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Short Note

(*S*)-4-[(3a*R*,4*S*,7a*R*)-4-Methoxy-6-methyl-3-methylene-2-oxo-2,3,3a,4,7,7a-hexahydrobenzofuran-5-yl]pentyl Acetate

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Abstract: A new etherified 1-*O*-acetylbritannilactone (ABL) analogue **2** was semi-synthesized by a mild etherification reaction. The structure of the newly synthesized analogue was identified by ^{1}H-NMR, ^{13}C-NMR and HR-ESI-MS analysis. **2** exhibited similar cytotoxicity against HCT116, SGC-7901 and HeLa human cancer cell lines with ABL.

Keywords: 1-*O*-acetylbritannilactone; etherification; cytotoxicity

1. Introduction

1-*O*-acetylbritannilactone (ABL, **1**, Scheme 1), a 1,10-*seco*-eudesmanolide sesquiterpene extracted from *Inula britannica* L. (Xuanfuhua in Chinese), has been reported to have a large number of biological effects including anti-inflammatory, antibacterial, antihepatitic, antidiabetes, and antitumor activities [1–6]. It has been shown to possess anticancer effects in various cancer cells [2,3,7–10], including anti-proliferation, cell cycle arrest, induction of apoptosis and increased sensitivity to apoptosis. The relative configuration of ABL at the C4-methyl and C6-hydroxyl group have been revised as 4*S* *, 6*S* * using X-ray diffraction analysis by us [11].

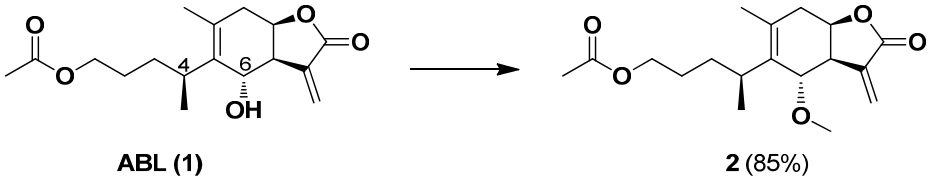
It has been reported that 6-hydroxy (6-OH) and α-methylene-γ-lactone of ABL act as important bioactive motifs [12,13]. Therefore, structure modifications for improving ABL bioactivity at both positions of ABL have been conducted by many medicinal chemists. Structural modifications of ABL have focused mainly on alterations at 6-OH moiety such as oxidation and acylation by Liu [12–14] and us [11,15]. However, etherified ABL analogues have not been synthesized so far, perhaps due to the complexity of its structure (sensitive to strong acid or base) and larger steric hindrance. Because of this, using ABL as the starting material, we semi-synthesized a new etherified analogue 1-*O*-acetyl-6-methoxybritannilactone (**2**) (aslo named (*S*)-4-[(3a*R*,4*S*,7a*R*)-4-methoxy-6-methyl-3-methylene-2-oxo-2,3,3a,4,7,7a-hexahydrobenzofuran-5-yl] pentyl acetate) by a mild etherification reaction with Ag_2O and dimethyl sulfide as 6-OH activation agents.

2. Results and Discussion

Ethers are usually prepared from alcohols or their conjugate bases. One important procedure, known as the Williamson Ether Synthesis, is proceeded by an SN_2 reaction of an alkoxide nucleophile with an alkyl halide. To synthesize etherified ABL analogues, a classic Williamson ether synthesis method was firstly carried out with ABL and CH_3I using NaH or NaOMe as organic base at room temperature. However, the reaction was not observed under aprotic solvent conditions including acetone, THF and DMSO but in the presence of phase transfer catalysis triethylbenzylaminium

chloride (TEBA). Finally, we used mild etherification reaction with Ag_2O and dimethyl sulfide as 6-OH activation agents to obtain the methyl etherified analogue **2** in 85% yield (Scheme 1). Full characterization spectra of **2** refers to the supplement material.

ABL and **2** were assayed for cytotoxicity against three human solid tumor cell lines, HCT116, SGC-7901 and HeLa by using a reported method [11], and the results (IC_{50}) are shown in Table 1. **2** showed similar cytotoxicity with ABL against HCT116, SGC-7901 and HeLa, revealing the etherification reaction may not be an effective strategy to improve cytotoxic activity for ABL.



Scheme 1. Synthesis of 1-*O*-acetyl-6-methoxybritannilactone (**2**). *Reagents and conditions*: CH_3I (20 eq.), Ag_2O (5 eq.), molecular sieve 4 Å (1.5 w%), CH_3SCH_3 (5 eq.), THF, rt, 24 h.

Table 1. Cytotoxic activities (IC_{50}) of 1-*O*-acetylbritannilactone (ABL) and etherified analogue **2**.

No.	IC_{50} [1] (μM)		
	HCT116	**SGC-7901**	**HeLa**
ABL (**1**)	36.1 ± 3.1	42.5 ± 6.1	32.6 ± 2.5
2	35.7 ± 5.2	37.5 ± 3.8	38.6 ± 2.9
VP-16	2.13 ± 0.23	6.56 ± 0.68	2.97 ± 0.25

[1] The IC_{50} values represent the concentration that causes 50% inhibition of cell viability. Cells were treated with ABL and **2** for 72 h. All data (mean ± SD) are the average of three or four determinations. Cancer cell lines: HCT116 (human colorectal cancer), SGC-7901 (human gastric cancer), HeLa (human cervix cancer). VP-16 represents etoposide (a positive control).

3. Materials and Methods

3.1. General

ABL was isolated from the EtOAc-soluble fraction of the ethanolic extract of the dried flowers of *I. britannica*, followed by repeated silica gel column chromatography purification [11]. Column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China). Compounds were visualized either in UV light (254 nm) and/or by staining with 5% phosphomolybdic acid followed by heating. All NMR spectra were recorded on a 500 MHz Bruker NMR spectrometer. HR-MS spectra were recorded on an Thermo Scientific LTQ Orbitrap XL spectrometer. Other chemicals used in this study were commercial analytical-grade reagents.

3.2. Synthesis of (S)-4-[(3aR,4S,7aR)-4-Methoxy-6-methyl-3-methylene-2-oxo-2,3,3a,4,7,7a-hexahydrobenzofuran-5-yl]pentyl Acetate (or Named: 1-O-Acetyl-6-methoxybritannilactone)

To a 5 mL round-bottomed flask containing ABL (0.1 mmol) in dry THF (3 mL) was added CH_3I (2.0 mmol), Ag_2O (0.5 mmol), Me_2S (0.5 mmol), molecular sieve 4 Å (1.5 weight % of THF). The resulting solution was stirred at room temperature for 24 h. After completely by TLC test, the solution was washed with water, extracted with EtOAC, dried with anhydrous Na_2SO_4, and further purified by the column chromatography to get the analogue **2** in the yield of 85%.

1-O-Acetyl-6-methoxybritannilactone (**2**): yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.31 (d, *J* = 2.7 Hz, 1H, H-13a), 5.63 (d, *J* = 2.2 Hz, 1H, H-13b), 4.98–4.92 (m, 1H, H-8), 3.99–3.86 (m, 2H, H-1), 3.63–3.54 (m, 1H, H-6), 3.38 (s, 3H, C$\underline{H}_3$O-6), 2.75 (dd, *J* = 16.0, 2.6 Hz, 1H, H-9a), 2.67 (dd, *J* = 12.8, 7.8 Hz, 1H, H-4), 2.38 (dd, *J* = 16.0, 1.8 Hz, 1H, H-9b), 2.04 (s, 3H, C$\underline{H}_3$CO-1), 1.74 (s, 3H, H-14), 1.46–1.33 (m, 1H, H-2a), 1.29–1.17 (m, 2H, H-2b and H-3a), 1.06–0.92 (m, 4H, H-3b and H-15); ^{13}C-NMR (126 MHz, $CDCl_3$)

δ 171.37 ($CH_3\underline{C}OO$-1), 170.00 (C-12), 137.53 (C-11), 134.92 (C-10), 131.08 (C-5), 123.39 (C-13), 78.17 (C-8), 76.25 (C-6), 64.48 (C-1), 56.40 ($\underline{C}H_3O$-6), 39.97 (C-7), 34.54 (C-9), 33.02 (C-4), 31.64 (C-3), 26.73 (C-2), 21.12 (CH_3COO-1), 20.44 (C-14), 18.83 (C-15). HRMS (ESI) m/z calcd for $C_{18}H_{27}O_5$ $[M + H]^+$ 323.18530, found 323.18524.

Supplementary Materials: ^{1}H-NMR, ^{13}C-NMR and HR-ESI-MS spectra are reported in the supplementary materials as Figures S1–S3. They and the molfiles are available online at http://www.mdpi.com/1422-8599/2016/1/M890.

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Conflicts of Interest: The authors declare no conflict of interest.

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