particle size 0.11 μm, carrier gas He, flow rate 0.5 ml/min, temperature gradient 225–275°, 5°/min, 275° isotherm for 10 min, injector temperature 300°, scan time per decade 1 sec.

SPECTRAL PROCEDURES.—Elemental composition of pure substances **1** and **2** was determined by hrms on Finnigan MAT, MAT 90 with direct insertion probe, resolution 12000, accelerator voltage 5.0 kV, ion source temperature 250°, probe temperature 20–200°. The data are summarized in Table 4.

Nmr spectra were recorded on VXR-400 Varian spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C. Protonated carbons were assigned by conventional heteronuclear correlation, and quaternary carbons were assigned by (^1H,^{13}C)-COSY optimized for the detection of long-range coupling constants (3) and by proton-coupled spectra.

TABLE 4. Mass Spectroscopic Data of Compounds **1** and **2**.

m/z	Composition	Compound[a]	
		1	**2**
258	$C_{15}H_{18}N_2O_2$	—	71.8
257	$C_{15}H_{17}N_2O_2$	—	70.1
242	$C_{15}H_{18}N_2O$	100	—
241	$C_{15}H_{17}N_2O$	96.3	17.7
240	$C_{15}H_{16}N_2O$	—	53.3
239	$C_{15}H_{15}N_2O$	—	45.7
227	$C_{14}H_{15}N_2O$	16.4	42.8
211	—	6.9	—
210	—	5.4	—
167	$C_{11}H_5NO$	—	12.5
159	$C_{10}H_9NO$	37.0	25.9

[a]Table entries are relative intensity (%).

The uv spectra were recorded on Shimadzu MPS-2000 multipurpose recording spectrophotometer. The following λ max values were found: **1**, 233; **2**, 235 nm.

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Received 6 June 1990