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The different conformations and crystal structures of dihydroergocristine

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ABSTRACT

The identification of different forms of dihydroergocristine (DHEC) was carried out by crystallization from different organic solvents. DHEC was identified as potential template for molecularly imprinted polymers (MIPs) for the epimeric specific analysis of ergot alkaloids (EAs) in food. DHEC was crystallized from different solvents in order to mimic the typical MIP synthesis conditions. Four new solvatomorphs of DHEC were obtained. All solvatomorphs contain a water molecule in the crystal structure, whereas three compounds contain an additional solvent molecule. Based on the conformation of DHEC a comparison with typical EA molecules was possible. The analysis showed that DHEC is a suitable template for MIPs for EAs.

1. Introduction

Fungi of the genus *Claviceps* are specialized parasites of grasses, rushes and sedges, including forage grasses, corn, wheat, barley, oats, rice, and rye [1]. The most prominent member of the genus *Claviceps* is *C. purpurea*. Infection with these species leads to the formation of dark purplish-black mycelia mass called *sclerotium*. The *sclerotium* itself contains inter alia a number of highly toxic secondary fungal metabolites, the so-called ergot alkaloids (EAs), which can cause severe diseases in humans and animals (e. g. gangrene, paresis → ergotism) after consumption of contaminated food and feed.

The main ergot alkaloids produced by *C. purpurea* are ergometrine, ergotamine, ergosine, ergocristine, α-ergocryptine, and ergocornine, along with their corresponding isomeric forms (-inine-forms) [2]. All these EAs are based on a tetracyclic ergoline ring which is methylated on the N-6 nitrogen atom, substituted on C-8 and possesses a double bond in C9–C10 position. They can be divided into simple lysergamides (ergometrine/-inine) and ergotpeptines (ergosine/inine, ergotamine/-inine, ergocornine/-inine, α-ergocryptine/inine and ergocristine/-inine). While the C8-(*R*)-isomers (suffix "-ine") show a high toxicity, the C8-(*S*)-isomers ("-inine") are considered as biologically less or not active [1,3].

In recent years EAs and their epimer-specific determination have gained increasing importance in preventing poisoning of livestock and consumers and economic losses.

For analysing EAs often methods based on the use of high-performance liquid chromatography in combination with fluorescence detection were used. This requires an efficient clean-up to remove matrix components from the raw extracts. Otherwise the quantification is exacerbated by spectral interferences especially when analysing processed cereal samples. In the literature some of the clean-up methods based on the removal of matrix components by binding the EAs on an adsorbent material [4–8], are described. Another approach is the binding of EAs to a resin, and subsequently eluting the EAs after removing matrix components by washing with a suitable solvent. Therefore, Chromabond® C18 ec SPE cartridges [9], EXtrelut® NT3 columns [10–13] or strong cation exchange (SCX) columns [14,15] were applied. A very selective sample clean-up procedure based on the use of molecularly imprinted polymers (MIPs) as solid-phase extraction materials for analysing EAs was described so far only once [16].

MIPs are synthetic polymers featuring receptor or catalytically active sites. Synthesis of MIPs is commonly based on the formation of reticulated polymers in the presence of templates, which could be the analyte itself or a structurally related substance. Because the EAs vary in their substituents at the C8-position it is reasonable to use a structurally related substance as template instead of using one of the priority EAs. Furthermore, using an EA analogue as template molecule for polymer synthesis leads to the avoidance of

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inaccuracies in measurements caused by residual template bleeding.

A feasibly and reasonably priced template for the synthesis of MIPs for EAs is Dihydroergocristine (DHEC), which can be obtained from commercial available Dihydroergocristine mesylate (DHEC mesylate, Fig. 1). To form suitable receptor or catalytically active sites for EAs during the imprinting process it is essential that DHEC possess the same configuration of the asymmetric centres as the EAs. Since it is not known in detail whether the configurations of the asymmetric centres are influenced during the synthesis steps, it is necessary to identify the absolute configuration of DHEC, based on single crystal X-ray data. Furthermore, only if the conformation of the DHEC reveals a high degree of shape similarity with those of the EAs a selective interaction between the MIP and the EAs can be ensured. Otherwise during imprinting of the polymer an arrangement of potential binding sites would be generated which is not suitable for the EAs. Therefore it is necessary to verify the shape similarity under the influence of different solvents used for the synthesis. A reliable possibility to prove this requirement approximately is based on the single crystal X-ray analysis. For answering these two questions the crystal structures of DHEC crystallized from different solvents were determined. Here we present the crystal structure of four different solvatomorphs of DHEC, the conformational relation among these structures, and prove the suitability of DHEC as a template for EAs for MIPs.

2. Experimental

2.1. Synthesis of dihydroergocristine

20.3 g (28.7 mmol) Dihydroergocristine mesylate (Teva Czech Industries s.r.o., Opava, Komárov, Czech Republic) were suspended in 200 mL of a 25% ammonia solution (p. A., Merck KGaA, Darmstadt, Germany). Then 1000 mL chloroform (p. A., neoLab Migge Laborbedarf-Vertriebs GmbH, Heidelberg, Germany) were added and the suspension was stirred at ambient temperature until the solid was dissolved. After phase separation the aqueous layer was extracted twice with chloroform (1000 mL each). The combined organic layers were washed with 1000 mL brine, subsequently dried over Na_2SO_4 (p. A., Merck KGaA), filtered and evaporated to dryness. The resulting white product was further dried 4 days at 50 °C in vacuo. Codestillation with acetone (CHEMSOLUTE®, Th. Geyer GmbH & Co. KG, Renningen, Germany) and water (Seralpure) was performed to remove small amounts of remaining solvent. Subsequently, the powder was dried again in vacuo for 2 days at 70 °C to give 15.1 g (24.7 mmol, 85.8%) dihydroergocristine.

M.p.: 180.7–181.6 °C. **Elemental composition**: calc. for $C_{35}H_{41}N_5O_5 \times H_2O$ (%): C, 66.75; H, 6.88; N, 11.12; found: C, 66.22; H, 6.85; N, 10.91. **HR-MS**: calc. for $C_{35}H_{42}N_5O_5^+$ 612.3181; found 612.3180 [M + H$^+$]. **^{1}H NMR** (**600 MHz**, **CD_3OD**, **ppm**) δ: 7.37–7.34 (m, 2H, H-18′/22′), 7.22–7.18 (m, 2H, H-19′/21′), 7.15 (dt, J = 8.2, 0.7 Hz, 1H, H-14), 7.14–7.10 (m, 1H, H-20′), 7.09 (dd, J = 8.1, 7.1 Hz, 1H, H-13), 6.91 (d, J = 1.5 Hz, 1H, H-2), 6.86 (ddd, J = 7.3, 1.4, 0.6 Hz, 1H, H-12), 4.68 (t, J = 5.8 Hz, 1H, H-5′), 3.83 (dd, J = 9.2, 6.8 Hz, 1H, H-11′), 3.59–3.52 (m, 1H, H-8′$_A$), 3.52–3.47 (m, 1HH-8′$_B$), 3.44 (dd, J = 14.6, 4.3 Hz, 1H, H-4$_A$), 3.33 (dd, J = 14.1, 6.1 Hz, 1H, H-16′$_A$), 3.21 (dd, J = 14.0, 5.5 Hz, 1H, H-16′$_B$), 3.04 (ddd, J = 11.5, 3.8, 2.0 Hz, 1H, H-7$_A$), 2.97–2.90 (m, 1H, H-10), 2.93–2.87 (m, 1H, H-8), 2.84–2.76 (m, 1H, H-9$_A$), 2.64 (ddd, J = 14.6, 11.1, 1.8 Hz, 1H, H-4$_B$), 2.49 (s, 3H, H-17), 2.46 (t, J = 11.6 Hz, 1H, H-7$_B$), 2.20 (ddd, J = 11.2, 9.9,4.3 Hz, 1H, H-5) 2.16–2.12 (m, 1H, H-13′), 2.13 (dd, J = 13.6, 6.8 Hz, 1H, H-10′$_A$), 2.12–2.08 (m, 1H, H-10′$_B$), 2.07–1.99 (m, 1H, H-9′$_A$), 1.92–1.80 (m, 1H, H-9′$_B$), 1.61 (q, J = 12.5 Hz, 1H, H-9$_B$), 1.12 (d, J = 6.7 Hz, 3H, H-14′/15′), 0.96 (d, J = 6.8 Hz, 3H, H-14′/15′). **^{13}C NMR** (**151 MHz**, **CD_3OD**, **ppm**) δ: 178.2 (C-18), 167.9 (C-3′), 167.2 (C-6′), 139.9 (C-17′), 135.1 (C-15), 132.8 (C-11), 131.0 (C-18′/C-22′), 129.0 (C-19′/C-21′), 127.3 (C-20′), 127.3 (C-16), 123.5 (C-13), 119.4 (C-2), 113.5 (C-12), 111.2 (C-3), 109.9 (C-14), 105.1 (C-12′), 92.2 (C-2′), 68.4 (C-5), 65.3 (C-11′), 59.8 (C-7), 58.2 (C-5′), 47.4 (C-8′), 43.3 (C-8), 43.2 (C-17), 40.9 (C-10), 40.3 (C-16′), 35.1 (C13′), 32.4 (C-9), 27.7 (C-4), 27.3 (C-10′), 23.1 (C-9′), 17.2 (C-14′/15′), 16.1 (C-14′/C-15′). **IR** (**microscope**, **cm^{-1}**): 3620, 3425, 3339, 2945, 2806, 1710, 1668, 1643, 1632, 1533, 1443, 1225, 1209, 1036, 1015.

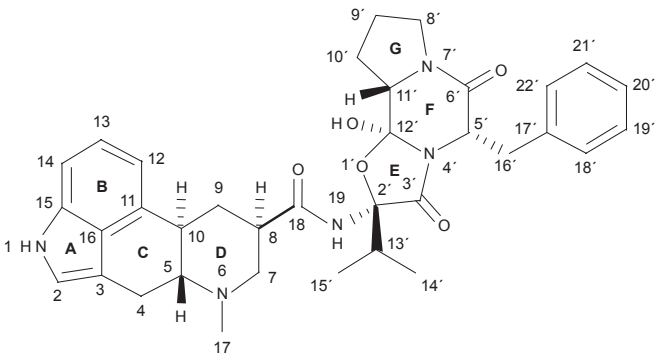

Fig. 1. Chemical structure of DHEC with the numbering system used for characterization.

2.2. Crystallization of dihydroergocristine

Dihydroergocristine was crystallized from various solvents (methanol, chloroform, dichloromethane, acetonitrile), for details see Table 1. Colourless crystals suitable for X-ray analysis were formed after several weeks on slow evaporation of the solvent at ambient temperature in the absence of light.

2.3. Instrumental

The melting point was determined by the capillary tube method using an automatic melting point meter (KSP I N, A. Krüss Optronic, Hamburg, Germany). Elemental analysis was conducted using a vario MACRO elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The NMR spectra were recorded on a Bruker Avance 600 MHz (Bruker Corporation, Billerica, USA) with the CD_3OD peak as internal standard. Carbons were assigned based on two dimensional NMR analysis (H,H-COSY, HSQC, HMQC). High-resolution mass spectra were obtained with an Exactive Benchtop Orbitrap™ mass spectrometer (Thermo Scientific™, Bremen, Germany, USA). Infrared spectra were recorded on a Bruker Equinox 55 FT-IR spectrometer (Bruker Corporation, Billerica, USA) in the range of 4.000–800 cm^{-1}. The single crystal X-ray data were collected at room temperature using a Bruker AXS SMART diffractometer with an APEX CCD area detector (Mo Kα radiation, graphite monochronator, λ = 0.71073 Å). Data reduction and adsorption correction were carried out using the Bruker AXS SAINT and SADABS packages. The structures were solved by direct methods and refined against F^2 by full-matrix least squares calculation using SHELX97 [17].

Table 1
Overview of solvents and masses of Dihydroergocristine used for crystallisation.

Solvent	m_{DHEC} [mg]	$V_{Solvent}$ [μL]
Methanol	4.1	200
Chloroform	5.6	500
Dichloromethane	2.6	1000
Acetonitrile	4.0	200