Tetrahedron Letters 51 (2010) 3449–3450

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Ashitabaol A, a new antioxidative sesquiterpenoid from seeds of *Angelica keiskei*

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ARTICLE INFO

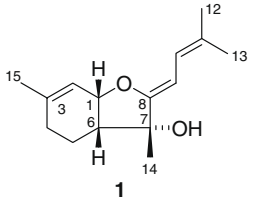
Article history:
Received 26 March 2010
Revised 25 April 2010
Accepted 28 April 2010
Available online 17 May 2010

Keywords:
Sesquiterpenoid
Angelica keiskei
Seed
ABTS free radical scavenging activity

ABSTRACT

A new sesquiterpenoid designated ashitabaol A was isolated from seeds of *Angelica keiskei*. The structure was determined by interpretation of spectroscopic data and was confirmed by X-ray crystallographic analysis. Ashitabaol A exhibited free radical scavenging activity.

Excessive generation of free radicals causes damage to intracellular and extracellular proteins, polyunsaturated fatty acids, and nucleic acids.[1] Seed germination process involves starting of respiration as well as imbibition. Free radicals are continuously produced during seed germination and cause the tissue of seed to be at risk of oxidative damage. It has been reported that the seed has abilities to protect the tissue against oxidative injury.[2] The capacity for scavenging free radicals increases significantly during germination.[3] In the course of the search for antioxidants from a Japanese herb, *Angelica keiskei* Koidzumi (Japanese name 'Ashitaba'),[4] we found that the MeOH extract of the seeds of *A. keiskei* exhibits potent free radical scavenging activity. An activity-guided fractionation of the MeOH extract led to the isolation of a new free radical scavenger designated ashitabaol A (**1**). This report describes the purification and the structure elucidation of **1**.



1

The seeds (355 g) of *A. keiskei* were extracted with MeOH. The concentrated MeOH extract was suspended in water and partitioned with hexane. The hexane-soluble portion (1.7 g) was fractionated repeatedly on a silica gel column employing EtOAc in hexane gradient mixtures and on an ODS column employing MeOH in H_2O gradient mixtures to afford **1** (33 mg, 0.009 %) as colorless needles, mp 88–90 °C, $[\alpha]_D^{25}$ +204 (*c* 0.316, MeOH).

Ashitabaol A (**1**) exhibited a molecular ion peak at *m/z* 234 in the EIMS. The molecular formula of **1** was established to be $C_{15}H_{22}O_2$ on the basis of high-resolution ESITOFMS data (*m/z* 257.1516 $[M+Na]^+$, Δ −0.1 mmu), indicating five degrees of unsaturation. The IR spectrum displayed absorption bands at 3508 (OH), 1670, and 1633 cm^{-1} (C=C). The UV (MeOH) absorption maximum at 257 nm (log ε 4.27) suggested the presence of conjugated double bonds. The ^1H NMR, ^{13}C NMR (Table 1), and ^{13}C–^1H COSY data of **1** revealed the presence of an aliphatic methyl (δ_H 1.50; δ_C 27.7), three olefinic methyls (δ_H 1.70, 1.75, and 1.76; δ_C 18.2, 25.9, and 23.7), an aliphatic methine (δ_H 1.92; δ_C 45.7), two aliphatic methylenes (δ_H 1.20, 1.80, 1.97, and 2.02; δ_C 19.2 and 29.0), an oxymethine (δ_H 4.51; δ_C 75.0), three sp^2 methines (δ_H 5.29, 5.70, and 6.03; δ_C 92.7, 118.2, and 118.0). The ^1H NMR spectra measured at 0, 10, 20, 25 °C revealed the presence of an exchangeable proton (δ_H 1.83 at 20 °C).

Interpretation of the ^1H–^1H COSY data led to a proton spin network system of H-2–H-1–H-6–H_2-5–H_2-4. The HMBC correlations from H-1 to C-8; from H_3-15 to C-2, C-3, and C-4; and from H_3-14 to C-6, C-7, and C-8 revealed the presence of a hexahydrobenzofuran skeleton. The presence of a 3-methylbut-2-enylidene unit was indicated on the basis of the HMBC correlations from H-9 to C-11; from H-10 to C-12 and C-13; from H_3-12 to C-10, C-11, and C-13; and from H_3-13 to C-10, C-11, and C-12. The HMBC correlations from H-9 to C-7 and from H-10 to C-8 demonstrated the attachment of the 3-methylbut-2-enylidene unit at C-8 of the hexahydrobenzofuran skeleton. The HMBC correlations from the hydroxyl proton to C-7, C-8, and C-14 established the attachment of the hydroxyl group at C-7.

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doi:10.1016/j.tetlet.2010.04.122

Table 1
NMR spectral data of **1** in $CDCl_3$ at 20 °C[a]

No	δ_C	δ_H multiplicity (J in Hz)	HMBC
1	75.0 d	4.51 br t (4.8)	C-2, C-3, C-5, C-6, C-8
2	118.2 d	5.70 br s	C-6, C-15
3	142.3 s		
4	29.0 t	2.02 ddd (17.6, 5.1, 2.2) 1.97 m	C-2, C-3, C-5, C-6, C-15
5	19.2 t	1.20 qd (13.2, 5.1) 1.80 dddd (13.2, 4.8, 2.2, 2.2)	C-1, C-3, C-4, C-6, C-7 C-1, C-4, C-6
6	45.7 d	1.92 ddd (13.2, 4.8, 4.8)	C-1, C-5, C-7, C-8, C-14
7	79.1 s		
8	159.4 s		
9	92.7 d	5.29 d (11.4)	C-7, C-8, C-11
10	118.0 d	6.03 dhept (11.4, 1.5)	C-8, C-12, C-13
11	130.4 s		
12	25.9 q	1.75 br s (3H)	C-10, C-11, C-13
13	18.2 q	1.70 br s (3H)	C-10, C-11, C-12
14	27.7 q	1.50 s (3H)	C-6, C-7, C-8
15	23.7 q	1.76 br s (3H)	C-2, C-3, C-4
7-OH		1.83 br s	C-7, C-8, C-14

[a] The ^1H and ^{13}C NMR were measured at 400 and 100 MHz, respectively. Chemical shifts were referenced to solvent (δ_H 7.26 and δ_C 77.0).

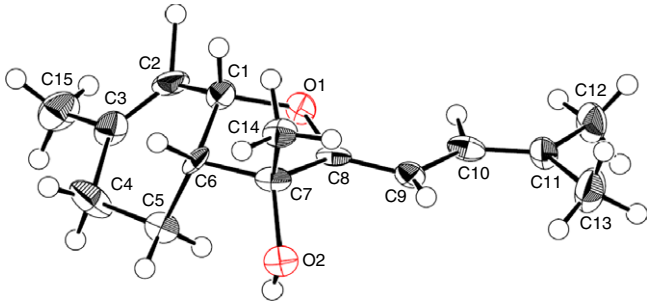

Figure 1. Perspective view of the crystal structure of **1**.

The relative stereochemistry of **1** was determined by NOE experiments. Irradiation of the methyl proton signal (H_3-14) resulted in enhancements of both the signals for the proton attached to C-1 (11%) as well as the proton on C-6 (6%), placing these protons on the same face of the hexahydrobenzofuran ring. The relative configuration was supported by the enhancement of 6% on the signal for H-6 upon irradiation of H-1. In addition, irradiation of H_3-14 led to a 7% increase in the signal corresponding to H-9 but no effect on the signal for H-10, indicating the geometry of Δ^8 to be *Z*. The structure of **1** was confirmed by X-ray crystallographic analysis (Fig. 1).[5] Consequently, the structure of ashitabaol A was elucidated as structure **1**. At present, the absolute configuration of **1** remains to be determined.

Although many bisabolane-type sesquiterpenoids have been isolated from natural sources,[6] sesquiterpenoids possessing a tetrahydro- or hexahydrobenzofuran skeleton with the 3-methylbut-2-enylidene unit are very rare in nature. The sole precedent example is bisabolangelone which has a tetrahydrobenzofuran-4(2*H*)-one skeleton,[7,8] while ashitabaol A (**1**) is a new structure without a carbonyl group.[9]

Ashitabaol A (**1**) exhibited ABTS free radical scavenging activity with SC_{50} = 13.8 μM.[10] While bisabolangelone at 100 μM did not exhibit ABTS free radical scavenging activity. Ashitabaol A (**1**) was found to be abundantly present in the seed coat throughout seed germination on the basis of HPLC analyses. These findings suggest that ashitabaol A (**1**) is involved in the protection of *A. keiskei* seed against oxidative damage by free radicals during seed germination.

Acknowledgments

We thank Masaaki Shirai, Nagahama Institute of Bio-Science and Technology, for technical assistance. This study was supported in part by the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan.

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5. CCDC 771163 contains the supplementary crystallographic data. The data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif from the Cambridge Crystallographic Data Centre.
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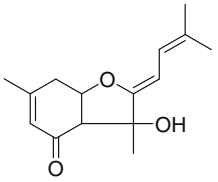
8. The structural formula of bisabolangelone is as follows.



9. A referee pointed out that ashitabaol A (**1**) is merely a reduced form of the known bisabolangelone. We, however, consider that the differences of the structural features of **1**, that is, lack of a carbonyl group and the position of the endocyclic double bond, from those of bisabolangelone significantly affect the potency of radical-scavenging activity. Bisabolangelone exhibits a diverse range of biological activities such as antifeedant,[11] cytotoxic,[12] anti-ulcer,[13] and anti-inflammatory[14] effects. Further, its total synthesis has been described.[15] The unique structure and biological activity of **1** will certainly attract interest among organic and medicinal chemists. It would also be of considerable interest to investigate the absolute configuration, the mechanism of radical-scavenging activity, and the biosynthetic pathway of **1**.
10. SC_{50} value is the concentration of the compound required to obtain 50% of the maximum scavenging capacity from the regression lines in the plot of % scavenging capacity versus concentration. Trolox was used as the positive control (SC_{50} = 9.8 μM).
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