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Structure and Biosynthesis of Chrysophysarin A, a Plasmodial Pigment from the Slime Mould *Physarum polycephalum* (Myxomycetes)

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Abstract—A yellow optically-active pigment, chrysophysarin A (**1**), has been isolated from microplasmodia of the slime mould *Physarum polycephalum*. The structure was established by means of 1D and 2D NMR spectroscopy and mass spectrometry. The absolute configuration was assigned by the synthesis of *N*-(3,3-dimethylacryloyl) derivatives of (*S*)- and (*R*)-leucine and by comparison of the corresponding CD spectra. The biosynthesis of the compound was elucidated by feeding labeled acetate to the plasmodia.

Introduction

For a long time, the myxomycete *Physarum polycephalum* has been the subject of intensive research in cell biology, physiology, genetics and also in chemistry by virtue of its distinctive feature, which is moving and sporulating under the influence of light.[1] The polyenes physarochrom A,[2] physarorubinic acid A and B,[3] and the polycephalins B and C[4] have already been isolated from plasmodia of the intensely yellow-colored slime mould.

Chrysophysarin A (1)

During further chromatographic separation, a substance, chrysophysarin A (**1**), with interesting signals in the NMR spectrum, attracted our attention. Now we wish to report on the structure and biosynthesis of this compound.

Suspension-cultured microplasmodia of *P. polycephalum* were completely extracted in the dark. Consecutive stepwise partition of the red-brown gum and repeated chromatography of the chloroform phase at 4°C on Sephadex LH-20 yielded chrysophysarin A (**1**) as an amorphous golden-yellow powder.

The structure of **1** was determined by spectroscopic analyses. The high resolution mass spectrum (HR-ESI) shows a molecular ion at 356.23, establishing the molecular formula $C_{21}H_{30}N_3O_2$. In the UV spectrum, the pigment exhibits absorption maxima at λ=262 and 355 nm.

Table 1. NMR data of chrysophysarin A (**1**) in $CD_3OD/CDCl_3$ 1:1 (v/v)

^{1}H	δ_H (ppm)	$J_{H,H}$ (Hz)	^{13}C	δ_C (ppm)
N-1–CH_3 (3H)	3.88 (s)		N-1–CH_3	34.05 Q
2-H (1H)	8.98 (s)		C-2	145.49 D
N-3–CH_3 (3H)	3.93 (s)		N-3–CH_3	36.44 Q
4-H (1H)	7.69 (s)		C-4	119.82 D
			C-5	134.81
6-H (1H)	6.47 (d)	15.7	C-6	114.72 D
7-H (1H)	6.98 (dd)	9.9, 15.7	C-7	136.42 D
8-H (1H)	6.60 (m)		C-8	134.82 D
9-H (1H)	6.60 (m)		C-9	137.20 D
10-H (1H)	6.68 (dd)	10.3, 14.3	C-10	139.30 D
11-H (1H)	6.52 (dd)	11.2, 14.3	C-11	133.62 D
12-H (1H)	7.25 (dd)	11.2, 15.0	C-12	141.30 D
13-H (1H)	6.16 (d)	15.0	C-13	124.89 D
			C-14	167.85
15-H (1H)	4.55 (dd)	4.3, 10.3	C-15	58.45 D
16-H (2H)	1.53 (m)			
	1.60 (m)		C-16	39.73 T
17-H (1H)	1.71 (m)		C-17	25.59 D
18-H (3H)	0.97 (d)	6.6	C-18	21.66 Q
19-H (3H)	0.93 (d)	6.6	C-19	23.40 Q
			C-20	209.52
21-H (3H)	2.21 (s)		C-21	26.86 Q

The ^{1}H NMR spectrum displays the signals of a tetraene system (δ 7.25–6.16). Two ^{1}H resonances at δ 8.89 and 7.69 are assigned to an imidazole ring according to their shift values and coupling constants (145.49 ppm, J=215 Hz; 119.82 ppm, J=195 Hz). Both nitrogens bear methyl groups with ^{1}H shift values at δ 3.88 and 3.93. ^{1}H–^{1}H COSY, HMBC and HMQC experiments connect signals at δ 4.55 and between 1.71 and 0.93 to a leucine

Keywords: slime mould; myxomycetes; *Physarum polycephalum*; natural products; polyene; imidazole.

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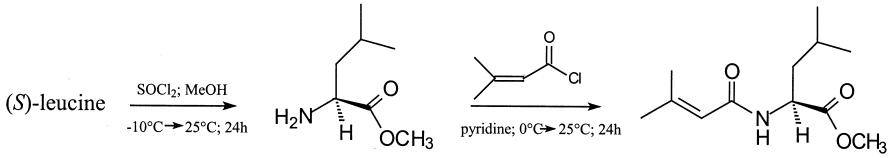
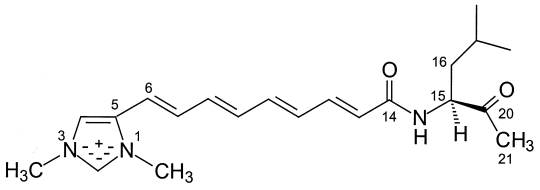


Scheme 1. Synthesis of *N*-(3,3-dimethylacryloyl)-(*S*)-leucine methylester (**2**).

residue, whose carboxy group is modified to an acetyl moiety. Long-range couplings in the ^{1}H and ^{13}C NMR spectra link the imidazole ring to a polyene chain and C-14 over the amide nitrogen to C-15 of leucine.

Therefore chrysophysarin A has the structure **1**.



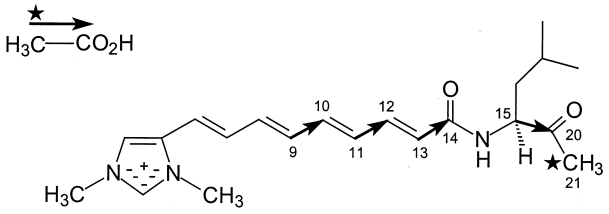
To identify the absolute configuration at position C-15 the *N*-(3,3-dimethylacryloyl) derivatives of (*S*)- and (*R*)-leucine methylester were synthesized as shown in Scheme 1 and their CD spectra compared.

The synthesis started with (*S*)- and (*R*)-leucine, respectively, which were transformed with thionyl chloride/MeOH into the corresponding methyl esters.[5] The (*S*)- and (*R*)-leucine esters were converted with 3,3-dimethylacryloyl chloride and pyridine to the corresponding amides, which were obtained as colorless crystals.

The CD spectra of chrysophysarin and of the (*S*)-leucine derivate (**2**) show an almost parallel course. Therefore evidence is given for the (*S*)-configuration in C-15 of chrysophysarin A (**1**).

Biosynthesis

For the investigation of the biosynthesis, chrysophysarin A (**1**) was isolated, on the one hand from plasmodia, which had been fed with [2-^{13}C]sodium acetate and, on the other hand, from plasmodia, which had been grown with [$^{13}C_2$–2-d_3]sodium acetate. The incorporation of the labeled precursors has been proven by 1D ^{13}C NMR and ^{13}C–^{13}C COSY NMR spectra. The ^{13}C–^{13}C COSY NMR spectrum shows the intact acetate units C9–C10, C11–C12 and C13–C14 with an incorporation rate of 4.5%. The carbons C-15 and C-20 originate from an additional acetate unit, whose incorporation rate is 1.4%. The incorporation of acetate in this position is in accordance with the common biosynthesis of leucine from valine and acetyl-CoA.[6]



An incorporation rate of 4.6% into carbon C-21, not only by feeding [$^{13}C_2$–2-d_3]sodium acetate but also by feeding [2-^{13}C]sodium acetate, shows that the methyl group C-21 originates from a fragmented acetate unit, whose carboxy group was presumably lost by decarboxylation. A similar procedure, but without fragmentation, is used to elongate the carbon chain by the insertion of histidine for the construction of the polyene system: histidine is transformed into the corresponding α,β-unsaturated acid and connected by means of its carboxy group to three acetate units.

Therefore L-leucine, acetate and histidine are proposed as biosynthetic building blocks for the biosynthesis of chrysophysarine A (**1**).

Experimental

General

NMR: Bruker AMX2-600 (600.28 MHz for ^{1}H and 150.9 MHz for ^{13}C; chemical shifts are given relative to the residual solvent signal CD_3OD: δ_H=3.35, δ_C=49.0; $CDCl_3$: δ_H=7.24, δ_C=77.0). – HR-EI MS, HR-ESI MS: Finnigan MAT 95Q (EI: DI, 210°C, 70 eV; ESI: 250°C, 2.5 kV). – IR: Perkin-Elmer Spectrum 1000. – UV/VIS: HP 8452A Diode Array Spectrophotometer. – CD: Instruments S. A. Jobin Yvon CD-6-Dichrograph. – Analytical TLC: aluminium sheets silica gel 60 F_{254} (Merck), 0.2 mm, benzene/ethyl formate/formic acid, 10:5:3). – CC:Sephadex LH-20 (Pharmacia); eluent: MeOH.

Culture conditions

Microplasmodia of the yellow wild type strain of *P. polycephalum* were cultured under axenic conditions according to the literature.[7] Cells were harvested after 7 days by centrifugation (2500 g) and subsequently washed with 1 mM $Ca(H_2PO_4)_2$/10 mM glucose.[8]

Application of labeled precursors

[2-^{13}C]sodium acetate or [$^{13}C_2$–2-d_3]sodium acetate were added to the standard medium before autoclavation. The concentration was 50 mg acetate in 100 ml medium (pH=4.75).

Extraction and isolation of chrysophysarin A (1)

All the steps of extraction and isolation were carried out in the dark because the *Physarum* pigments are very light sensitive. The wet biomass (250 g) was completely extracted with acetone/methanol/chloroform/ethyl acetate 3:2:1:1 (v/v/v/v) in a blender at room temperature, and the solvent was removed by evaporation. The crude extract (5.5 g, oil) was partitioned first between 200 ml methanol/water 9:1 (v/v) and *n*-hexane (3×100 ml). The combined *n*-hexane phases were washed once with methanol/water 9:1 (100 ml). The methanol/water layers were combined, the solvent was evaporated, and the residue was dissolved again in 200 ml of water and extracted with chloroform (3×100 ml). Repeated chromatography of the chloroform phase (337 mg) at 4°C on Sephadex LH-20 with methanol (80 cm×6 cm, i.d.) yielded a faint yellow fraction, which gave on evaporation pure chrysophysarin A (**1**) (26 mg, 0.000104%) as an amorphous golden-yellow powder.

Chrysophysarin A (1). TLC: R_f=0.12. – UV/VIS (MeOH): λ_{max} (log ϵ)=262 nm (0.40), 341 (1.28), 355 (1.55), 372 (1.15). – CD (MeOH): λ_{max} ($\Delta\epsilon$)=206 (−0.63), 223 (+0.08), 250 (−0.17), 297 (+0.03), 354 (+0.23). – ^{1}H NMR and ^{13}C NMR see Table 1. – IR (KBr): $\tilde{\nu}$ = 3400 (s), 2923 (s), 2852 (m), 1711 (w), 1619 (s), 1466 (m), 1396 (m), 1016 (w), 951 (w). – HR-ESI MS: *m*/*z*= 356.2327 (356.2338 calcd. for $C_{21}H_{30}N_3O_2$) [M^+].

***N*-(3,3-Dimethylacryloyl)-(*S*)-leucine methylester (2).** 10 ml thionyl chloride was carefully added dropwise to 5.0 g (38.1 mmol) L-leucine in 50 ml dry methanol at −10°C. The solution was allowed to warm up and then stirred at room temperature for 24 h. After evaporation and recrystallisation from ether, the leucine methylester was obtained as colorless crystals (yield: 5.43 g, 29.9 mmol, 78.5%). The methylester (500 mg, 2.75 mmol) was dissolved in 20 ml absolute pyridine and 0.5 ml 3,3-dimethylacryloyl chloride was added at 0°C. The resulting mixture was stirred for 24 h at room temperature, poured into water and extracted three times with ethyl acetate. The combined organic phases were washed with water, sodium bicarbonate (pH=8), citric acid (pH=3), water and brine and dried over magnesium sulfate. The *N*-(3,3-dimethylacryloyl)-(*S*)-leucine methylester (**2**) was obtained as colorless crystals (yield: 443 mg, 1.95 mmol, 70.9%). – TLC: R_f=0.45. – UV/VIS (MeOH): λ_{max} (log ϵ)=219 nm (1.09). – CD (MeOH): λ_{max} ($\Delta\epsilon$)=208 (−4.41), 221 (−2.02), 232 (−2.66), 267 (+0.06). – ^{1}H-NMR ($CD_3OD/CDCl_3$ (1:1 (v/v)): δ=5.75 (m, 1H), 4.52 (m, 1H), 3.73 (s, 3H), 2.12 (d, 3H, *J*=1.1 Hz), 1.87 (d, 3H, *J*=1.1 Hz), 1.69 (m, 1H), 1.62 (m, 2H), 0.96 (d, 3H, *J*=6.6 Hz), 0.94 (d, 3H, *J*=6.6 Hz). – ^{13}C NMR ($CD_3OD/CDCl_3$, 1:1 (v/v)): δ=174.71, 168.77, 152.27, 118.49, 52.45, 51.38, 41.18, 27.31, 25.42, 23.07, 21.85, 20.03. – IR (KBr): $\tilde{\nu}$=3435 (m), 3302 (s), 3072 (w), 2958 (m), 2871 (m), 1745 (s), 1664 (s), 1634 (s), 1545 (s), 1270 (m), 1215 (s), 1183 (s), 1153 (m), 980 (w). – HR-EI MS: *m*/*z*=227.1518 (227.1521 calcd. for $C_{12}H_{21}NO_3$) [M^+].

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