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NEOSARTORIN, AN ERGOCHROME BIOSYNTHESIZED BY *NEOSARTORYA FISCHERI*

B. PROKSA,*† D. UHRÍN,‡ T. LIPTAJ§ and M. ŠTURDÍKOVÁ¶

†Slovakofarma a.s., SK-920 27 Hlohovec, Slovakia; ‡Department of Chemistry, University of Edinburgh, Edinburgh, United Kingdom; §Central Laboratories, Faculty of Chemistry, Technical University, Bratislava, Slovakia; ¶Department of Biochemical Technology, Faculty of Chemistry, Technical University, Bratislava, Slovakia

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Key Word Index—*Neosartorya fischeri*; fungus; ergochrome, 2,4′-bistetrahydroxanthone; neosartorin.

Abstract—The yellow pigment, named neosartorin was isolated from the mycelium of *Neosartorya fischeri*. Its structure, representing an asymmetric ergochrome, was deduced from spectral data, mainly NMR experiments.

INTRODUCTION

Neosartorya fischeri (*Trichocomaceae*), a soil mould phylogenetically related to *Aspergillus fumigatus* [1], is an agent participating in fruit fouling [2]. *N. fischeri* fermented on various media biosynthesized tryptoquivaline mycotoxins fiscalins [3]; terreine, fumitremorgins, verruculogen [4] or verruculogen-like ardeemins [5]. However, some strains of *N. fischeri* biosynthesized antifungal metabolites, which are derivatives of xanthocillin [6].

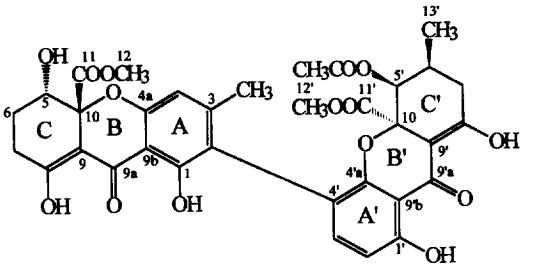
We identified a new yellow pigment, denoted neosartorin (**1**) in the mycelium of *N. fischeri*. Production, isolation and structural elucidation of compound **1** is described in this paper.

RESULTS AND DISCUSSION

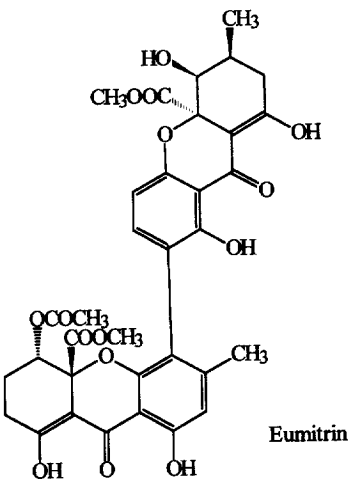
A new metabolite, named neosartorin (**1**), was identified in the mycelium of *N. fischeri* grown on glucose/glycerol medium. The maxima observed at 279 and 336 nm in the UV spectrum are typical of those of a chromanone chromophore. The absorption band at 336 nm was shifted to 351 nm in methanolic KOH, indicating phenolic hydroxyls. The IR spectrum of neosartorin (**1**) confirmed the presence of a hydroxyl hydrogen bonded to a carbonyl group, and of carboxymethyl and acetoxy carbonyls attached to a benzene ring. The mass spectrum with M^+ (*m/z* 680), M-59, M-59-42, M-59-60, and M-59-60-60 was characteristic of ergochromes, e.g. secalonic acid [7] or eumitrin A_1 [8]. Neosartorin (**1**) is an isomer of the latter as determined from the analysis of its NMR spectra. The one-bond C-H correlations were established in a HSQC experiment [9], while the long-range coupling constants were detected in a ge-HMBC experiment [10]. The last experiment was crucial for the structure elucidation of compound **1**. A summary of the long-range proton-carbon correlations observed in a HMBC spectrum optimized for an 8 Hz coupling constant is given in Table 1 together with the relative intensities of cross peaks. ^{1}H-^{1}H chemical shift correlation and coupling constants were determined from a series of 1D COSY spectra [11] and spatial proximities were estimated on the basis of 1D NOESY experiments [12]. Proton and carbon chemical shifts are summarized in Table 2 and Table 3, respectively.

Contrary to eumitrin A_1 (**2**), where the connection of C-4—C-2′ was identified, both xanthene nuclei in structure **1** are coupled through C-2—C-4′. This was unequivocally determined by the presence of long-range coupling constants between H-3′ and C-2 (s, strong), C-1 (w, weak) and C-3 (w), together with a weak ^{4}J(H-4, C-4′) coupling constant and coupling constants between protons of C-3—CH_3 and C-4′a (w), C-3′ (w) and C-4′ (w). Compounds **1** and **2** also have different substituents on rings C and C′: the acetoxy group is attached to C-5′ of neosartorin (**1**), while eumitrin A_1 (**2**) has an acetylated C-5—OH. The relative configuration of compound **1** was determined on the basis of ^{1}H-^{1}H coupling constants. Coupling constants *J*(H-5, H-6ax) = 2.0 Hz and *J*(H-5, H-6eq) = 4.0 Hz are consistent with an equatorial orientation of H-5. The same orientation was also found for the C′ ring. The large coupling constant *J*(H-6′, H-

*Author to whom correspondence should be addressed.



Neosartorin (1)



Eumitrin A1 (2)

7′ax) = 10.0 Hz suggests that the H-6′ proton is axial, thereby the C-6′—CH_3 must be in the equatorial position. The signal of H-6′ is split by a 1.5 Hz coupling constant to proton H-5′ which therefore is equatorial. Carboxymethyl moieties at C-10 and C-10′ are in a *cis*-position with respect to protons H-5 and H-5′, respectively, as shown by the presence of NOEs between CH_3 protons of these groups and protons H-5 and H-5′. Minimum energy conformers a *trans*-position of these protons and their respective carboxymethyl groups show dihedral angles which are inconsistent with experimental proton-proton coupling constants in ring C and C′. The relative configuration of both substructures of neosartorin (**1**) is therefore the same and identical to that of ergochrome AA (secalonic acid A) or its antipode ergochrome EE (secalonic acid D). The observed NOEs between the H-12′ protons of the carboxymethyl group and C-1—OH and C-8—OH protons as well as between C-5′—$COOCH_3$ protons and C-3—CH_3 suggested that the rings A,A′ are not coplanar.

So far ergochromes were detected as secondary metabolites of *Claviceps* spp. , *Penicillium oxalicum*, *Aspergillus ochraceus* and various lichen species [13]. This is the first isolation of an ergochrome from the mycelium of *Neosartorya fischeri*.

EXPERIMENTAL

Elemental analysis: EA 1108 (Fisons); IR and UV: FT-IR MAGNA 750 (Nicolet) and UV-VIS (Zeiss) spectrometer, respectively; EI-MS: SSQ 700 (Finnigan) spectrometer, 70 eV, 180°; NMR: INOVA 600 (Varian) spectrometer equipped with a 5 mm z-gradient triple-resonance inverse probe. Spectra were run at 25° using 20 mg of sample dissolved in 600 μl of $CDCl_3$. The $CHCl_3$ signal was used for referencing at 77.06 ppm for ^{13}C and 7.26 ppm for ^1H. The optical rotation: model 121 (Perkin Elmer) polarimeter. TLC: Silufol UV-254 sheets (Kavalier); HPLC: Separon SGX C-18 7 μm 150 × 3 mm column (Tessek), MeOH – H_2O 4:1, 0.8 ml/min. Column chromatography: Kieselgel 60, 0.063 – 0.200 mm (Merck), 25 × 3 cm column. MM2 calculations were performed with Chem3D program (Cambridge Soft Corporation).

Microbial strain and cultivation

N. fischeri isolated from the river Vah sediments in Slovakia and deposited in the Culture Collection of the Department of Biochemical Technology, Bratislava, was used. The culture was maintained on Czapek Dox agar at 28°. Inoculation medium consisted of (g/l): sucrose (40), corn steep liquor (10), $NaNO_3$ (2), KH_2PO_4 (1), KCl (0.5), $MgSO_4.7H_2O$ (0.5), $FeSO_4.7H_2O$ (0.01); pH adjusted to 6.5. This medium inoculated with spores of *N. fischeri* was cultivated for 24 hr at 28°, then 10 ml of vegetative inoculum was transfered into 500 ml flasks containing 100 ml of production medium composed of (g/l): glycerol (60), glucose (30), corn steep liquor (10), $NaNO_3$ (2), KH_2PO_4 (1), KCl (0.5), $MgSO_4.7H_2O$ (0.5), $FeSO_4.7H_2O$ (0.01); pH adjusted to 6.5. The inoculated medium was cultivated at 28° on a rotary shaker (3.7 Hz) for 168 hr.

Isolation of neosartorin

Mycelium obtained after filtration of fermentation beer (pH 5.6, 1.5 l) was extracted with Me_2CO (3 × 300 ml) and the extracts conc. A final extraction was carried out using EtOAc (3 × 100 ml). The organic layers were then combined, dried and conc *in vacuo*. The residue was triturated with Et_2O and the insoluble portion (1.8 g) was dissolved in $CHCl_3$ and chromatographed on a column of silica gel, eluted with $CHCl_3$ – MeOH (19:1). Elutant fractions were analysed by TLC on Silufol sheets in $CHCl_3$ – C_6H_6 – MeOH (5:5:1); detection at 254 nm and spraying with $FeCl_3$ soln(dark brown spot). Fractions containing compound with R_f 0.55 were concd. and the residue, crystallized from Me_2CO – Et_2O (1:1), afforded yellow crystals of compound **1** (740 mg) which gave the single peak (R_t = 5.5 min) in HPLC. For $C_{34}H_{32}O_{15}$ (M_r = 680.63), calc.: C: 60.00, H 4.74; found C: 60.14, H 4.90; $[\alpha]_{20,D} = -197°$ (c = 1, $CHCl_3$). UV: λ_{max} (ϵ, $m^2\,mol^{-1}$)/MeOH: 265 (674), 279 (762), 286 (719), 336

Table 1. Summary of the intra-residue[a] long-range proton-carbon interactions as observed in the HMBC spectrum of neosartorin (**1**)

	C															
H	1	2	3	4	4a	5	6	7	8	9	9a	9b	10	11	C-3-CH_3	
4	w	s			s						m	s			s	
5							w			m	w		m	w		
6ax								w								
6eq						s		s	s				s			
7ax						w	s		s	s	w		w			
7eq						s	s		s				w			
12													w	s		
C-1-OH	s	s	m								m	s			w	
C-3-CH_3	w	s	s	s	w						v		m			
C-8-OH							m	s	s	s	m		v			
	C															
H	1′	2′	3′	4′	4′a	5′	6′	7′	8′	9′	9′a	9′b	10′	11′	13′	C-5′CO
2′	s		w	s	m							s				
3′	s	m			s							m				
5′								s		s			s		s	s
6′						m			m	m			m	m	s	
7′ax,eq						s		s	m	s			m		s	
12′													w	s		
13′						s		s	w				w			
C-1′-OH	s	s	m									m	s			
C-8′-OH							m	s	s	s	m		v			

[a] inter residue interactions are given in the text; s — strong, m — medium, w — weak, v — very weak.

Table 2. ^{1}H NMR data of neosartorin (**1**) in $CDCl_3$

H	δ/ppm	$J_{H,H}$
4	6.47 *s*	
5	4.36 *dd*	5,6ax = 1.7; 5,6eq = 4.1
6ax	1.97 *m*	6ax,6eq = 14.9; 6ax,7ax = 11.4; 6ax,7eq = 6.9
6eq	2.14 *m*	6eq,7ax = 7.0; 6eq,7eq = 1.0
7ax	2.83 *m*	7ax,7eq = 19.1
7eq	2.37 *m*	
12	3.78 *s*	
C-1—OH	11.53 *s*	
C-3—CH_3	2.07 *s*	
C-8—OH	13.90 *s*	
2′	6.58 *d*	2′,3′ = 8.5
3′	7.12 *d*	
5′	5.28 *d*	5′,6′ = 1.4
6′	2.31 *m*	6′,13′ = 6.5; 6′,7′ax = 11.3; 6′,7′eq = 6.5
7′eq	2.37 *m*	7′ax,7′eq = 18.8
7′ax	2.37 *m*	
12′	3.64 *s*	
13′	0.92 *d*	
C-1′—OH	13.78 *s*	
C-5′-$OCOCH_3$	1.92 *s*	
C-8′—OH	11.36 *s*	

Table 3. ^{13}C NMR data of compound **1** in $CDCl_3$

C	δ/ppm	C	δ/ppm
1	159.9	1′	161.8
2	118.4	2′	110.0
3	148.5	3′	139.8
4	108.9	4′	114.5
4a	156.7	4′a	155.3
5	66.8	5′	69.2
6	22.9	6′	27.6
7	24.2	7′	32.4
8	178.5	8′	177.7
9	100.4	9′	100.0
9a	187.7	9′a	187.8
9b	104.7	9′b	106.7
10	83.8	10′	81.9
11	171.0	11′	170.5
12	53.6	12′	53.2
C-3—CH_3	20.8	13′	17.2
		C-5′–OCO	169.0
		C-5′–$OCOCH_3$	20.3

(3146), λ_{max} (ϵ, $m^2 mol^{-1}$)/0.1 M KOH: 287 (360), 351 (3416). IR $\nu_{max}^{KBr} cm^{-1}$ 3384, 1742, 1617, 1588, 1560, 1483, 1434. MS *m/z* (rel. int.) 680 (19, M^+), 637 (1, M-43), 621 (100, M-59), 579 (3, M-59-42), 561 (38, M-59-60), 501 (4, M-59-60-60), 455 (2), 377 (8), 281 (9), 251 (10), 193 (4) 169 (3), 137 (8), 109 (6), 60 (5), 43 (14).

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