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ERGOT ALKALOID GLYCOSIDES FROM SAPROPHYTIC CULTURES OF CLAVICEPS, II. CHANOCLAVINE I FRUCTOSIDES

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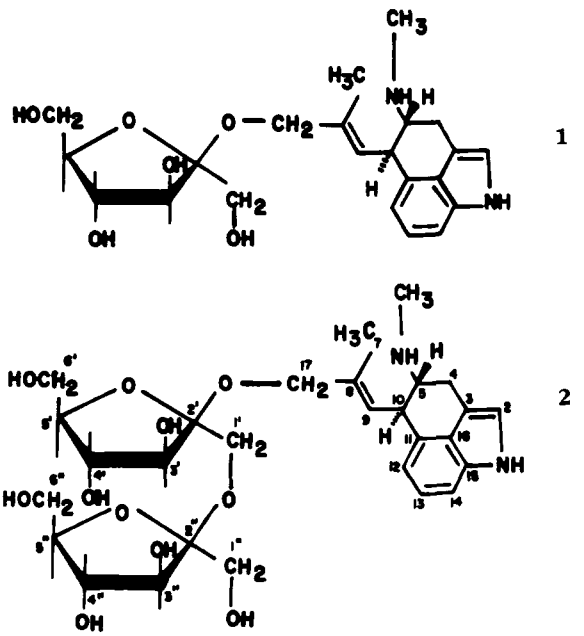
ABSTRACT.—Submerged culture of *Claviceps fusiformis* supplemented with chanoclavine I produced chanoclavine I *O*-β-D-fructofuranoside [**1**] and chanoclavine I *O*-β-D-fructofuranosyl-(2↦1)-*O*-β-D-fructofuranoside [**2**]. The structures of the two new glycosides were deduced from mass and nmr spectral data.

Recently, we have reported the isolation of two elymoclavine fructosides from the culture media of two *Claviceps* strains (1). These compounds are formed by the action of β-D-fructofuranosidase (EC 3.2.1.26) present mainly in the periplasmatic space of the producer cells (2). During screening of different *Claviceps* strains for this enzymic activity, we found the strain of *Claviceps fusiformis* W1 exhibiting high fructosylation ability towards elymoclavine. The alkaloid mixtures (4.2 g/liter) normally produced in a submerged cultivation of this strain are composed of elymoclavine, agroclavine, and the two abovementioned elymoclavine fructosides (1). Chanoclavine I (known as an elymoclavine precursor) is under these conditions produced in traces only. To test the fructosylation ability towards chanoclavine I, the culture medium was supplemented with this compound (1.5 g/liter). We observed the formation of chanoclavine I aldehyde, agroclavine, elymoclavine, and two additional alkaloids (Figure 1). These compounds, accounting for 15% of the total alkaloid content, were separated by reversed-phase liquid chromatography, and their structures were determined by spectroscopic methods.

Uv spectra of these compounds were characteristic for alkaloids having a C-8–C-9 double bond (3). Their cims display molecular ions at *m/z* 419 and 581, to-



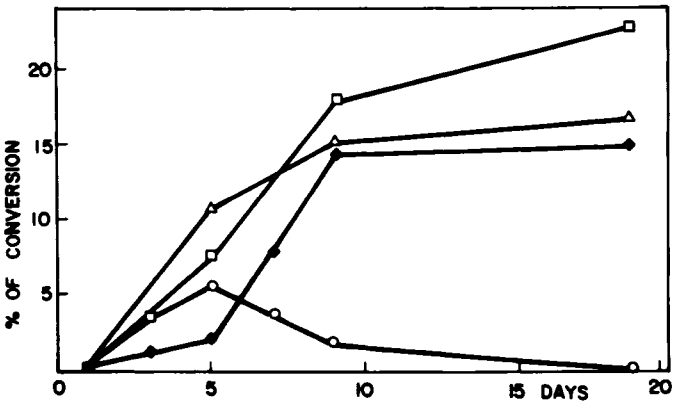


FIGURE 1. Conversion of chanoclavine I by *Claviceps fusiformis* W1: o-o chanoclavine I aldehyde, □-□ agroclavine, Δ-Δ elymoclavine, ♦-♦ chanoclavine I fructosides (total).

gether with intense ions *m/z* 256 and 257, corresponding to the chanoclavine I moiety. Chanoclavine I was also found as the only basic product of their acid hydrolysis. All chanoclavine I protons and carbons were found in ^{1}H- and ^{13}C-nmr spectra (Tables 1–3). Therefore, both **1** and **2** are chanoclavine I derivatives. The

TABLE 1. ^{13}C-nmr Chemical Shifts (ppm) of **1**, **2**, and Chanoclavine I.

Carbon	Compound		
	Chanoclavine I	**1**	**2**
C-2	119.95	120.35	120.78
C-3	111.01	n.o.[a]	n.o.
C-4	26.70	26.00	25.13
C-5	63.02	62.75	62.94
N-Me	44.04	43.52	42.87
C-7	14.75	15.17	15.20
C-8	140.59	138.82	139.05
C-9	127.03	127.31	126.70
C-10	33.98	33.52	32.91
C-11	132.33	n.o.	132.67
C-12	116.72	116.98	117.20
C-13	123.69	123.86	124.04
C-14	110.22	110.47	110.79
C-15	135.79	n.o.	135.97
C-16	127.61	127.42	127.13
C-17	68.94	67.97	67.93
C-1′		62.75	62.39
C-2′		105.82	105.50
C-3′		78.91	79.39
C-4′		77.41	77.10
C-5′		83.82	83.80
C-6′		64.63	63.95
C-1″			62.94
C-2″			105.06
C-3″			79.20
C-4″			76.61
C-5″			83.74
C-6″			63.65

[a]n.o. = not observed.

TABLE 2. ^{1}H-nmr Signals of **1**, **2**, and Chanoclavine I.

Proton	Compound		
	Chanoclavine I	**1**	**2**
H-2	6.917	6.966	6.957
H-4α	2.718	2.823	2.802
H-4β	3.277	3.310[a]	3.330[a]
H-5	2.818	3.005	2.976
H-7	1.874	1.891	1.896
H-9	5.399	5.450	5.456
H-10	3.894	3.979	3.967
H-12	6.647	6.664	6.661
H-13	7.006	7.031	7.032
H-14	7.131	7.157	7.153
H-17d[b]	4.588	4.260	4.278
H-17u[c]	4.588	4.108	4.116
N-Me	2.445	2.536	2.523
H-1′d		3.699	3.900
H-1′u		3.571	3.667
H-3′		4.148	4.161
H-4′		3.985	3.992
H-5′		3.769	n.d.[d]
H-6′d		3.745	n.d.
H-6′u		3.615	n.d.
H-1″d			3.650
H-1″u			3.560
H-3″			4.110
H-4″			4.035
H-5″			n.d.
H-6″d			n.d.
H-6″u			3.618

[a]Overlap by the solvent signal. Assignment by COSY and delayed-COSY.
[b]d = downfield.
[c]u = upfield.
[d]n.d. = not determined.

^{13}C-nmr spectrum of **1** contains six additional signals, consisting of one O-C-O, three OCH, and two OCH_2 type carbons. The corresponding protons in the ^{1}H-nmr spectrum of **1** (so far not identified) form one AB system and one five-spin system. Two such systems are present in the ^{1}H-nmr spectrum of **2**. These facts indicate that the moiety attached to chanoclavine I in **1** is a ketohexose. Comparison of ^{13}C-nmr data with those of complete set of methyl hexulosides (4,5) [i.e., looking for the maximum lines with the smallest (<1 ppm) chemical shift difference for signals having the same multiplicity], allowed us to reject all pyranose forms and some isomers. The best fit was found for β-fructofuranoside. The observed values of vicinal proton-proton couplings $J_{3,4}$ and $J_{4,5}$ among the furanose protons agree well with those reported (6) (Table 4). The C-17 protons are magnetically equivalent in the ^{1}H-nmr spectrum of the parent alkaloid but not with **1** or **2**. That indicates a hindered rotation around the C-17–O bond, due to a fructose unit attached at this site. Therefore, **1** is chanoclavine I *O*-β-D-fructofuranoside. The second set of six carbon resonances in the ^{13}C-nmr spectrum of **2** also corresponds to a β-fructofuranoside moiety. Because one AB system of the sugar H-1 protons in the ^{1}H-nmr spectrum of **2** is shifted upfield with respect

TABLE 3. Selected Coupling Constants (Hz) of the Ergolene Part.

Proton	Compound		
	Chanoclavine I	**1**	**2**
H-2, -4α	1.4	1.3	> 0
H-4α, -4β	−14.7	−14.9	−14.9
H-4α, -5	8.8	8.8	8.7
H-4β, -5	4.0	n.d.[a]	n.d.
H-5, -10	8.0	n.d.	n.d.
H-7, -9	1.4	1.2	1.3
H-9, -10	9.9	9.9	9.9
H-9, -17d[b]	1.3	1.1	1.1
H-9, -17u[c]	1.3	1.1	0.9
H-12, -13	8.2	8.1	8.0
H-12, -14	0.9	0.6	0.7
H-13, -14	7.1	7.1	7.1
H-17d, -17u	—	−12.1	−12.1

[a]n.d. = not determined.
[b]d = downfield.
[c]u = upfield.

to that of **1**, the second fructose is 2↦1 linked. Thus, **2** is chanoclavine I *O*-β-D-fructofuranosyl-(2↦1)-*O*-β-fructofuranoside.

EXPERIMENTAL

STRAIN.—*C. fusiformis* W1 used for chanoclavine I bioconversion (7) is deposited in the Collection of Microorganisms of the Institute of Microbiology, Czechoslovak Academy of Sciences, Prague. The strain was maintained on a T2 agar medium (8). Active biomass was prepared by washing spores from a 25-day-old agar culture into medium TI and subsequent cultivation on a rotary shaker under conditions described elsewhere (8). A 14-day-old culture was harvested under sterile conditions by centrifugation, washed with sterile saline, and transferred into an NPY medium up to a concentration of 20 g/liter. Composition of NPY medium (g/liter): sucrose 100, yeast extract 0.1, citric acid monohydrate 16.8, $CaCl_2$ 1.2, KCl 0.12, $MgSO_4 \cdot 7H_2O$ 0.25, $FeSO_4 \cdot 7H_2O$ 0.02, $ZnSO_4 \cdot 7H_2O$ 0.015, pH 5.5 (NaOH).

Chanoclavine I (Galena, Opava, Czechoslovakia) was dissolved in 2% succinic acid and filter-sterilized, and stock solution (30 mg/ml) was added to the culture media (60 ml) up to a final concentration of 1.5 g/liter. The bioconversion was run under the same conditions as the cultivation.

TABLE 4. Coupling Constants (Hz) of Fructose Protons.

Proton	Compound	
	1	**2**
H-1d[a], -1u[b]	−11.8	−10.2 −11.8
H-3, -4	8.1	8.1 8.2
H-4, -5	7.8	7.8 7.5
H-5, -6d	3.0	3.1 3.0
H-5, -6u	7.7	7.3 n.d.[c]
H-6d, -6u	−12.2	−12.1 n.d.

[a]d = downfield.
[b]u = upfield.
[c]n.d. = not determined.

ALKALOID SEPARATION.—Alkaloids were separated from the culture broth by adsorption on bentonite (1). The MeOH solution was repeatedly loaded on Separon SGX C18 column (25 × 0.8 cm i.d., particle size 7 μm) and eluted with MeOH-H_2O-concentrated NH_3 (76:24:0.044). The column effluent was monitored by uv (225 nm). The Separon SGX C18 column (15 × 0.33 cm i.d., particle size, 7 μm) with the same mobile phase was also used for purity checking. The following capacity factors were found: for elymoclavine, 1.5; for **1**, 2.6; for **2**, 2.2; for chanoclavine I, 5.0.

DEGRADATION PROCEDURE.—Each fructoside (0.1 mg) was hydrolyzed with 1 N HCl for

30 min at 60°. The only product was chanoclavine I.

GENERAL SPECTRAL PROCEDURES.—Uv, nmr, and cims were measured as published earlier (1). Cims of **1**: *m/z* (rel. int. %) 419 (100), 418 (46), 257 (81), 256 (25), 239 (69), 237 (50), 208 (8), 183 (61), 154 (19), 127 (6). Cims of **2**: *m/z* (rel. int. %) 581 (1), 419 (100), 418 (31), 257 (61), 256 (18), 239 (82), 237 (48), 223 (12), 208 (26), 183 (51), 154 (20), 127 (23).

LITERATURE CITED

1. M. Flieger, N.F. Zelenkova, P. Sedmera, V. Křen, J. Novák, V. Rylko, P. Sajdl, and Z. Řeháček, *J. Nat. Prod.*, **52**, 506 (1989).
2. A.G. Dickerson, *Biochem. J.*, **129**, 263 (1972).
3. A. Hofmann, "Die Mutterkornalkaloide," Ferdinand Enke Verlag, Stuttgart (1964).
4. P.C.M.H. DuPenhoat and A.S. Perkin, *Carbohydr. Res.*, **36**, 111 (1974).
5. S.J. Angyal and G.S. Bethell, *Aust. J. Chem.*, **29**, 1249 (1976).
6. R.D. Guthrie, I.D. Jenkins, and R. Yamasaki, *Aust. J. Chem.*, **25**, 1019 (1982).
7. V. Křen, S. Chomátová, J. Břemek, J. Pilát, and Z. Řeháček, *Biotechnol. Lett.*, **8**, 327 (1986).
8. V. Křen, S. Pažoutová, V. Rylko, P. Sajdl, M. Wurst, and Z. Řeháček, *Appl. Environ. Microbiol.*, **48**, 826 (1984).

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