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Three pyrrolyloctatetraenyl-α-pyrones from *Auxarthron conjugatum*

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Abstract

The red pigments, auxarconjugatins A, B and C, were isolated from mycelia of *Auxarthron conjugatum*, an ascomycetous fungus belonging to the Onygenaceae, in which the causative fungi of severe mycoses also belong. The structures of auxarconjugatins A, B and C, including the stereochemistry of the conjugated tetraene, were established by spectroscopic analyses. These compounds were in equilibrium with a few double bond stereoisomers of the double bonds when dissolved in MeOH or MeCN. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Onygenaceae; Pathogenic fungus; *Auxarthron conjugatum*; Auxarconjugatin; Pyrrolyloctatetraenyl-α-pyrone

1. Introduction

The causative fungi of severe mycoses (e.g. coccidiodomycosis caused by *Coccidioides immitis* Rixford & Gilchrist, paracoccidiodomycosis by *Paracoccidioides brasiliensis* (Splendore) Almeida, blastomycosis by *Blastomyces dermatitidis* Gilchrist & Stokes (teleomorph: *Ajellomyces dermatitidis* McDonough & Lewis), and histoplasmosis by Histoplasma capsulatum Dahling [teleomorph: *Ajellomyces capsulatus* (Kwon-Chang) McGinnis & Katz]), are all dimorphic hyphomycetes, the teleomorph of which should belong to the family Onygenaceae, in the order Onygenales (De Hoog & Guarro, 1995, see also pp. 121–128). *Auxarthron conjugatum* (Kuehn) Orr & Kuehn (anamorph: *Malbranchea* sp.) also belongs to the Onygenaceae and is taxonomically close to the above pathogenic fungi. This fact prompted us to investigate the chemical constituents of *A. conjugatum* (strain CBS 247.58), isolated from soil from Arizona in the USA (Centraalbureau voor Schimmelcultures, 1995). Three new red pigments, auxarconjugatins A (**1**) to C (**3**), were isolated from the mycelium of this fungus and their structures elucidated.

2. Results and discussion

2.1. Structure of auxarconjugatin A (*1*)

The molecular formula of auxarconjugatin A (**1**) was confirmed as $C_{19}H_{18}NO_3Cl$ by high resolution EIMS and elemental analysis. The ^{1}H-NMR spectrum of **1** showed 14 signals, which were assigned to one singlet methyl signal (δ 1.82), one methoxy signal (δ 3.91), one NH signal (δ 11.38) and 11 olefinic methine protons (δ 6.12–7.48). The ^{13}C-NMR spectrum, however, showed signals for 19 carbons (Table 1) and a DEPT experiment assigned these signals to 2 methyl groups, 11 sp^2 methines, and 6 quaternary carbons including one ester carbonyl carbon and 2 oxygenated sp^2 carbons.

The ^{1}H–^{1}H COSY spectrum established a conjugated tetraene structure for C-1′ to C-8′. The above double bonds apparently possessed a *E*-configuration at C-1′, C-5′ and C-7′ and *Z*-configuration at C-3′

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Table 1
^{1}H and ^{13}C NMR chemical shifts of auxarconjugatins (1–4) in DMSO–d_6

Carbon	1		1a		2		3		4	
No.	δ_C	δ_H (Hz)	δ_C	δ_H (Hz)	δ_C	δ_H (Hz)	δ_C	δ_H (Hz)	δ_C	δ_H
2	163.4		163.4		162.6		163.1		163.4	
3	100.8		100.7		88.4	5.58 d (2.0)	100.8		100.3	
3-Me	8.8	1.82 s	8.9	1.81 s			8.5	1.82 s	8.5	1.92 s
4	165.9		166.0		170.8		165.7		165.7	
4-OMe	56.7	3.91 s	56.7	3.89 s	56.3	3.80 s	56.4	3.90 s	56.6	3.95 s
5	96.8	6.72 s	96.5	6.62 s	100.7	6.23 d (2.0)	96.0	6.58 s	96.2	6.50 s
6	156.9		157.1		158.4		157.0		159.1	
1′	123.0	6.37 d (15.3)	122.1	6.31 d (15.3)	121.6	6.29 d (15.2)	121.3	6.29 d (15.3)	124.8	
1′-Me									21.1	2.05 s
2′	129.1	7.48 dd (15.3, 12.5)	134.6	7.06 dd (15.3, 11.3)	135.2	7.04 dd (15.2, 11.6)	134.5	7.06 dd (15.3, 11.6)	134.7	6.47 d
3′	126.8	6.19 dd (12.5, 11.3)	130.7	6.49 dd (14.6, 11.3)	130.5	6.47 dd (14.3, 11.6)	129.6	6.44 dd (14.6, 11.6)	128.9	7.16 dd
4′	134.4	6.38 t (11.3)	138.6	6.71 dd (14.6, 11.3)	138.9	6.72 dd (14.3, 11.3)	138.5	6.70 dd (14.6, 11.3)	137.9	6.54 dd
5′	126.5	6.90 dd (14.5, 11.3)	131.2	6.40 dd (14.6, 11.3)	131.1	6.40 dd (14.7, 11.3)	129.6	6.35 dd (14.6, 11.3)	131.3	6.37 dd
6′	136.9	6.63 dd (14.5, 11.3)	136.8	6.61 dd (14.6, 11.0)	136.9	6.61 dd (14.7, 11.3)	136.9	6.55 dd (14.6, 10.6)	135.9	6.59 dd
7′	125.0	6.88 dd (15.6, 11.3)	124.9	6.76 dd (15.6, 11.0)	124.7	6.76 dd (15.6, 11.3)	123.1	6.65 dd (15.6, 10.6)	124.8	6.75 dd
8′	120.9	6.53 d (15.6)	120.8	6.52 d (15.6)	120.8	6.52 d (15.6)	125.0	6.60 d (15.6)	120.2	6.49 d
1″ (NH)		11.38 br s		11.39 br s		11.46 br s		11.01 br s		11.44 dd
2″	126.0		126.1		126.0		130.4		125.9	
3″	111.8		112.0		111.9		109.7	6.24 brs	111.5	
4″	109.1	6.12 dd (2.9, 2.6)	109.2	6.11 dd (2.8, 2.4)	109.2	6.12 dd (2.7, 2.4)	109.2	6.07 ddd (3.0, 2.3)	109.0	6.13 dd
5″	120.4	6.89 dd (3.2, 2.9)	120.5	6.87 dd (3.1, 2.8)	120.5	6.89 dd (3.1, 2.7)	120.3	6.81 ddd (3.0, 2.6)	120.2	6.90 dd

from the value of coupling constants ($J_{1',2'} = 15.3$ Hz, $J_{3',4'} = 11.3$ Hz, $J_{5',6'} = 14.5$ Hz, and $J_{7',8'} = 15.6$ Hz). Moreover, the configuration at C-3′ was confirmed by nuclear Overhauser enhancement (NOE) at 4′-H (δ 6.38) on irradiation of 3′-H (δ 6.19) in the differential NOE spectrum (Fig. 1).

HMBC correlations (Fig. 1) were observed between 3-CH_3 (δ 1.82) to C-2 (δ 163.4), C-3 (δ 100.8) and C-4 (δ 165.9), between the 4-OCH_3 (δ 3.91) to C-4, and between 5-H (δ 6.72) to C-4 and C-6 (δ 156.9). Consideration of the number of oxygen atoms in **1** and the chemical shifts of C-2 and C-6, one oxygen atom must be inserted between C-2 and C-6 to form a lactone ring. Thus **1** contains a 4-methoxy-3-methyl-α-pyrone residue which is connected to the tetraene moiety at C-1′, since HMBC correlations between 5-H and C-1′ (δ 123.0), 1′-H (δ 6.37) and C-6, and 2′-H (δ 7.48) and C-6 were observed.

In a decoupling experiment irradiating at the imine proton (δ 11.38), the two methine protons of 4″-H [δ 6.12 (dd, J = 2.9 and 2.6 Hz)] and 5″-H [δ 6.89 (dd, J = 3.2 and 2.9 Hz)] were changed into doublets. The following correlations were observed in the HMBC spectrum: 4″-H to C-5″ (δ 120.4), 5″-H to C-3″ (δ 111.8) and C-4″ (δ 109.1) (Fig. 1). These data indicated the presence of a 2,3-disubstituted pyrrole ring. The HMBC correlations from H-7′ (δ 6.88) to C-2″ and C-3″, and H-8′ (δ 6.53) to C-2″ (δ 126.0), indicated that the pyrrole ring was connected to C-8′ of the tetraene residue. Auxarconjugatin A (**1**) is therefore (1′*E*,3′*Z*,5′*E*,7′*E*)-6-(8-(3-chloro-1*H*-pyrrol-2-yl)-1,3,5,7-octatetraenyl)-4-methoxy-3-methyl-2*H*-pyran-2-one. From the various NOE experiments (Fig. 1), the conformation of **1** in DMSO was assumed to be as shown in Fig. 1.

Compound **1a** was also isolated from the mycelial extract of *A. conjugatum* along with **1**. From the analysis of the various spectra, it was clear that **1a** was a stereoisomer of **1**. From the detailed analysis of the HMQC and HMBC spectra observed as similar correlations to those of **1** in Fig. 1, the structure of **1a** was confirmed to be (1′*E*,3′*E*,5′*E*,7′*E*)-6-(8-(3-chloropyrrol-2-yl)-1,3,5,7-octatetraenyl)-4-methoxy-3-methyl-2*H*-pyran-2-one. Compounds **1** and **1a** are stereoisomers of auxarconjugatin A, the 3′-*cis*-form and all-*trans*-form respectively.

When **1** was dissolved in MeCN or MeOH, **1a** slowly formed and became the main component along with two other minor compounds after several days. The structure of auxarconjugatin A (**1** and/or **1a**) was not changed in the solid state or in DMSO solution. From analysis of the time course by HPLC, it is clear that auxarconjugatin A is in equilibrium with four

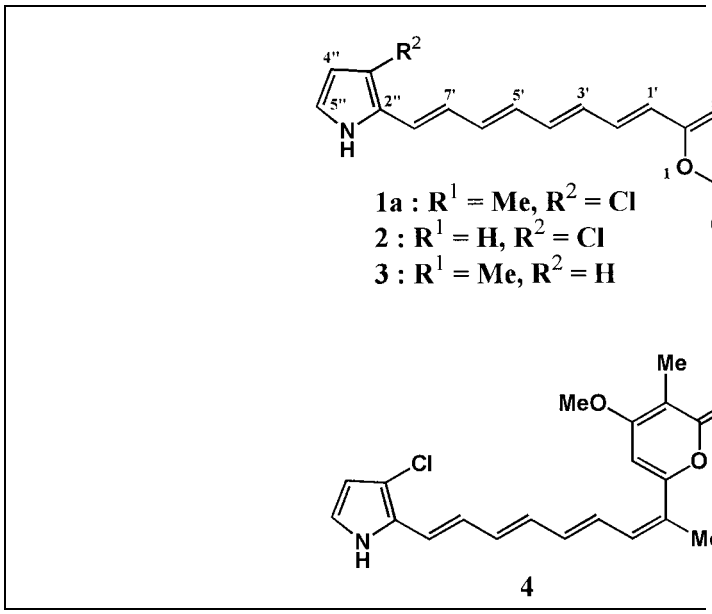
double bond stereoisomers and that the all-*trans*-form (**1a**) is the most stable and main component.

2.2. Structure of auxarconjugatins B (2) and C (3)

The molecular formulae of auxarconjugatins B (**2**) and C (**3**) were confirmed as $C_{18}H_{16}NO_3Cl$ and $C_{19}H_{19}NO_3$, respectively, by high resolution EIMS. The ^{1}H-NMR spectra of **2** and **3** were similar to those of **1a** (Table 1), except that two olefinic protons [δ 5.58 (d, J = 2,0 Hz) and 6.23 (d, J = 2,0 Hz)] appeared in the α-pyrone ring of two instead of one olefinic proton [δ 6.62 (s)] in **1a** and the methyl signal (δ 1.81 in **1a**) in **2** had disappeared. In addition three olefinic protons (δ 6.07, 6.24 and 6.81) were presented in the pyrrole ring of **3** instead of two olefinic proton (δ 6.11 and 6.87) in **1a**. The above results and the molecular formulae suggested that **2** and **3** should be the 3-demethyl derivative and the 3″-dechloro derivative of **1**, respectively.

The structures of auxarconjugatins B (**2**) and C (**3**) were confirmed from the detailed analysis of the HMQC and HMBC spectra, observed as similar HMBC correlations to those of **1** in Fig. 1, to be 6-(8-(3-chloropyrrol-2-yl)-1,3,5,7-octatetraenyl)-4-methoxy-2*H*-pyran-2-one and 6-(8-(pyrrol-2-yl)-1,3,5,7-octatetraenyl)-4-methoxy-3-methyl-2*H*-pyran-2-one, respectively. The stereochemistry of the tetraene moieties of **2** and **3** was determined as all-*trans* by consideration of the coupling constants of the vinyl protons ($J_{1',2'}$ = 15.2 Hz, $J_{3',4'}$ = 14.3 Hz, $J_{5',6'}$ = 14.7 Hz and $J_{7',8'}$ = 15.6 Hz for **2**; $J_{1',2'}$ = 15.3 Hz, $J_{3',4'}$ = 14.6 Hz, $J_{5',6'}$ = 14.6 Hz and $J_{7',8'}$ = 15.6 Hz for **3**). From HPLC analysis, auxarconjugatins B (**2**) and C (**3**) were also the main products of the equilibrium between 3 or 4 isomers in MeCN, though these minor isomers have not been isolated.

A pyrrolyloctatetraenyl-α-pyrone, rumbrin (**4**), was recently isolated from the mycelium of *Auxarthron umbrinum* (Yamagishi, Shindo & Kawai, 1993), as a cytoprotective substance and antioxidant (Yamagishi, Matsuoka, Odagawa, Kato, Shindo & Mochizuki, 1993). Compound **4** has one *cis*-double bond at C-1′ in the conjugated tetraene residue, but this compound might be in equilibrium with all-*trans* form as in the case of **1** and **1a**. It is assumed that auxarconjugatins A (**1**) to C (**3**) will have similar biological activity to that of **4**.



3. Experimental

3.1. General

Mps: uncorr; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR: TMS as int. standard; LPLC: Ultra Pack ODS-40B column (Amazon, 300 × 26 i.d. mm); HPLC: Senshu Pack PEGASIL-ODS prepacked column (250 × 20 i.d. mm) for isolation and a Capcell-Pack C18 UG120 prepacked column (250 × 4.6 i.d. mm) for analysis, monitored at 433 nm; TLC: precoated Kieselgel 60 F_{254} plates, detection by their own colouration.

3.2. Isolation of auxarconjugatins A (1), B (2) and C (3)

A. conjugatum, strain CBS 247.58, was cultivated at 25°C on shaking for 7 days in 90 flasks containing 200

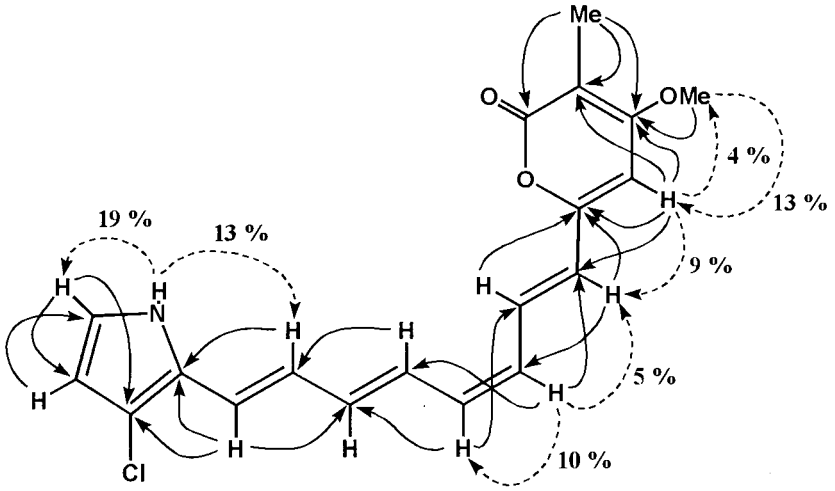


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Fig. 1. Correlations in the HMBC spectrum and differential NOE observations of auxarconjugatins A (**1**).

The arrow indicates the HMBC correlation from proton H_A to carbon C_B. Arrow with dotted line indicates the NOE on proton H_B irradiated at proton H_A.

ml of yeast-malt extract medium in each flask. The mycelium was extracted with MeOH. The organic layer was dried over Na_2SO_4 and then evaporated in vacuo. The obtained residue (8.2 g) was chromatographed on silica gel with $CHCl_3$–Me_2CO (50:1), followed by LPLC (80% MeOH) and then HPLC (70% MeCN) to give auxarconjugatins C (**3**) (3 mg), B (**2**) (5 mg) and A(**1**) (13 mg) (3.5 mg as **1a**-form).

Auxarconjugatin A (**1**): Red crystalline powder, mp 123–125°C (from H_2O–MeOH). Anal. Calcd for $C_{19}H_{18}NO_3Cl$: C, 66.45; H, 5.29; N, 4.08. Found: C, 66.34; H, 5.31; N, 4.21. EIMS (probe) 70 eV, *m/z* (rel. int.): 345.0964 $[M+2]^+$ (345.0946 for $C_{19}H_{18}NO_3{}^{37}Cl$, 35), 343.0958 $[M]^+$ (343.0975 for $C_{19}H_{18}NO_3{}^{35}Cl$, 100), 308 $[M\text{-}Cl]^+$ (11), 168 (12), 139 (35). UV λ_{max}^{MeOH} nm (logε): 206 (4.36), 268 (4.36). 329 sh (4.56), 341 (4.61), 440 (4.83). IR ν_{max}^{KBr} cm^{-1}: 3450, 3220 (OH, NH), 1660 (C═O). The assignments of ^1H- and ^{13}C-NMR spectroscopic signals are summarized in Table 1.

Auxarconjugatin A (**1a**-form): Red powder, mp >300°C (from H_2O–MeOH). EIMS (probe) 70 eV, *m/z* (rel. int.): 345.0965 $[M+2]^+$ (345.0946 for $C_{19}H_{18}NO_3{}^{37}Cl$, 35), 343.0990 $[M]^+$ (343.0975 for $C_{19}H_{18}NO_3{}^{35}Cl$, 100), 308 $[M\text{-}Cl]^+$ (10), 167 (15), 139 (42), 83 (24). UV λ_{max}^{MeOH} nm (log): 205 (4.19), 222 (4.14), 267 (4.34). 327 sh (4.25), 340 (4.31), 442 (4.91). IR ν_{max}^{KBr} cm^{-1}: 3220 (OH, NH), 1680 (C═O). The assignments of ^1H- and ^{13}C-NMR spectroscopic signals are summarized in Table 1.

Auxarconjugatin B (**2**): Red prisms, mp 203–205°C (from H_2O–MeCN). EIMS (probe) 70 eV, *m/z* (rel. int.): 331.0789 $[M+2]^+$ (331.0789 for $C_{18}H_{16}NO_3{}^{37}Cl$, 33), 329.0819 $[M]^+$ (329.0819 for $C_{18}H_{16}NO_3{}^{33}Cl$, 100), 294 $[M\text{-}Cl]^+$ (10), 204 (8), 168 (12), 125 (64), 62 (62). UV λ_{max}^{MeOH} nm (logε): 220 (3.60), 261 (3.75), 321 sh (3.60), 331 (3.66), 435 (4.33). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH, NH), 1680 (C═O). The assignments of ^1H- and ^{13}C-NMR spectroscopic signals are summarized in Table 1.

Auxarconjugatin C (**3**): Red prisms, mp >300°C (from H_2O–MeOH). EIMS (probe) 70 eV, *m/z* (rel. int.): 309.1364 $[M]^+$ (309.1365 for $C_{19}H_{19}NO_3$, 100), 264 (13), 236 (20), 168 (20), 139 (27), 117 (29), 83 (44). UV λ_{max}^{MeOH} nm (logε): 204 (4.27), 224 (4.17), 266 (4.38), 328 sh (4.16), 340 (4.21), 448 (4.91). IR ν_{max}^{KBr} cm^{-1}: 3250 (OH, NH), 1690 (C═O). The assignments of ^1H- and ^{13}C-NMR spectroscopic signals are summarized in Table 1.

*3.3. Equilibrium between (1′E,3′E,5′E,7′E)-auxarconjugatin A (**1a**) and (1′E,3′Z,5′E,7′E)-auxarconjugatin A (**1**)*

All-*trans*-form (**1a**) and 3′-*cis*-form (**1**) of auxarconjugatin A were dissolved in MeCN and MeOH. The time course of isomerisation was investigated using HPLC (60% MeCN) analyses.

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