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ERGOT ALKALOID GLYCOSIDES FROM SAPROPHYTIC CULTURES OF *CLAVICEPS*, I. ELYMOCLAVINE FRUCTOSIDES

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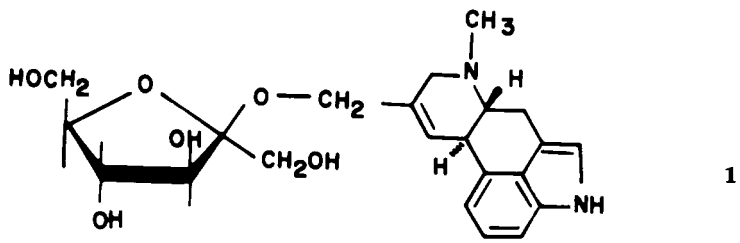
ABSTRACT.—The structures of elymoclavine-*O*-β-D-fructofuranoside [**1**] and a new elymoclavine-*O*-β-D-fructofuranosyl-(2↦1)-*O*-β-D-fructofuranoside [**2**] produced in saprophytic cultures of strains *Claviceps* sp. SD 58 and *Claviceps purpurea* 88 EP on sucrose medium are described. The structures have been determined on the basis of uv, ms, and 2D-nmr data and a degradation procedure.

Elymoclavine-*O*-β-D-fructoside has been isolated from a saprophytic culture of *Claviceps* strain SD 58 by Floss *et al.* (1). This alkaloid was formed from elymoclavine and from the sucrose present in the medium by the action of the enzyme invertase present in the fungal mycelia. Invertase transferred the fructose moiety from sucrose to the hydroxyl group of elymoclavine. The invertase (fructosyl transferase) activity was most probably responsible for the formation of oligosaccharides in submerged cultures of *C. purpurea* grown on a sucrose-ammonia medium in aerated fermentors (2). These oligosaccharides contained D-glucose as the reducing unit and one, two, and three *O*-β-D-fructofuranosyl units.

The present report deals with structure determination and production of natural elymoclavine fructosides **1** and **2** under conditions of submerged fermentation.

EXPERIMENTAL

STRAINS.—The strain *Claviceps* sp. SD 58 (ATCC 26019), deposited in the Collection of Microorganisms, Institute of Microbiology, Czechoslovak Academy of Sciences, was obtained from Prof. J.A. Anderson (Texas Tech University, Lubbock). Cultivations were performed in 300-ml Erlenmeyer flasks (60



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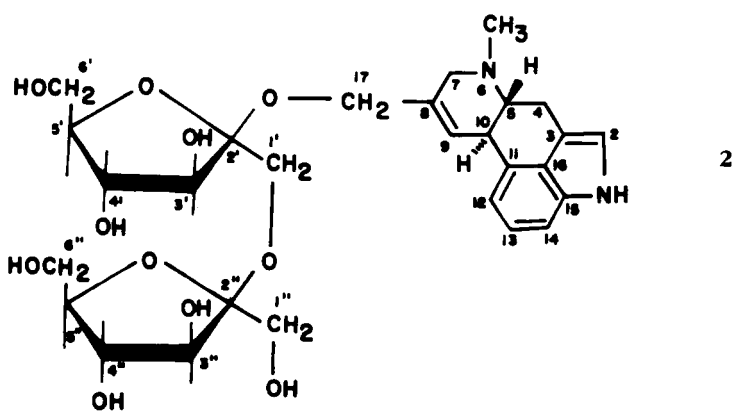

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ml of medium) on a rotary shaker (250 rpm, eccentricity 5.5 cm) at 24°. A 5-day-old inoculum was prepared in medium NL 611 (3), and 1.2 ml was used to inoculate production medium NL 720 (4).

The strain *Claviceps purpurea* 88 EP (Fr.) Tul. from the Collection of Microorganisms, Institute of Microbiology, Czechoslovak Academy of Sciences, was obtained from an ergotoxine-producing isolate by multiple mutagenesis (5). The submerged culture was cultivated on TI (inoculum) and CS2 (production) media under conditions described elsewhere (6).

ALKALOID SEPARATION.—Alkaloids were separated from the culture broth (pH adjusted to 7.5 with concentrated NH_3) by adsorption on bentonite (Lachema, Brno, Czechoslovakia) (2 × 50 g/500 ml) and desorbed with MeOH (3 × 100 ml), and the crude alkaloid solution was concentrated to a final volume of 10 ml under low pressure conditions. The MeOH solution was loaded (5 × 2 ml) on a Separon SGX C 18 column (35 g, 25 × 2.0 cm i.d., particle size 50 μm), and eluted with MeOH-H_2O-concentrated NH_3 (30:70:0.34). The column effluent was monitored by uv (288 nm). The first alkaloid fraction contained a mixture of all elymoclavine fructosides.

CHROMATOGRAPHY.—A mixture of elymoclavine fructosides was repeatedly loaded on Separon SGX C18 column (25 × 0.8 cm i.d., particle size 7 μm) and eluted with the above-mentioned mixture. A baseline separation of all fructosides was reached. 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. Column effluent was recorded by uv at 224 nm. The following capacity factors were found: for **1**, 15.42; for **2**, 10.77; for elymoclavine, 51.12.

DEGRADATION PROCEDURE.—A small amount (0.2 mg) of each fructoside was hydrolyzed with 1 N HCl for 30 min at 60°. The resulting mixtures were analyzed by hplc.

GENERAL SPECTRAL PROCEDURES.—The uv spectra were recorded on a Shimadzu MPS-2000 multipurpose recording spectrophotometer.

Nmr spectra were recorded on VXR-400 Varian spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C. ^1H-nmr data were collected on 32K, and FIDs were zero-filled to 64K prior to Fourier transformation. All chemical shifts are expressed in ppm downfield from TMS. Assignments were checked through suitable decoupling and 2D-nmr experiments.

Cims was taken on a Finnigan Mat MAT 90 instrument by dici and dci techniques; temperature on

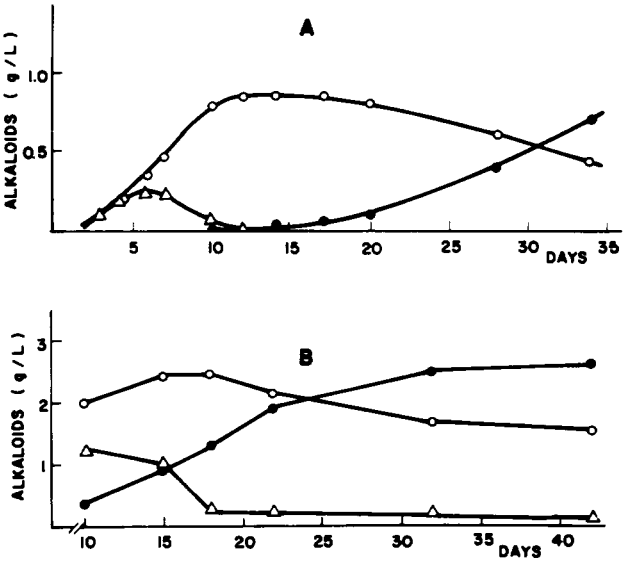

FIGURE 1. Formation of elymoclavine fructosides during submerged fermentation of *Claviceps* sp. SD 58 (A) and *Claviceps purpurea* 88 EP (B). Δ, Agroclavine; ○, elymoclavine; ●, elymoclavine fructosides (total).

the inlet system was 250°, probe temperature rose from 25 to 300° at a rate of 1°/sec. Ion current 0.2 mA, acceleration voltage 5.0 kV, ci gas NH_3. Cims of **1**: *m/z* (rel. int. %) 417 (33), 416 (22), 254 (23), 253 (30), 237 (100), 236 (85), 223 (9), 207 (6), 167 (9), 154 (6), 127 (1). Cims of **2**: *m/z* (rel. int. %) 579 (25), 578 (8), 416 (25), 254 (60), 253 (45), 237 (100), 236 (93), 223 (7), 207 (3), 167 (9), 154 (10), 127 (24).

RESULTS AND DISCUSSION

Reversed-phase liquid chromatography of 30-day-old submerged culture fermentation broths of the strains 88 EP and SD 58 reveals, besides the normal alkaloidal composition, four substances with exceptionally high polarity. Their uv spectra resemble those of alkaloids with double bond in position C-8–C-9, and the substances are characterized by formation in the later production or postproduction phase (Figure 1). Acid hydrolysis of all compounds afforded elymoclavine as the only basic product. Fragmentation diagnostic for elymoclavine (7) was also observed in the cims of these alkaloids. Elymoclavine was therefore used as a model compound. ^{1}H- and ^{13}C-nmr spectra of its acetate were already published (8). A convenient starting point for the ^{1}H-nmr spectrum assignment is the resonance of H-2. In the presence of CD_3OD its only coupling identifies H-4α, from which the whole coupling network can be traced by COSY (9) and delayed-COSY (10) experiments (Table 1). Similar networks were found for both **1**

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

Carbon	Compound		
	Elymoclavine[a]	**1**[b]	**2**[b]
2	119.09	119.98	120.07
3	111.45	111.58	111.30
4	27.17	27.56	27.38
5	64.76	65.87	65.82
N(6)-Me	41.15	41.02	40.81
7	57.20	58.13	58.02
8	134.37	134.63	134.13
9	121.55	123.34	123.62
10	40.98	41.65	41.43
11	131.74	131.91	131.58
12	112.83	113.51	113.58
13	123.12	123.74	123.76
14	109.60	110.35	110.44
15	136.12	135.61	135.60
16	126.88	127.74	127.67
17	65.06	65.01	64.84
1′	—	62.41	62.54
2′	—	105.79	105.52
3′	—	78.85	79.21
4′	—	77.26	77.06
5′	—	83.82	83.80
6′	—	64.88	64.77
1″	—	—	62.85
2″	—	—	105.00
3″	—	—	80.01
4″	—	—	76.50
5″	—	—	83.67
6″	—	—	63.91

[a]Solvent $CDCl_3/CD_3OD$; assignment based on COSY, delayed COSY, APT, DEPT, and HETCOR.

[b]Solvent CD_3OD.

TABLE 2. Coupling Constants (Hz) of Fructose Protons.

Protons	Compound	
	1	**2**
H-1 d, H-1 u[a] . . .	−11.9	−11.8[b] −10.1[c]
H-3,4	8.2	8.2[b] 8.3[c]
H-4,5	7.6	7.2[b] 7.4[c]
H-5,6 d	2.9	n.d.[d]
H-5,6 u	7.1	n.d.
H-6 d, H-6 u	−11.8	−11.8[b]

[a]d = downfield; u = upfield.
[b]First fructose moiety.
[c]Second fructose moiety.
[d]n.d. = not determined.

TABLE 3. ^{1}H-nmr Signals for Compounds **1**, **2**, and Elymoclavine.

Proton	Compound		
	Elymoclavine[a]	**1**[b]	**2**[b]
H-2	6.922	6.937	6.949
H-4α	2.798	2.797	2.841
H-4β	3.357	3.379	3.404
H-5	2.622	2.691	2.797
H-7α	3.026	3.174	3.230
H-7β	3.444	3.631	3.676
H-9	6.464	6.553	6.557
H-10	3.798	3.854	3.851
H-12	6.967	6.950	6.966
H-13	7.121	7.163	7.073
H-14	7.191	7.143	7.151
H-17 u[c]	4.106	4.112	4.131
H-17 d[d]	4.141	4.327	4.328
N-Me	2.523	2.588	2.648
H-1′ u	—	3.590	3.577
H-1′ d	—	3.717	3.649
H-3′	—	4.154	4.165
H-4′	—	4.008	4.012
H-5′	—	3.779	n.d.
H-6′ u	—	3.628	3.627
H-6′ d	—	3.734	n.d.[e]
H-1″ u	—	—	3.703
H-1″ d	—	—	3.906
H-3″	—	—	4.126
H-4″	—	—	4.046
H-5″ and H-6″ . . .	—	—	n.d.

[a]Solvent $CDCl_3/CD_3OD$.
[b]Solvent CD_3OD.
[c]u = upfield.
[d]d = downfield.
[e]n.d. = not determined.

and **2**. Furthermore, all signals belonging to the elymoclavine moiety are present in ^{13}C-nmr spectra of these compounds (Table 1). Thus **1** and **2** are elymoclavine derivatives.

Compound **1** exhibits a molecular ion *m/z* 417 or 416 ($C_{22}H_{28}N_2O_6$) in its cims. Six signals remained in the ^{13}C-nmr spectrum upon identifying the elymoclavine carbons (Table 1). They consist of one quarternary carbon (-O-C-O- type), three oxymethines, and two oxymethylenes. Apart from the elymoclavine protons two other spin systems were found by COSY: an AB system (CH_2OH) and four-spin system (-CH(OH)CH(OH)CH(O)-CH_2OH). These facts point to a ketohexose. Comparison of ^{13}C-nmr data with those of known methyl hexulosides (in D_2O) (11,12) ruled out all pyranose forms and most of the furanoses; the best fit was obtained for β-fructofuranose. Indeed, the observed coupling constants (Table 2) are very close to those reported in DMSO (13). Hence **1** is elymoclavine-*O*-β-D-fructofuranoside, a compound already reported by Floss *et al.* (1).

The molecular ion *m/z* 579 or 578 ($C_{28}H_{38}N_2O_{11}$) in the cims of **2** indicates the presence of two sugar units. All six carbon signals assigned to fructose are accompanied by others within 1 ppm (Table 1). The similarity of the extracted proton chemical shifts and the coupling constants (Tables 2,3) also indicates that the second monosaccharide is the same, i.e., β-D-fructofuranose. Nmr was used to establish the mode of its attachment. Formation of a glycosidic bond causes an upfield shift of the involved protons and a downfield shift of the participating carbons. The latter effect was not observed, probably because of a compensating effect due to the mobility of the five-membered ring system. However, an upfield shift of one AB system, assigned to H-1 protons, allows us to propose a 2↦1 linkage for this compound. Thus compound **2** is elymoclavine-*O*-β-D-fructofuranosyl-(2↦1)-*O*-β-D-fructofuranoside.

Two other isolated compounds are most probably higher glycosides of elymoclavine. Unfortunately, the rapidly decreasing solubility of these compounds and the very low quantity produced under submerged conditions did not allow us to determine the composition and structure of these alkaloids.

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