Waol A, *trans*-dihydrowaol A, and *cis*-dihydrowaol A: polyketide-derived γ-lactones from a *Volutella* species

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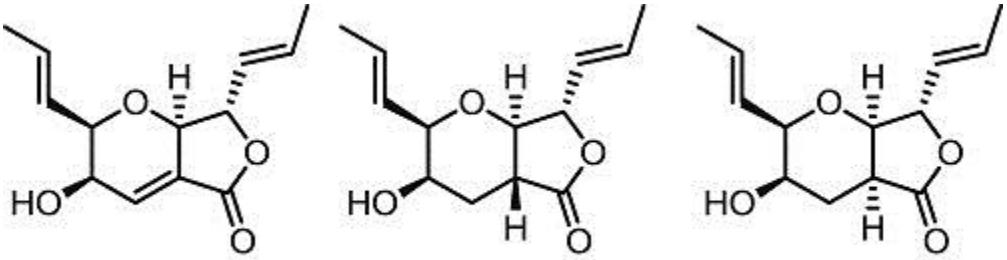
Abstract:

An organic extract of a filamentous fungus (MSX 58801), identified as a Volutella sp. (Hypocreales, Ascomycota), displayed moderate cytotoxic activity against NCI-H460 human large cell lung carcinoma. Bioactivity-directed fractionation led to the isolation of three γ-lactones having the furo[3,4-b]pyran-5-one bicyclic ring system [waol A (1), trans-dihydrowaol A (2), and cis-dihydrowaol A (3)]. The structures were elucidated using a set of spectroscopic and spectrometric techniques; the absolute configuration of 2 was established via a modified Mosher's ester method. Compounds 1 and 2 were evaluated for cytotoxicity against a human cancer cell panel.

Graphical Abstract:



Keywords: Polyketide | Cytotoxicty | γ-Lactone | Filamentous fungi | Waol A

Article:

In pursuit of structurally diverse anticancer leads from nature,[1 and 2] our group has been investigating filamentous fungi, particularly the Mycosynthetix library, representing over 55,000 accessions.[3, 4, 5, 6, 7,8 and 9] Fungi represent an exciting reservoir of bioactive natural products, as they are an underexplored and renewable resource.[10, 11 and 12]

An organic extract of the filamentous fungus MSX 58801, which was isolated from leaf litter in 1991, displayed moderate cytotoxic activity against NCI-H460 human large cell lung carcinoma (~86% inhibition of cell growth when tested at 20 μ g/mL).[3] Bioactivity-directed fractionation using flash chromatography followed by preparative RP-HPLC resulted in the isolation of three γ-lactones (**1**–**3**) containing a furo[3,4-*b*]pyran-5-one bicyclic ring system, with >95% purity for compounds **1** and **2** according to UPLC (Fig. S1, Supplementary data). Compounds **1** and **2** were evaluated for cytotoxicity against a human cancer cell panel.

Compound **1** (2.46 mg), which was obtained as a colorless oil, had a molecular formula of $C_{13}H_{16}O_4$ as determined by HRESIMS. The NMR (Fig. S2, Supplementary data), HRMS, and optical rotation data identified **1** as the known compound, waol A (FD-211; Fig. 1). First isolated in 1995 from a fermentation of *Myceliophthora lutea* TF-0409, [13] the structure of **1** was revised in 2003. [14 and 15]

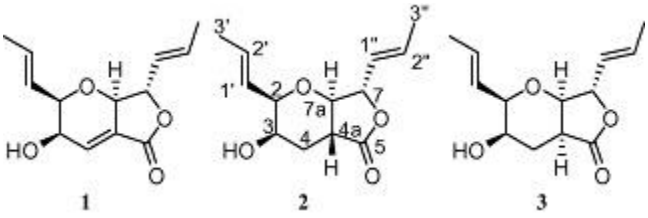


Figure 1. Structures of compounds **1**–**3**.

Compound **2** (9.67 mg) was also obtained as a colorless oil.[16] The molecular formula was determined as $C_{13}H_{18}O_4$ via HRESIMS, establishing an index of hydrogen deficiency of 5. The NMR data suggested structural similarity with compound **1**. However, compound **2** lacked the olefinic proton at δ_H 6.90, which was replaced by three aliphatic protons (δ_H 1.79, 2.43, and 2.91). These data suggested a difference between **1**and **2** of a double bond, as supported by a 2 amu difference in the HRMS data. The ^{1}H NMR data of **2**revealed the presence of four olefinic protons, corresponding to two *trans*-disubstituted olefins (δ_H 5.52, ddq,*J* = 15.5, 8.0, 1.7; 5.55, ddq, *J* = 15.5, 5.2, 1.7; 5.91, dqd, *J* = 15.5, 6.9, 1.7; and 5.99, dq, *J* = 15.5, 6.9, for H-1″, H-1′, H-2′, and H-2″, respectively), four oxymethines (δ_H 3.48, dd, *J* = 12.0, 8.6; 3.84, bq, *J* = 2.9; 4.03, ddd, *J* = 5.2, 2.9, 1.7; and 4.67, dd, *J* = 8.6, 8.0, for H-7a, H-3, H-2, and H-7, respectively), one methine (δ_H2.91, ddd, *J* = 12.6, 12.0, 3.4, for H-4a), one methylene (δ

$_{H}$ 1.79, ddd, J = 13.2, 12.6, 2.9; and 2.43, ddd, J = 13.2, 3.4, 2.9, for H-4 α and H-4 β, respectively), two equivalent methyls (δ_H 1.77, dd, J = 6.9, 1.7, for H-3′ and H-3′′), and one exchangeable proton (δ_H 1.84, for 3-OH). The ^{13}C NMR data revealed 13 carbons, consistent with the HRMS data and indicative of one carbonyl (δ_C 173.5 for C-5), four olefinic carbons (δ_C125.7, 126.4, 130.6, and 134.3, for C-1′′, C-1′, C-2′, and C-2′′, respectively), five methines (δ_C 39.0, 66.3, 81.2, 82.1, and 82.4 for C-4a, C-3, C-2, C-7a, and C-7, respectively), one methylene (δ_C 30.0 for C-4), and two methyls (δ_C 18.1 and 18.2 for C-3′ and C-3′′, respectively), (see Supplementary Figs. S3 and S4 for the ^{1}H and ^{13}C NMR spectra and Table S1). The two double bonds and the carbonyl group accounted for three degrees of unsaturations, leaving the remaining two accommodated by the bicyclic ring system. COSY data identified one spin system as H_3-3′/H-2′/H-1′/H-2/H-3/H_2-4/H-4a/H-7a/H-7/H-1′′/H-2′′/H_3-3′′ (Fig. 2a). The following key HMBC correlations were observed: H_3-3′→C-1′, H_3-3′′→C-1′′, H-2→C-2′, H-7→C-2′′, H-3→C-4a, H-7a→C-4, H-4a→C-7, and H-4a→C-5 (Fig. 2a). NOESY correlations from H-1′′ to H-7a, from H-7a to H-2, and from H-2 to H-3 and H-2′ indicated that H-1′′, H-7a, H-2, H-3, and H-2′ were all on the same face. Alternatively, NOESY correlations observed from H-4a to H-7 indicated that these two protons were on the same side of the molecule but opposite to the previous set (Fig. 2b). Comparing all of these data with those for **1** yielded the structure of **2** (Fig. 1), which was ascribed the trivial name *trans*-dihydrowaol A. The absolute configuration of **2** was assigned via a modified Mosher's ester method, [17] establishing the configuration as 2*R*, 3*R*, 4a*R*, 7*S*, and 7a*R* (Fig. 3). [18]

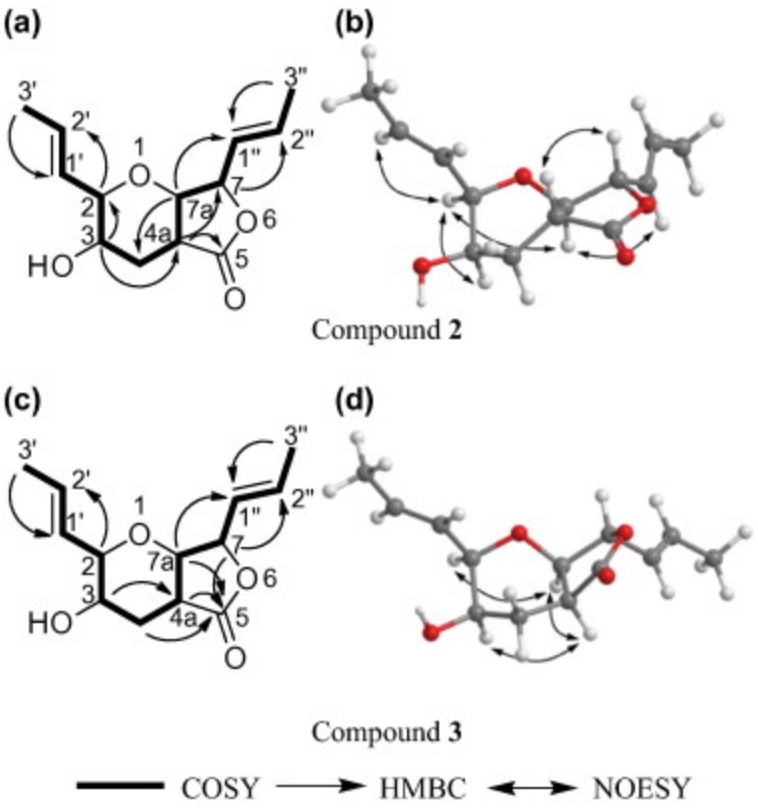


Figure 2. Key HMBC, COSY, and NOESY correlations of **2** and **3**.

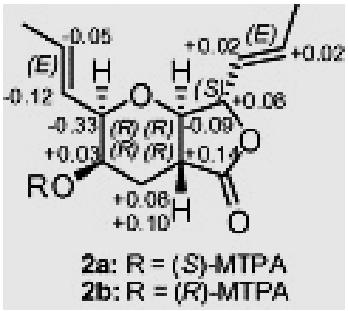


Figure 3. $\Delta\ \delta_H$ values [$\Delta\ \delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively), of *trans*-dihydrowaol A (**2**) in pyridine-d_5.

Compound **3** (1.45 mg) was obtained as a colorless oil.[19] The molecular formula was determined as $C_{13}H_{18}O_4$ via HRESIMS, and was the same as compound **2**. The NMR data (Table S1 and Figs. S5 and S6) suggested structural similarity with **2**. Key differences were a coupling constant of 0.6 Hz between H-4a (δ_H2.58, ddd, J = 7.5, 2.3, 0.6) and H-7a (δ_H 4.17, dd, J = 4.6, 0.6) in **3** versus 12 Hz in **2**, and a NOESY correlation from H-4a to H-7a in **3** versus H-4a to H-7 in **2** (Fig. 2d). These data implied a pseudoaxial/pseudoequatorial *cis* orientation of H-4a/H-7a. NOESY correlations were also observed from H-2 to H-7a and H-4a, and from H-4a to H-3, indicating that those protons were on the same face (Fig. 2d). These data suggested an inversion

in the configuration at C-4a in **3** relative to **2**, establishing the structure of**3** as an epimer of **2** (Fig. 1). The trivial name, *cis*-dihydrowaol A (**3**), was ascribed to this compound. The relative configuration of **3** was assigned by comparison with **2** as 2*R*, 3*R*, 4a*S*, 7*S*, and 7a*R*. An attempt to establish the absolute configuration via a modified Mosher' s ester method [17] was unsuccessful.

Compounds **1** and **2** were tested against two cancer cell lines, MDA-MB-435 (human melanoma) and SW-620 (human colon cancer), using methods described previously;[3] due to paucity of sample, compound **3** was not tested. While compound **1** showed moderate cytotoxic activity against the SW-620 cancer cell line, compound **2** was inactive against both cancer cell lines (Table 1), suggesting the importance of the double bond for cytotoxicity. Compound **1** was reported by Nozawa et al[13] to have broad spectrum activity against cultured tumor cell lines, including adriamycin-resistant HL-60 cells. Several compounds having the furo[3,4-*b*]pyran-5-one bicyclic ring system have been reported from fungi with diverse biological activities, including antibacterial and cytotoxic activities. [20, 21, 22, 23, 24 and 25]

Table 1.Cytotoxicity of compounds **1** and **2** against two human tumor cell lines[a]

Compound	IC_{50} values in μM	
	MDA-MB-435[b]	SW-620[c]
Waol A (**1**)	39.6	13.8
trans-Dihydrowaol A (**2**)	>40	>40

a Positive controls were vinblastine and bortezomib. Vinblastine was tested at concentrations of 3 ng/mL and 1 ng/mL: MDA-MB-435 cells had 37% and 99% viable cells; SW620 cells had 76% and 90% viable cells; respectively. Bortezomib was tested at concentrations of 5 nM and 2.5 nM: MDA-MB-435 cells had 90% and 91% viable cells; SW620 cells had 79% and 71% viable cells, respectively.

b Melanoma and tumor cell lines were tested using published protocols.

c Colon tumor cell lines were tested using published protocols.[3 and 20]

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North Carolina at Greensboro. Sequence data were generated at the Mycology laboratory of Dr. Andrew N. Miller, Illinois Natural History Survey, University of Illinois at Urbana-Champaign.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.06.008.

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16. trans-Dihydrowaol A (2): colorless oil; ½a_25
D _56_ (c = 0.1, MeOH); 1H NMR
(CDCl3, 500 MHz) and 13C NMR (CDCl3, 125 MHz) (see Supplementary data);
HRESIMS m/z 239.1278 [M+H]+ (calcd for C13H19O4 239.1278).

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18. Preparation of the (R)- and (S)-MTPA ester derivatives of trans-dihydrowaol A (2): To 0.75 mg of compound 2 was added 400 lL of pyridine-d5 and transferred into an NMR tube. To initiate the reaction, 10 lL of S-(+)-amethoxy-a-(trifluoromethyl)phenylacetyl (MTPA) chloride was added into the NMR tube with careful shaking and then monitored immediately by 1H NMR at the following time points 0, 15, 30, and 60 min. The reaction was found to be complete within 30 min, yielding the mono (R)-MTPA ester derivative (2b) of 2. 1H NMR data of 2b (500 MHz, pyridine-d5): 5.93 (1H, m, H-20), 5.89 (1H, m, H-10), 5.69 (1H, m, H-20 0), 5.60 (1H, m, H-10 0), 5.53 (1H, bq, J = 2.3, H-3), 4.81 (1H, dd, J = 8.6, 8.0, H-7), 4.48 (1H, d, J = 5.7, H-2), 3.94 (1H, dd, J = 9.2, 8.6, H-7a), 2.69 (1H, m, H-4a), 2.67 (1H, m, H-4b), 2.29 (1H, m, H-4a), 1.63 (3H, d, J = 6.9, H3-30), and 1.55 (3H, d, J = 6.3, H3-30 0). In an analogues manner, 0.75 mg of compound 2 dissolved in 400 lL pyridine-d5 was reacted in a second NMR tube with 10 lL (R)-(_)-a-MTPA chloride for 30 min, to afford the mono (S)-MTPA ester (2a). 1H NMR data of 2a (500 MHz, pyridine-d5): dH 5.88 (1H, m, H-20), 5.77 (1H, m, H-10), 5.70 (1H, m, H-20 0), 5.60 (1H, m, H-10 0), 5.56 (1H, bq, J = 3.4, H-3), 4.89 (1H, dd, J = 8.6, 8.0, H-7), 4.15 (1H, d, J = 6.9, H-2), 3.85 (1H, m, H-7a), 2.84 (1H, m, H-4a), 2.77 (1H, m, H-4b), 2.37 (1H, m, H-4a), 1.53 (3H, d, J = 6.3, H3-30), and 1.50 (3H, d, J = 6.3, H3-30 0).

19. cis-Dihydrowaol A (3): colorless oil; ½a_25
D _32_ (c = 0.1, MeOH); 1H NMR (CDCl3,
500 MHz) and 13C NMR (CDCl3, 125 MHz) (see Supplementary data); HRESIMS m/z 239.1280 [M+H]+ (calcd for C13H19O4 239.1278).

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