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Identification of Sterols in Submerged Cultures of Different *Claviceps* Species

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Summary

The occurence of sterols was studied by gas chromatography — mass spectrometry in the lipid fraction of saprophytic cultures of *Claviceps purpures*, *C. paspali*, *C. fusiformis* and *C.* sp. SD-58. A total of 15 sterols were found out of which 11 have not yet been described in the genus *Claviceps*. Comparison of biogenetic pathways of sterols was used to assess the relatedness of the strains under study.

Introduction

The need for elucidation of sterol occurrence and metabolism in the fungus *Claviceps* is documented by the fact that the basic precursor of sterols, mevalonic acid, is one of the precursors of ergot alkaloids (Review: Řeháček 1985).

The occurrence, biosynthesis and metabolism of sterols have been studied in different fungal species including Ascomycetes. Ergosterol (5,7,22-ergostatrienol, VI) was the first to be discovered in sclerotia of *Claviceps purpurea* by Tanret (1889). Apart from Tanret (1889) only a few authors dealt with the identification of sterols in *Claviceps* (Tanret 1908; Heyl and Swoap 1930; Heyl 1932; Wieland and Coutelle 1941; Seitz and Pomeranz 1983). In sclerotia of *C. purpurea* were identified 7,22-ergostadienol (VIII) (Heyl and Swoap 1930; Heyl 1932), 7,22-ergostadiene-3β,5α,6β-triol (cerevisterin), squalene (Wieland and Coutelle 1941) and 7-ergosterol (XI) (fungisterol) (Tanret 1908). Seitz and Pomeranz (1983) discovered ergosterol and its oxidation products in cereals infected with ergot.

This study concerns the occurrence of sterols in the lipid fraction of a submerged mycelium of seven saprophytic strains of *Claviceps* with regard to the relative abundance of individual sterols, their biosynthesis and possible application in taxonomy.

Materials and Methods

Strains and cultivation conditions

Strains *Claviceps purpurea* 129, *C. purpurea* CP/7/5/35, *C. purpurea* PLA-4, *C. fusiformis*, *C. species* SD-58, *C. paspali* MG-6, *C. paspali* FA (CCM F-731) were from the collection of this Institute. Strain *C. purpurea* CP/7/5/35 (Řeháček et al. 1984) was obtained by a 4-stage UV-muta-

Table 1. *Percentage content of sterols in the mycelium of submerged cultures of Claviceps*

Sterol	*C. purp.* 129	*C. purp.* CP/7/5/35	*C. purp.* PLA-4	*C. fusiformis*	*C.* sp. SD 58	*C. paspali* MG 6	*C. pasp.* FA
X_1	1.3	1.2	0.5	0.2	0.1	0.5	0.4
squalene (I)	0.5	4.5	12.7	2.2	1.2	0.6	0.6
X_2	0	0.8	0	0.4	0	0.8	1.7
X_3	0	0	2.5	0	0.4	0	0
8(14)-cholestenol (II)	0.8	19.0	5.2	10.9	0.2	1.3	0.3
5,22-ergostadienol (III)	1.8	3.7	50.8	65.2	5.9	11.0	3.0
4-methyl-8(14)-cholestenol (IV)	38.5	3.3	8.5	10.2	0	1.9	7.5
5,8,22-ergostatrienol (V)	25.6	2.7	1.7	0.4	0	1.3	0
5,7,22-ergostatrienol(VI)	12.8	53.0	3.4	6.5	71.2	32.3	75.5
5,24(28)-ergostadienol (VII)	0	0	0	0	1.1	2.6	3.4
7,22-ergostadienol (VIII)	17.9	7.2	0	0	0	0.3	1.4
5,7,22,24(28)-ergosta-tetraenol (IX)	0	0	0	0	17.5	4.5	0
8,24(28)-ergostadienol (X)	0	0	0	0.2	0	0.3	0.7
7-ergostenol (XI)	0	2.3	0	0.2	0.2	13.6	2.1
4-methyl-7-ergostenol (XII)	0	0	3.2	0.7	0.6	1.9	1.6
24-methyl-8,24(28)-lanostadienol (XIII)	0.8	1.4	0	0	0	0.1	1.0
24-methyl-8-lanostenol (XIV)	0	0.9	6.5	0	1.3	25.7	0.3
4-methyl-7,24-cholestadienol (XV)	0	0	0	0	0	1.3	[illegible]

Table 2. *Number of sterols commonly present or absent in different strains of Claviceps*[1]) (derived from Table 1)

	MG-6	129	35	C. fus.	SD-58	FA	PLA-4
MG-6	—						
129	10	—					
35	13	15	—				
C. fus.	12	12	13	—			
SD-58	10	7	9	40	—		
FA	16	10	13	12	10	—	
PLA-4	9	13	11	13	13	9	—

[1]) Abbreviations of strains:

MG-6	*C. paspali* MG-6
129	*C. purpurea* 129
35	*C. purpurea* CP/7/5/35
C. fus.	*C. fusiformis*
FA	*C. paspali*-FA
PLA-4	*C. purpurea* PLA 4

genesis of *C. purpurea* 129. All strains were cultivated on CS2 medium under conditions described previously (KŘEN et al. 1985a; KŘEN et al. 1986).

After 10-day cultivation the mycelium was collected by centrifugation and washed with water.

Analytical methods

Lipid extraction: Washed and centrifuged mycelium was hydrolyzed by 20% KOH (3 h, 100 °C). The unsaponified fraction was extracted 3 times with a mixture petroleum ether-diethylether (1 : 1). Acetates of sterols were prepared by reaction with acetanhydride in pyridine (24 h, 20 °C). The mixture was analyzed by gas chromatography — mass spectrometry on a HP 5995 B instrument (Hewlett Packard, USA) with capillary fused silica column 25 m × 0.20 mm Ultra 1 (HP) (methylsilicon), flow rate 1.1 ml He/min, column temperature 250—300 °C, gradient 1 K/min, injector 300 °C, ionizing voltage 70 eV.

Results

Occurrence and relative proportion of sterols in the mycelium of the *Claviceps* cultures under study are summarized in Table 1. The chromatography of the unsaponifiable fraction yielded a total of 18 peaks out of which 15 corresponded to sterols as determined by mass spectroscopy.

Numerical cluster analysis of the data on the contents of sterols, used previously for evaluating the content of fatty acids in *Claviceps* (KŘEN et al. 1985b) did not yield consistent results. A certain significance can be attached to the comparison of the qualitative proportion of sterols in different strains, which compared some closer relatedness between the strains.

The highest number of different sterols among the strains under study was found in *C. paspali* MG-6 and *C. paspali* FA. The two strains also harboured 16 out of the 18 compounds to be detected. A similar relatedness was displayed by strains of *C. pur-*

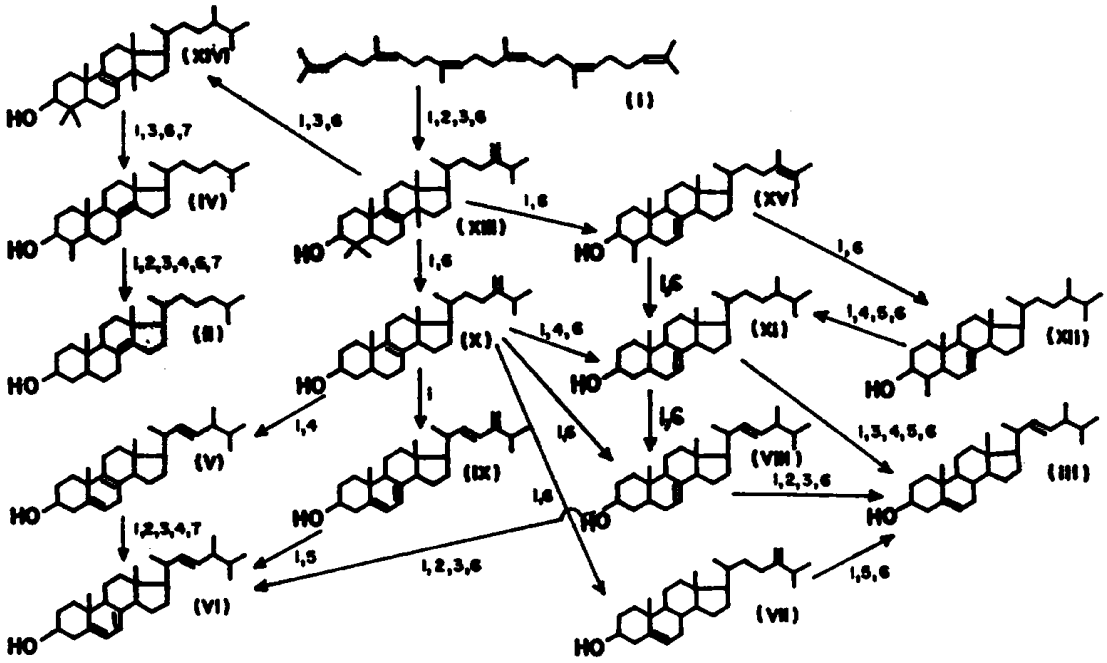

Fig. 1. Hypothetical scheme of the pathways of sterol biogenesis in different strains of Claviceps. 1 — *C. paspali* MG-6; 2 — *C. purpurea* 120; 3 — *C. purpurea* CP/7/5/35; 4 — *C. fusiformis*; 5 — *C.* sp. SD-58; 6 — *C. paspali*-FA; 7 — *C. purpurea* PLA 4.

Table 3. *Numbers of common pathways of sterol biosynthesis in different strains of Claviceps*[1]), according to Fig. 1

	MG-6	129	35	C. fus.	SD-58	FA	PLA 4
MG-6	21						
129	1	5					
35	3	5	3				
C. fus.	5	1	3	6			
SD-58	3	0	1	3	4		
FA	16	4	7	4	3	16	
PLA 4	3	2	3	2	0	2	3

[1]) Abbreviations vis Table 2.

purea 129 and CP/5/7/35 that exhibited the common occurrence or absentions of 15 sterols (Table 2).

The proposed biogenetic scheme of sterol synthesis in the strains under investigation (Fig. 1) is based on analyses of sterols assumed to be potential intermediates of appropriate pathways.

However, one must admit, that the occurrence or non-occurrence of an intermediate does not imply its biogenetic importance.

Fig. 1 indicates that the majority, i.e. 16 common biogenetic pathways of sterol biosynthesis, is displayed by the two strains of *C. paspali*. A significant agreement was also found between the strains of *C. purpurea*, 129 and CP/7/5/35 (Table 3).

Discussion

The elucidation of metabolism of sterols in *Claviceps* is in our opinion an important part of the study of physiology of formation of ergot alkaloids (Review: Řeháček 1985), mainly due to the fact that mevalonic acid is a common precursor of sterols and ergot alkaloids. Dimethylallylpyrophosphate is the substrate of an enzymic allylation of tryptophan to 4-dimethylallyltryptophan that forms the basis of the ergoline skeleton of ergot alkaloids. Whereas in proliferating cells of a submerged culture of *C. purpurea* CP/7/5/35 mevalonate is utilized for both the formation of sterols and the formation of alkaloids in differentiated cells it is preferentially used for alkaloid formation (Křen et al. 1986).

The biosynthetic pathway of sterols in *Claviceps* is more resistant to mutagenic treatments than the pathway of fatty acids whose spectrum changes radically after UV-irradiation (Křen et al. 1985b). Our study documents that *C. purpurea* CP/7/5/35 which is the result of a mutation improvement of *C. purpurea* 129 retains a marked similarity with the parent strains as concerns the composition of sterols.

The results of a novel identification of 11 sterols in three species of *Claviceps* will be used in a detailed study of the biosynthetic relationships between ergot alkaloids and sterols. A possibility was pointed out of a chemotaxonomical study of the genus *Claviceps* based on comparison of the biogenetic pathways of sterols.

References

Heyl, F. W.: The sterols of ergot III. The occurrence of an isomer of alpha-dihydroergosterol. J. Am. Chem. Soc. **54**, 1074—1076 (1932).

Heyl, F. W., and Swoap, O. F.: The occurrence of dihydro-ergosterol. J. Am. Chem. Soc. **52**, 3688—3690 (1930).

Křen, V., Řezanka, T., and Řeháček, Z.: Occurrence of ricinoleic acid in submerged cultures of various *Claviceps* species. Experientia **41**, 1476—1477 (1985a).

Křen, V., Řezanka, T., Sajdl, P., and Řeháček, Z.: Identification of fatty acids in submerged cultures of *Claviceps* species. FEMS Microbiol. Lett. **30**, 359—363 (1985b).

Křen, V., Pažoutová, S., Rylko, V., and Řeháček, Z.: Saprophytic production of clavine alkaloids and activity of 3-hydroxy-3-methylglutaryl coenzyme-A reductase. Folia Microbiol., in press (1986).

Řeháček, Z.: Biotechnology of ergot alkaloids. Trends Biotechnol. **2**, 166—172 (1985).

Řeháček, Z., Pažoutová, S., Kozová, J., Sajdl, P., Křen, V., a Fliegr, M.: Nový produkční kmen mikroorganismu *Claviceps purpurea* CP/7/5/35. PV-7761-84, Cs. pat. 12. 3. 1984.

Seitz, L. M., and Pomeranz, Y.: Ergosterol, Ergosta-4,6,8(14),22-tetraeno-3-on, ergosterol peroxide, and chitin in ergoty barley, rye, and other grasses. J. Agric. Food Chem. **31**, 1036—1038 (1983).

Tanret, M. C.: Sur un nouveau principe immédiat de l'ergot de siegle, l'ergostérine. C. R. Hebd. Seances Acad. Sci. **108**, 98—103 (1889).

TANRET, M. C.: Sur l'ergostérine et la fongistérine. C. R. Hebd. Seances Acad. Sci. 147, 75—77 (1908).

WIELAND, H., and COUTELLE, G.: Zur Kenntnis des Fungisterins und anderer Inhaltsstoffe von Pilzen. Liebig Ann. Chem. 548, 270—283 (1941).

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