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Occurrence of ricinoleic acid in submerged cultures of various *Claviceps* sp.

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Summary. Ricinoleic acid was found in different *Claviceps* sp., indicating that it is neither specific chemotaxonomic marker of *Claviceps purpurea*, nor a specific indicator of peptide alkaloid synthesis.
Key words. Claviceps; ricinoleic acid; ergot alkaloids; fatty acids.

Mycelial differentiation of the fungus *Claviceps* involves morphological and biochemical changes. In parasitic cultures a conspicuous feature of differentiation is the formation of sclerotium and alkaloid synthesis. An analogous pattern is found also in saprophytic *Claviceps* strains. In these strains the production of alkaloids under conditions of submerged cultivation is accompanied by sporulation[2] and differentiation of the culture into the sclerotial type of mycelium[3]. Sclerotial cells are assumed to be the dominant producers of alkaloids[3,4]. Another indicator of differentiation of the culture into the sclerotial type is abundant production of lipids (triglycerides)[4-6].

In sclerotial cells lipids are found in substantially higher

amounts than in cells of a sphacelial mycelium and are unique in that they contain ricinoleic acid ([R-(Z)]-12-hydroxy-9-octadecenoic acid). Ricinoleic acid has so far been considered an important biochemical indicator of morphological and biochemical differentiation of *C. purpurea* mycelium. Its level reaches a maximum closely before the onset of peptide alkaloid formation[7] and exceeds the mean level found during fermentation by as much as 49%[7]. High-producing strains of *C. purpurea* contain in their lipid fraction up to 40 mol% ricinoleate[5]. Total cell fatty acids show an analogous pattern during fermentation[7]. In contrast to production strains of *C. purpurea*, nonproduction strains contain no ricinoleic acid and their content of total lipids is substantially lower[5]. Biosynthesis of ricinoleic acid and ergot peptide alkaloids probably have a common regulatory mechanism[5,6]. Among the *Claviceps* sp. under study, i.e. *C. purpurea*, *C. paspali* and *C.* sp. SD-58 (probably *C. fusiformis*)[6], the relationship between mycelial differentiation and the occurrence of ricinoleic acid was proved only in *C. purpurea* producing peptide alkaloids[6]. These findings formed the basis of the hypothesis that ricinoleic acid is a specific chemotaxonomic marker of *C. purpurea* strains producing peptide alkaloids[6].

We studied the content of fatty acids in submerged cultures of six species of *Claviceps* deposited in the collection of this institute: *C. purpurea* 129/35, *C. purpurea* PLA 4, *C. fusiformis*, *C.* sp. SD-58, *C. paspali* MG-6, *C. paspali* FA (CCM F-731). Inoculation medium was T1[8]. A bioreactor (3 l) with medium CS2[8] (1.5 l) was inoculated with a 7-day-old submerged inoculum (120 ml). The paddle impeller speed in the bioreactor was 500 rpm, aeration rate 1 l/min, temperature 24 ± 1 °C. After 10-day cultivation the mycelium was collected by centrifugation and washed with water. Methyl esters of fatty acids were prepared by method No. 3 described by Marberry[9]. A mixture of the methyl esters was analyzed by gas chromatography-mass spectrometry on a HP 5995B (Hewlett Packard, USA) instrument on a capillary fused silica column under conditions described previously[10]. The content and composition of the alkaloid mixture was analyzed by high performance liquid chromatography[11].

In contrast to reports in the literature, ricinoleic acid was found in the mycelium of different *Claviceps* sp. (table) and is thus not a specific chemotaxonomic marker of *C. purpurea*, nor a specific indicator of synthesis of peptide alkaloids. In general, no significant correlation was found between the rate of production of alkaloids in different *Claviceps* sp. and the cellular level of ricinoleate. However, a valuable finding was that such a relationship was exhibited solely by *C. purpurea* strains producing peptide alkaloid and that the correlation between an increased level of ricinoleate in the mycelium and the differentiation of the mycelium to the sclerotial type was characteristic solely of the species *C. purpurea*.

Ricinoleic acid in mycelium of submerged cultures of various *Claviceps* sp. producing alkaloids

	Alkaloids Type	Production (μg/ml)	Ricinoleic acid*
C. purpurea 129/35	chano-I-, agro-, elymoclavine	1000–4000	1.90
C. purpurea PLA 4	peptides (cyclols)	7–10	3.24
C. paspali MG-6	lysergic acid α-hydroxyethylamide	20–40	0.30
C. paspali FA	lysergic acid α-hydroxyethylamide	1000	0.20
C. fusiformis	chano-I-, agro-, elymoclavine	1000–2000	4.39
C. sp. SD-58	chano-I-, agro-, elymoclavine	1000–2000	4.11

* Percentage of fatty acids mixture.

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