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Aromatic butenolides produced by a soil ascomycete *Auxarthron* sp. KCB15F070 derived from a volcanic island

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ABSTRACT

LC/MS-based chemical screening of fungal extract fraction library led to identification of three 2,3-aryl substituted furanone metabolites (**1**–**3**), including one known butenolide glycoside (**1**) whose stereochemistry remained unsolved and two new compounds gotjawaside and gotjawalide (**2** and **3**), from *Auxarthron* sp. KCB15F070, a fungus isolated from a soil sample of the volcanic island Jeju, Korea. Their planar structures were elucidated by 1D- and 2D-NMR spectroscopic and HRESIMS spectrometric techniques, and the absolute configurations of three compounds were solved using a combination of chemical derivatizations and computational analysis of vibrational circular dichroism (VCD) spectra.

Introduction

Fungal secondary metabolites have been recognized as a rich source of structurally divergent small molecules that can be exploited for the discovery of novel bioactive substances [1]. In our efforts to investigate a unique diversity of secondary metabolites produced by fungi inhabiting terrestrial and marine environments of the Korean Peninsula, we have been performing chemical (LC/MS-based dereplication) and bioassay-guided screening of fungal crude extracts, which led to the isolation of a series of new compounds including highly oxygenated azaphilones geumsanols [2], diketopiperazine alkaloid haenamindole [3], and phenylspirodrimane stachybotrysin [4]. In order to increase the probability of discovering previously undiscovered fungal metabolites, we constructed the fraction library by reversed-phase octadecyl silica gel (ODS) fractionation of culture extracts from fungi isolated from the Gotjawal areas of the volcanic island Jeju, Korea, a biodiversity hot spot harboring a unique ecosystem and diverse microbial communities [5]. Each ODS fraction sample was chemically analyzed by the LC/MS-based dereplication strategy using in-house and existing commercial databases to prioritize fractions for further investigation. Using this approach, we detected promising ion peaks in the LC/MS spectra (Fig. S1) and consequently isolated furanone metabolites (**1**–**3**) including one known and two new compounds from the extract fractions of *Auxarthron* sp. KCB15F070. Only limited classes of metabolites have been reported from members of the genus *Auxarthron*, including polyenylpyrroles rumbrin [6], auxarconjugatins [7], and aromatic alkaloids methylpenicinoline and amauromine [8]. Compound **1** was identified as a previously reported metabolite

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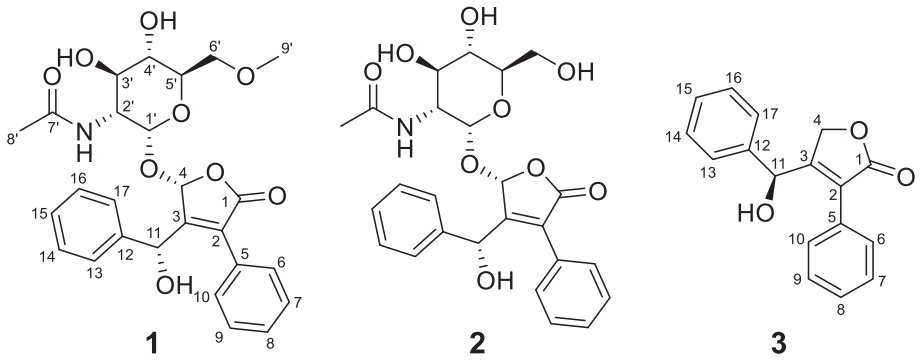

malfilamentoside B whose stereochemistry was unassigned in the original report [9]. Malfilamentoside B and its dehydroxylated derivative malfilamentoside A isolated from *Malbranchea filamentosa* are the only previously known example of furanone glycosides produced by the fungal sources. We herein report the structures of three furanone glycosides including their absolute configurations defined by spectroscopic and chemical methods as well as VCD calculations.

Results and discussion

Compound **1** was isolated as a white amorphous powder that gave a $[M + Na]^+$ ion in the HRESIMS at *m/z* 522.1739, indicating a molecular formula of $C_{26}H_{29}NO_9Na$. Structure determination using a combination of 1D and 2D NMR techniques, including COSY, HSQC, and HMBC, revealed that compound **1** has the identical planar structure with malfilamentoside B which possesses a 2,3-aryl substituted furanone backbone with aminosugar linked at C-4 (Fig. 1) [9]. The relative configuration of a sugar unit was determined by analysis of vicinal ^1H-^1H coupling ($^3J_{H,H}$) constants in conjunction with the ROESY spectroscopic data (Fig. 2). The large vicinal coupling constant of $J_{H\text{-}2',H\text{-}3'}$ (10.5 Hz) assigned axial orientations. This interpretation was further supported by the ROESY data in which the 1,3-diaxial cross-peaks were observed at H-2′/H-4′ and H-3′/H-5′. The equatorial position of H-1′ was established by the small coupling constant of $^3J_{H\text{-}1',H\text{-}2'}$ (3.4 Hz) and the ROESY correlation between H-1′ and H-2′. The sugar moiety was therefore defined to be 6-*O*-methyl-*N*-acetyl-α-glucosamine. The absolute stereochemistry at C-11 was assigned by the modified Mosher ester analysis [10]. Because the difference in the ^1H NMR chemical shifts between *S*- and *R*-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters ($\Delta\delta_H = \delta_S - \delta_R$) were positive for H-4 and negative for H-13/17, a 11*R* configuration was determined (Fig. 3). Although another secondary alcohol at C-3′ in the sugar moiety was also esterified with MTPA, it was not possible to unambiguously assign the absolute configuration of C-3′ due to the same positive $\Delta\delta_H$ sign for both substituents H-2′ and H-4′ (nonhomogeneous distributions). The absolute configuration of the sugar moiety was determined to be D on the basis of the sugar derivatization experiments using **2** as discussed below.

Next, we attempted to establish the absolute stereochemistry at C-4 in **1** by theoretical electronic circular dichroism (ECD) analysis. An empirical ECD helicity rule has been proposed for the absolute configuration of 5-substituted 2(5*H*)-furanones [11]. This rule uses the ECD signs of n-π* and π-π* transitions of α,β-unsaturated lactone chromophore. The corresponding n-π* and π-π* transitions in **1** are not easily attributable because of overlapping of the π-π* transitions of the phenyl groups, which prevents us from applying this rule. As the carbonyl group in furanones exhibits a characteristic strong absorption band in the IR region [12–14], we measured the IR and vibrational circular dichroism (VCD) spectra of **1** to gain an insight into the absolute configuration at C-4. As shown in Fig. 4a, two intense absorption bands were observed in the C=O stretching region. The higher wavenumber band at ca. 1770 cm^{-1} is attributable to the C=O stretching band of the lactone carbonyl group, whereas the lower band at ca. 1660 cm^{-1} can be assigned to the amide C=O stretching band. A positive and negative VCD signals were observed for the lactone and amide C=O stretching bands, respectively. Fig. 4b shows the calculated VCD spectra of optimized structures of (4*R*)-**1** and (4*S*)-**1** in the carbonyl region. The spectral data for (4*R*)-**1** matched well with the experimental data. The positive VCD signal was also calculated for the (4*R*)-furanone without the sugar moiety (Fig. S29). From these results, the absolute configuration at C-4 was assigned to be *R*.

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was deduced as $C_{25}H_{27}NO_9$ based on the analysis of HRESIMS in combination with NMR data (Table 1). The ^1H and ^{13}C NMR spectra of **2** exhibited chemical shifts and splitting patterns highly comparable to those of **1**. The interpretation of the 2D NMR data, including COSY, HSQC, and HMBC spectra, led to the construction of the planar structure of **2** (Fig. 1). The cross-peaks of H-1′/H-2′/H-3′/H-4′/H-5′/H_2-6′ in the COSY spectrum showed the connectivity through C-1′ to C-6′. The connectivity of an *N*-acetyl group was established by HMBC correlations of H-2′ and H_3-8′ to the amide carbon C-7′ (δ_C 173.7). The determination of the aglycone structure of **2** was mainly achieved by interpretation of HMBC correlations. Key HMBC correlations from one distinctive proton H-4 for a acetal moiety ($\delta_{C/H}$ 103.4/6.31) to the ester carbonyl C-1 and two olefinic non-protonated carbons C-2 and C-3 suggested the presence of an α, β-unsaturated γ-lactone ring. Two mono-substituted benzene rings whose presence was revealed by a combination of the 2D NMR data were then connected to the aglycone moiety by three-bond HMBC correlations. HMBC correlations from H-6/10 to C-2 placed one benzene ring at C-2 as a substituent. The remaining benzene was proved to be linked to C-3 via an oxymethine bridge C-11 from analysis of the HMBC cross-peaks from H-13/17 to C-11 and from H-11 to carbons in the aglycone moiety (C-2, C-3, and C-4). Lastly, obvious HMBC correlations from H-1′ to C-4 positioned the sugar moiety at C-4. Thus, the planar structure of **2** was established as a demethylated derivative of **1**.

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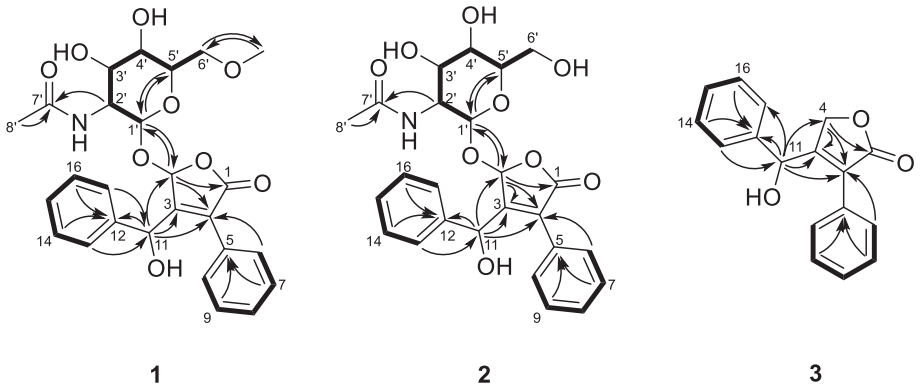

Fig. 1. ^{1}H-^{1}H COSY and HMBC correlations of **1**–**3**.

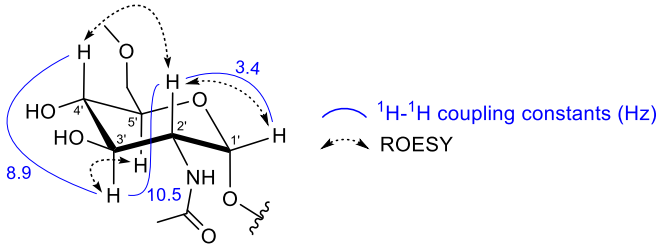

Fig. 2. ^{1}H-^{1}H coupling constants (Hz) and key ROESY correlations of the sugar unit in **1**.

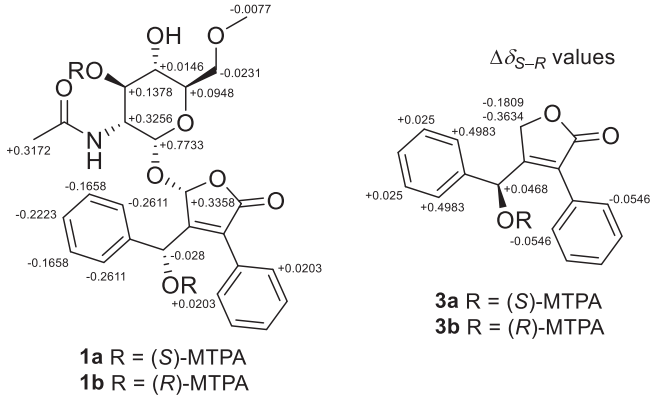

Fig. 3. $\Delta\delta_{S-R}$ values (ppm) for (*S*)- and (*R*)-MTPA esters for **1** and **3**.

The relative configuration of the sugar in **2** was determined to be identical to **1** by analysis of $^{3}J_{H,H}$ coupling constants and ROESY correlations. Because the authentic sample of *N*-acetyl-α-D-glucosamine is commercially available, sugar derivatization experiments and chromatographic comparison were performed to assign the absolute configuration of the sugar moiety in **2** [15]. The acid hydrolysate derivatized with L- and D-cysteine methyl esters and *σ*-tolyl isothiocyanate showed peaks for thiocarbamoyl-thiazolidine carboxylate products whose retention times matched with the L- and D-cysteine derivatives of the authentic D-*N*-acetylglucosamine (Fig. S30), respectively. The sugar unit of **2** was therefore proved to be D-*N*-acetylglucosamine. Compound **1**, which is the 6′-*O*-methyl analog of **2**, showed the similar specific rotation value (+10 for **1**; +17 for **2**) as well as highly comparable 1D NMR shifts and splittings to **2**, indicating that two compounds share the same stereochemistry. The absolute configuration of the sugar unit of **1** was therefore deduced to be same as that of **2**.

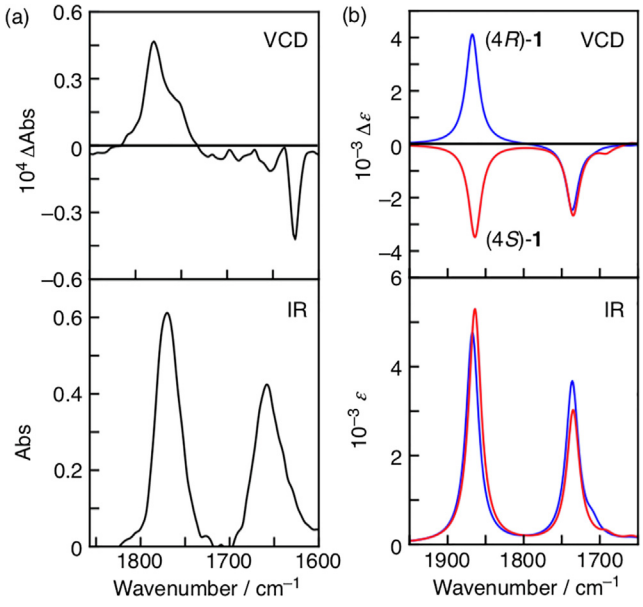

Fig. 4. (a) Experimental VCD (top) and IR (bottom) spectra of **1** in $CHCl_3$ at room temperature. (b) Calculated VCD (top) and IR (bottom) spectra of (4*R*)-**1** (blue) and (4*S*)-**1** (red) at the B3LYP/6-31G(d,p) level.

Likewise, the stereochemistry at C-4 and C-11 of **2** was assigned to be identical to that of **1**.

Compound **3** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{17}H_{14}O_3$ by the HRESIMS and NMR data. ^{1}H and ^{13}C NMR spectra of **3** revealed similar structural features to **1**, except for the missing sugar signals and the appearance of one methylene signal (δ_C 70.9). Key HMBC correlations from newly observed methylene protons (δ_H 5.14 and 4.85) to carbons in the α, β-unsaturated γ-lactone ring (C-1, C-2, and C-3) indicated that the acetal proton at C-4 in **1** was replaced with an oxymethylene in **3** (Fig. 1). The planar structure of **3** was confirmed by analysis of COSY and HMBC data, which led to the assignment of all carbon and proton resonances (Table 1). The absolute configuration of C-11 was then determined by the modified Mosher's method. The chemical shift differences ($\Delta\delta_{S-R}$) between MTPA esters (**3a** and **3b**) assigned the 11*S* configuration (Fig. 3). The ECD spectra of **3** were opposed to **1** and **2** (Fig. S31), which is explainable by the opposite stereochemistry at C-11 or absence of a stereocenter C-4 in the γ-lactone chromophore.

While some members possessing the 4-benzyl-3-phenylbutenolide motif have been reported to exhibit interesting biological activities [16], those of glycosylated derivatives have not yet

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Table 1
^{13}C and ^{1}H NMR spectroscopic data for **2** and **3** in CD_3OD.

No.	**2**		**3**	
	δ_C[a], type	δ_H (J in Hz)[b]	δ_C[c], type	δ_H (J in Hz)[d]
1	172.0, C		175.6, C	
2	131.4, C		128.0, C	
3	160.4, C		165.6, C	
4	103.4, CH	6.31, s	70.9, CH_2	5.14, d (18.2) 4.85, dd (18.2, 0.2)
5	130.5, C		131.3, C	
6/10	130.3, CH	7.35, ovl[e]	129.9, CH	7.44, ovl[e]
7/9	129.5, CH	7.35, ovl[e]	130.3, CH	7.44, ovl[e]
8	130.4, CH	7.34, ovl[e]	130.3, CH	7.41, m
11	71.1, CH	5.95, s	70.2, CH	5.93, s
12	142.7, C		142.4, C	
13/17	128.8, CH	7.46, d (7.5)	127.3, CH	7.30, m
14/16	130.1, CH	7.41, t (7.7)	129.9, CH	7.33, m
15	129.6, CH	7.34, ovl[e]	129.3, CH	7.29, m
1′	101.7, CH	5.23, d (3.5)		
2′	55.3, CH	3.91, dd (3.5, 10.6)		
3′	73.2, CH	3.38, dd (9.0, 10.5)		
4′	71.6, CH	3.49, t (9.3)		
5′	75.3, CH	3.77, m		
6′	62.1, CH_2	3.85, m 3.79, m		
7′	173.7, C			
8′	23.0, CH_3	1.74, s		

[a] Recorded at 200 MHz.
[b] Recorded at 800 MHz.
[c] Recorded at 225 MHz.
[d] Recorded at 900 MHz.
[e] Overlapped with other signals.

been revealed. Compounds **1**–**3** were evaluated for their antibacterial and antifungal activities against *Escherichia coli, Bacillus cereus*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Alternaria brassicicola*, *Aspergillus flavus*, and *Fusarium oxysporum*, but all compounds were inactive at 50 μg/disk. In cytotoxicity tests employing cancer cell lines originated from various tissues (HeLa, MDA-MB-231, A549, Neuro-2a, and HL-60 cell lines), any significant cytotoxic activities were not observed. Compounds were also subjected to several in-house cell-based assays including cell migration and autophagy assays, but none of them showed pronounced activities. It was previously reported that whereas gymnoascolide A isolated from *Gymnoascus reessii* possessing a phenyl and benzyl substituted γ-butenolide, comparable to the aglycone part of **1** and **2**, exhibit vasodilatory and selective antifungal activities [17,18], γ-butenolide glycosides malfilamentosides A and B (**1**) from *Malbranchea filamentosa*, do not show any relevant activities [9]. The lack of bioactivities of glycosylated derivatives in our assay system supports significantly different properties of glycosides with their aglycone motifs, warranting further evaluation of bioactivities.

Conclusion

Two new aromatic butenolides possessing the 4-benzyl-3-phenylbutenolide motif, together with one known butenolide which had been previously reported without stereochemistry, were isolated from a soil ascomycete *Auxarthron* sp. KCB15F070. Their structures including absolute configurations were determined by spectroscopic and chemical means as well as computational analysis. To date, only two γ-butenolide glycosides, namely malfilamentosides A and B, were found from fungi [9].

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151227.

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