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Isolation, structure elucidation, and biological evaluation of the unusual heterodimer chrysoxanthone from the ascomycete IBWF11-95A

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ABSTRACT

Chrysoxanthone, an unusual heterodimer of blennolide A and 2-hydroxychrysophanol linked through a diaryl ether bridge, was isolated from mycelia of the ascomycete IBWF11-95A grown in submerged culture. Its structure was elucidated by two-dimensional NMR spectroscopy. The metabolite shows antibacterial activity against different species with MIC values between 2.5 and 20 μg/mL while also inhibiting the growth of several fungi.

During our efforts to identify novel biologically active natural products of fungal origin, the ascomycete IBWF11-95A was found to produce an intensely yellow colored compound (**1**) with antibacterial and antifungal properties. Structure elucidation by spectroscopic methods revealed it to be an unprecedented heterodimer of the tetrahydroxanthone blennolide A and the anthraquinone 2-hydroxychrysophanol. Blennolide A (or ergochrome B) and its stereoisomers are known as the constituents of the homo- and heterodimeric ergochromes or secalonic acids since the 1950s. These pigments were first isolated from ergot, the sclerotia of *Claviceps purpurea*.[1,2] The monomeric ergochromes B and E have only recently been described as the discrete natural products blennolide A and B.[3] Anthraquinones such as emodin are known to be the biogenetic precursors of the ergochromes,[4] and 2-hydroxychrysophanol is a metabolite of *Myrsine africana*[5] and *Hemerocallis fulva*.[6]

The sterile filamentous ascomycete IBWF11-95A was isolated from a twig collected in Oberjoch, Germany, and shows no morphological characteristics and no significant ITS homology to a known species. The ITS sequence is available online (GenBank Accession No. FJ896405). Mycelial cultures are deposited in the culture collection of the Institute of Biotechnology and Drug Research (IBWF e.V.), Kaiserslautern, Germany. For maintenance, the fungus was grown on YMG agar slants (yeast extract 4 g/L, malt extract 10 g/L, glucose 10 g/L, the pH value was adjusted to 5.5 before autoclaving). Solid media contained 2% of agar. IBWF11-95A was cultivated in 0.5 L of malt medium (malt extract 40 g/L, the pH value was adjusted to 5.5 before autoclaving) in a 1 L Erlenmeyer flask at 22–24 °C on a rotary shaker. For inoculation, 1 cm^2 of a well grown agar culture was used. After 14 days the glucose was used up and the mycelium was separated from the fluid. The mycelium (18.5 g wet weight) was extracted two times with MeOH/acetone (1:1) and the combined extracts were dried. Upon dissolving the crude extract (710 mg) in MeOH, a yellow precipitate was obtained which was washed two times with MeOH, three times with H_2O, again once with MeOH, once with ethyl acetate, and finally twice with MeOH. This procedure yielded 70 mg of a yellow colored mixture of structurally related compounds. Preparative HPLC (Macherey & Nagel, SP 250/21 Nucleosil 100 C18, 25 mL/min, isocratic 66% MeCN, 34% water containing 0.1% H_3PO_4) was performed on 16 mg of this mixture to afford 69 mg of **1**.

Chrysoxanthone (**1**)[7] had an elemental composition of $C_{31}H_{24}O_{11}$ as suggested by HRMS. Broad UV absorption bands along with the intense yellow color indicated an extensively conjugated chromophore. The compound was only soluble in DMSO-d_6 in which NMR spectroscopic analyses were therefore carried out. ^{1}H NMR spectra showed two aromatic spin systems along with aliphatic fragments; one aromatic spin system was part of a 2,3-disubstituted phenol, as became apparent from 2D NMR experiments, and a benzo-γ-pyrone partial structure was deduced from

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the chemical shifts of the quaternary carbons in the phenol with resonances at 109.3 and 157.0 ppm. The proton at 3.98 ppm from the aliphatic spin system showed an HMBC correlation to the pyrone carbonyl at 178.7 ppm and to a quaternary carbon at 101.9 ppm. This carbon turned out to be the α-center of an enolized 1,3-diketone moiety. Successive HMBC analysis showed that the third ring was an anellated cyclohexenol. The enol OH resonance was found at 16.20 ppm due to hydrogen bonding with the enol carbon at 187.0 ppm. This tricyclic fragment additionally contained an angular carbomethoxy group on one of the bridging quaternary centers and was identical to blennolide A as judged by comparison of the recorded NMR data with published values.[3] The presence of the same tautomer was assumed based on the matching values for the chemical shift of C-7 and the geminal coupling constant of the methylene protons. However, the other tautomer cannot be ruled out since no HMBC correlations were found for the proton at 16.20 ppm.

The second fragment also contained the proton spin system of a 2,3-disubstituted phenol with its resonances being located at lower field compared to the xanthone fragment. The quaternary carbon resonances were found at 116.1 and 133.3 ppm, leading to the conclusion that this phenol contained acceptor substituents in positions 2 and 3. HMBC correlations allowed to identify the two ketone carbonyls as part of an anthraquinone scaffold. The third ring of the anthraquinone was found to be a methyl-substituted catechol.

The discussed data led to the assumption that the link between the two units was a diaryl ether connecting C-1 to one of the oxygenated centers of the anthraquinone. Two hydroxyl resonances at 12.00 and 11.82 ppm gave evidence for two phenolic protons hydrogen-bonded symmetrically to the central carbonyl, thus ruling out the possibility of substitution at these positions. The positions of the hydroxyl protons were confirmed by HMBC correlations to the neighboring carbon atoms. In addition, the respective chemical shifts were in good accordance with reported examples,[8] placing the diaryl ether between C-1 and C-2′. While no HMBC correlations between the two fragments were found to substantiate the proposed constitution, it was ultimately proven by an ROE contact between H_3-11′ and H-2 (Fig. 1).

The relative configuration of the stereogenic centers could be determined by analysis of the scalar coupling constants and by ROE correlations (Fig. 2). The planar conjugated part of the tricyclic system requires the angular carbomethoxy group to be axial. The axial methylene proton at C-7 exhibited a ^3J coupling constant of 11.0 Hz, placing CH_3-11 in equatorial position. The axial H-6 showed merely weak scalar coupling but strong ROE correlations to H-5 which in turn has to be equatorial. This relative configuration is in accordance with that of blennolide A, the reported coupling constants of which are matched while those of the epimeric blennolide B differ significantly.[3] Strong UV and visible absorption precluded the measurement of the optical rotation, however CD spectroscopy gave positive bands at 333 and 265 nm, which is in accordance with the data of blennolide A.[3]

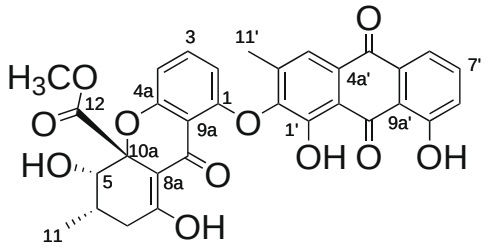

Figure 1. Structure of chrysoxanthone (**1**).

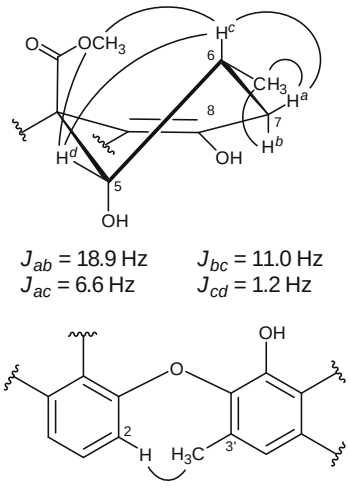

Figure 2. Coupling constants and ROE correlations in **1**.

Chrysoxanthone (**1**) possesses antimicrobial activity against different species with MIC values between 2.5 and 20 μg/mL. The most sensitive species with a MIC value of 2.5 μg/mL was *Arthrobacter citreus*. The growth of some fungi was also inhibited. These data are shown in Table 2. No antifungal activity up to 50 μg/mL was observed against *Penicillium islandicum*, *Zygorhynchus moelleri*, *Ascochyta pisi*, *Fusarium oxysporum*, *Fusarium fujikuroi*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, and *Nematospora coryli*.

Table 1
^{1}H (400 MHz) and ^{13}C NMR (101 MHz, DMSO-d_6) data of **1**

Position	δ_H	δ_C	HMBC
1	—	159.0 (s)	—
2	6.27 (dd, 8.4, 0.7)	108.2 (d)	1, 4, 4a, 9a
3	7.37 (t, 8.4)	136.1 (d)	1, 4a, 9a
4	6.77 (dd, 8.4, 0.7)	111.9 (d)	1, 2, 4a, 9, 9a
4a	—	156.9 (s)	—
5	3.98 (dd, 4.4, 1.2)	70.1 (d)	6, 7, 8a, 9, 10a, 11, 12
6	1.97 (dtd, 11.0, 6.6, 1.2)	28.4 (d)	11
7	2.48 (dd, 18.9, 6.6)	34.3 (t)	5, 6, 8, 8a
	2.31 (dd, 18.9, 11.0)		6, 8, 8a, 11
8	—	187.0 (s)	—
8a	—	101.9 (s)	—
9	—	178.7 (s)	—
9a	—	109.3 (s)	—
10a	—	84.6 (s)	—
11	1.03 (d, 6.6)	17.6 (q)	5, 6, 7, 8
12	—	171.7 (s)	—
13	3.65 (s)	53.3 (q)	12
1′	—	154.0 (s)	—
2′	—	145.3 (s)	—
3′	—	140.5 (s)	—
4′	7.73 (s)	121.7 (d)	1′, 2′, 3′, 10′
4a′	—	129.5 (s)	—
5′	—	181.0 (s)	—
5a′	—	133.3 (s)	—
6′	7.74 (dd, 7.5, 1.0)	119.5 (d)	5′, 8′, 9a′
7′	7.83 (dd, 8.3, 7.5)	137.6 (d)	5a′, 9′, 9a′
8′	7.40 (dd, 8.3, 1.0)	124.5 (d)	6′, 9′, 9a′, 10′
9′	—	161.3 (s)	—
9a′	—	116.1 (s)	—
10′	—	191.7 (s)	—
10a′	—	116.0 (s)	—
11′	2.27 (br s)	16.4 (s)	2′, 3′, 4′
5-OH	5.82 (d, 4.4)	—	—
8-OH	16.20 (s)	—	—
1′-OH	12.00 (s)	—	1′, 2′, 10a′
9′-OH	11.82 (s)	—	8′, 9′, 9a′

Coupling constants (J) are given in Hz. ^{13}C multiplicities were determined indirectly by HSQC. HMBC correlations are from proton(s) stated to the indicated carbons.

Table 2
Minimum inhibition concentrations of **1** against several organisms

Organism	Minimum inhibition concentration (μg/mL)
Micrococcus luteus	10
Bacillus brevis	5
Bacillus subtilis	10
Arthrobacter citreus	2.5
Corynebacterium insidiosum	5
Mycobacterium phlei	20
Enterobacter dissolvens	>50
Pseudomonas fluorescens	10
Escherichia coli	20
Absidia glauca (+)	20
Absidia glauca (−)	20
Aspergillus ochraceus	5
Paecilomyces variotii	20
Penicillium notatum	2.5
Mucor miehei	20

The cytotoxicity was moderate with IC_{50}-values of 50 μg/mL for Jurkat, L-1210, and Colo-320 cells. For HeLa-S3 the IC_{50} exceeded 50 μg/mL.

Chrysoxanthone (**1**) shows antibacterial and antifungal properties in accordance with its monomers. Anthraquinones such as 2-hydroxychrysophanol possess various biological activities among which are antibiotic and cytotoxic properties.[9] For blennolide A, antifungal activity against *Microbotryum violaceum* and antibacterial activity against *Escherichia coli* and *Bacillus megaterium* in agar diffusion assays have been described.[3]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.008.

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7. *Chrysoxanthone* (**1**). yellow solid, mp 235–236 °C; measurement of optical rotation was precluded by optical absorption of the sample; CD (CH_2Cl_2, *c* $1.75 \cdot 10^{-5}$ mol/L) λ (Δε) 333 (+9.9), 265 (+8.2 $M^{-1}\,cm^{-1}$) nm; UV (MeOH) λ_{max} nm (log ε) 225 (4.58), 260 (4.40), 290 (4.12), 322 (4.15), 427 (4.06); λ_{min} nm (log ε) 243 (4.27), 284 (4.08), 300 (4.03), 378 (3.87) nm; IR (KBr): ν = 3442, 1738, 1618, 1466, 1414, 1373, 1259, 1092, 1005, 760 cm^{-1}; NMR data see Table 1; APCI-MS (+): *m/z* (%) 573.3 $[M+H]^+$ (100); APCI-MS (−): *m/z* (%) 571.2 $[M-H]^-$ (100); HRFAB-MS *m/z* = 573.1401 (calcd for $[C_{31}H_{24}O_{11}+H]^+$ 573.1397).
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