Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2019–2021
Bioorganic & Medicinal Chemistry Letters

Anti-tumor abietane diterpenes from the cones of *Sequoia sempervirens*

Kwang-Hee Son, Hyun-Mi Oh, Sung-Kyu Choi, Dong Cho Han and Byoung-Mog Kwon*

Korea Research Institute of Bioscience and Biotechnology, University of Science and Technology in Korea, 52 Uendong Yoosunggu Taejeon, 305-600 Republic of Korea

Received 30 January 2005; revised 17 February 2005; accepted 18 February 2005
Available online 10 March 2005

Abstract—A new abietane, namely, 20-hydroxyferruginol (**1**), together with known ferruginol (**2**), 18-hydroxyferruginol (**3**), sugiol (**4**), and 6α-hydroxysugiol (**5**), were isolated from the cones of *Sequoia sempervirens*. Their structures were elucidated through spectral data. Compounds **1** and **5** strongly inhibited colon, lung, and breast human tumors and oncogene transformed cells with GI_{50} 2–5 μg/mL.

Cancer remains the second leading cause of death in most of the countries and as a result there is a need for effective compounds. In continued screening of plant extracts for potential anticancer agents,[1–5] we found that the methanol extracts of the cones of *Sequoia sempervirens* inhibited the growth of human tumor cells. The methanol extract of the dried cones of *S. sempervirens* was fractionated by silica gel flash chromatography eluting with 10–40% ethylacetate in hexane.[6] Active fractions were further purified by silica and C-18 column chromatography. Finally, the colorless solid a new abietane, 20-hydroxyferruginol (**1**), and four known compounds, ferruginol (**2**),[7] 18-hydroxyferruginol (**3**),[7] sugiol (**4**),[8] and 6α-hydroxysugiol (**5**),[9] were obtained by preparative reverse phase HPLC (YMC J'sphere ODS-H80, 250 × 20 mm ID column). In this communication, we report the structure of **1** and anti-tumor activities of the isolated compounds.

Abietane diterpenes are widely distributed, naturally occurring products in the plant kingdom. Many of them were isolated from *Taiwania cryptomerioides*, *Thuja standishii*, *Cedrus atlantica*, *Juniperus formosana*, *Cephalotaxus harringtonia*, *Cryptomria japonica*, *Vitex rotundifolia*, and *Salvia* species. They exhibit significant biological activities including anti-biotic, anti-tumor, anti-tuberculous, anti-malarial, and anti-oxidant effects.[10–12] Due to their wide distribution and interesting biological activities, many studies of isolation and identification of the compounds have been reported.[13,14]

The structure of **1** was elucidated by interpretation of NMR and mass spectral data. Analysis of HRFABMS ($[M]^+$, *m/z* 302.2246) and ^{13}C NMR spectrum of **1** led to a molecular formula $C_{20}H_{30}O_2$, which indicated six degrees of unsaturation (see Table 1 for ^1H and ^{13}C NMR spectral data). The ^{13}C NMR of **1** exhibited 20 carbons, which revealed carbon signals for four methyls, six methylenes (one bearing oxygenated), four methines (two aromatic hydrogens), and six quaternary carbons. The HMQC experiment and DEPT spectrum served in identifying the protons attached to a specific carbon.

The existence of cyclohexane and aromatic moieties shown in the partial structure A (Fig. 1) was determined as follows. Proton–proton connectivity, H-1 through H-3, and H-6 through H-7 were observed in the ^1H–^1H COSY spectrum. The chemical shifts of C-20 (51.05 ppm) suggested that it was substituted with oxygen. In the DEPT experiment, the carbon (C-20) was assigned as a methylene group. In the HMBC experiment, correlations of a nonprotonated carbon C-10 (72.20 ppm) with H-7 and H-20, a quaternary carbon C-4 (34.38 ppm) with H-3 and H-5, and that of an aromatic C-9 (135.02 ppm) with H-7 and H-20 were observed. Hence, the structure of isolated compound **1** was thought to be an abietane diterpene. Two- and

Keywords: Anti-tumor; Abietane diterpene; Cancer cell.
* Corresponding author. Tel.: +82 42 860 4557; fax: +82 42 861 2675; e-mail: kwonbm@kribb.re.kr

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.02.057

Table 1. NMR data of 20-hydroxyferruginol (**1**, 400 MHz, in $CDCl_3$)

Atom no	δ_C	δ_H	HMBC (C → H)
1	41.63	1.61 (m)	3, 20
		1.94 (m)	
2	18.72	1.47 (m)	3
		1.87 (m)	
3	42.36	1.30 (m)	1, 2
		1.47 (m)	
4	34.34		3, 5
5	58.01	1.41 (m)	7, 20
6	24.35	1.21 (m)	7
		2.06 (m)	
7	35.28	2.70 (m)	6, 14
		2.78 (m)	
8	132.72		7, 14
9	135.02		7, 11, 20
10	72.20		7, 20
11	118.69	6.74 (s)	20
12	152.07		11, 14
13	133.32		15
14	126.62	6.91 (s)	7, 15
15	26.47	3.22 (sept)	14
16	22.88	1.22 (d)	15
17	22.55	1.21 (d)	15
18	32.25	0.95 (s)	
19	21.74	0.92 (s)	
20	51.05	3.05 (d)	11
		2.61 (d)	

three-bond HMBC correlations between the methyl groups and their respective neighboring carbons also supported the partial structure B in Figure 2. C-13 and the isopropyl group were connected, which was confirmed by the HMBC correlation C-13 (68.14 ppm) and H-15 (3.22 ppm).

The relative stereochemistry of **1** was determined by the analysis of NOESY experiments in which strong NOEs between H-20 and H-11 and between H-5 and H-2 were observed.

As already mentioned, isolated compounds belong to an abietane diterpene, which exhibits a wide range of biological activities. Even though Gao and Han reported cytotoxic abietane diterpenes,[15] until this report, there

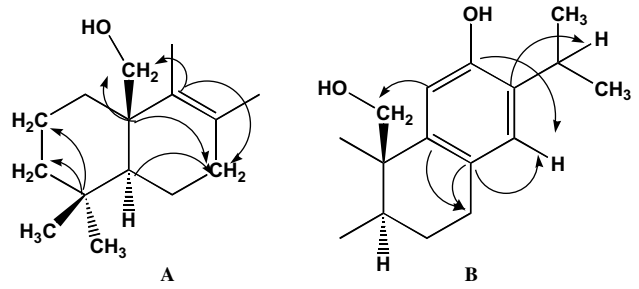
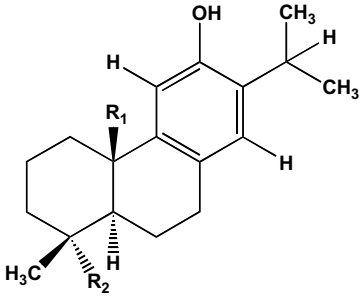


Figure 2. Selected HMBC correlations of compound **1**.

Table 2. Growth inhibitory activity of compounds **1**–**5** against human tumor cell lines[a]

Cell line	Compound	GI_{50} (μg/mL)
Colon (SW620)	**1**	8.22 ± 0.41[b]
	2	< 50
	3	< 50
	4	< 50
	5	3.98 ± 0.65
Colon (HCT116)	**1**	4.34 ± 0.33
	2	< 50
	3	< 50
	4	< 50
	5	2.52 ± 0.42
Breast (MDA-MB-231)	**1**	8.34 ± 0.25
	2	< 50
	3	< 50
	4	< 50
	5	3.64 ± 0.65
Lung (NCI-H23)	**1**	2.8 ± 0.37
	2	< 50
	3	< 50
	4	< 50
	5	2.21 ± 0.61
Lung (A549)	**1**	2.9 ± 0.37
	2	< 50
	3	< 50
	4	< 50
	5	1.42 ± 0.71

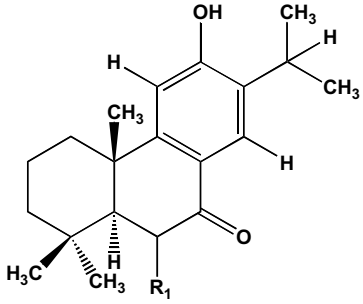
[a] Anti-tumor agent, adriamycin was used as control of cytotoxicity evaluation (GI_{50}, μg/mL).
[b] Results are expressed as mean ± SD.



1 $R_1 = CH_2OH$, $R_2 = CH_3$

2 $R_1 = CH_3$, $R_2 = CH_3$

3 $R_1 = CH_3$, $R_2 = CH_2OH$



4 $R_1 = H$,

5 $R_1 = OH$

Figure 1. Structure of isolated compounds **1**–**5**.

has been no report concerning the anti-tumor effects of ferruginols and sugiols.

To determine the inhibitory activity of tumor cell growth of isolated compounds, compounds **1**–**5** were treated with several human tumor cell lines including SW620 (colon), MDA-MB-231 (breast), HCT116 (colon), NCI-H23 (lung), and A549 (lung). Growth inhibitory activities of the compounds were summarized in Table 2.[16] As shown in Table 2, growth inhibitory activity of 20-hydroxyferruginol (**1**) and 6-hydroxysugiol (**5**) with a hydroxyl group is more than 10 times stronger than that of ferruginol (**2**) and sugiol (**4**). However, 18-hydroxyferruginol (**3**) having a hydroxyl group did not inhibit any human tumor cells. Therefore, it is very hard to tell about the structure–activity relationship from this study. In the experiments with oncogenic H-*ras* transformed cells, compounds **1** and **5** strongly inhibited H-*ras* transformed rat2 cells in a dose-dependent manner with GI_{50} 2.3 and 3.5 μg/mL, respectively.

In summary, we isolated a new abietane diterpene **1** and elucidated the structure using NMR and mass spectral data. Anti-tumor activities of compounds **1** and **5** in human tumor and oncogene transformed cells support that the compounds will be a good lead molecule for the development of anti-tumor agents.

Acknowledgements

This work was supported by Plant Diversity Research Center (PF0320506-0) of the 21st Century Frontier Research Program and the KRIBB Research Initiative Program.

References and notes

1. Lee, S. H.; Kim, H. K.; Kang, H. M.; Seo, J. M.; Son, K. H.; Lee, H. S.; Kwon, B. M. *J. Org. Chem.* **2002**, *67*, 7670–7675.
2. Kim, J. H.; Kim, H. K.; Jeon, S. B.; Son, K. H.; Kim, E. H.; Kang, S. K.; Sung, N. D.; Kwon, B. M. *Tetrahedron Lett.* **2002**, *43*, 6205–6208.
3. Lee, S. H.; Kang, H. M.; Song, H. C.; Lee, H.; Lee, U. C.; Son, K. H.; Kim, S. H.; Kwon, B. M. *Tetrahedron* **2000**, *56*, 4711–4715.
4. Seo, J. M.; Kang, H. M.; Son, K. H.; Kim, J. H.; Lee, C. W.; Kim, H. M.; Chang, S. I.; Kwon, B. M. *Planta Med.* **2003**, *69*, 218–222.
5. Lee, S. H.; Lee, M. Y.; Kang, H. M.; Han, D. C.; Son, K. H.; Yang, D. C.; Sung, N. D.; Lee, C. W.; Kim, H. M.; Kwon, B. M. *Bioorg. Med. Chem.* **2003**, *11*, 4545–4549.
6. 500 g of cones of *Sequoia sempervirens* was collected from a coast redwood in Santa Cruz, CA, USA. Dried cones were extracted with methanol and the crude extracts were purified by column chromatography. 20-Hydroxyferruginol (**1**): white amorphous powder; mp 97–98 °C; UV (CH_3OH) λ_{max} 211 ($\log \varepsilon = 3.03$), 283 ($\log \varepsilon = 2.05$) nm; $[\alpha]_D^{25}$ +70.58 (*c* 0.5, CH_3OH); EI *m*/*z* 302 (100%), 284 (10%), 270 (15%), 177 (90%), 164 (82%).
7. Fang, J. M.; Lee, C. K.; Cheng, Y. S. *Phytochemistry* **1993**, *33*, 1169–1172.
8. Li, A.; She, X.; Zhang, J.; Wu, T.; Pan, X. *Tetrahedron* **2003**, *59*, 5737–5741.
9. Su, W. C.; Fang, J. M.; Cheng, Y. S. *Phytochemistry* **1994**, *35*, 1278–1284.
10. Politi, M.; Braca, A.; Tommasi, N. D.; Morelli, I.; manunta, A.; Battinell, L.; mazzanti, G. *Planta Med.* **2003**, *69*, 468–470.
11. Iwamoto, M.; Minami, T.; Tokuda, H.; Ohtsu, H.; Tanaka, R. *Planta Med.* **2003**, *69*, 69–72.
12. Ulubelen, A.; Topcu, G.; Johansson, C. B. *J. Nat. Prod.* **1997**, *60*, 1275–1280.
13. Chyu, C. F.; Lin, H. C.; Kuo, Y. H. *Chem. Pharm. Bull.* **2005**, *53*, 11–14.
14. Barrero, A. F.; Moral, J. F. Q.; Herrador, M. M.; Arteaga, J. F.; Akssira, M.; Benharref, A.; Mohamed Dakir, M. *Phytochemistry* **2005**, *66*, 105–111.
15. Gao, J.; Han, G. *Phytochemistry* **1997**, *44*, 759–761.
16. The cell lines used were obtained originally from ATCC and were maintained in RPMI 1640 (Gibco/BRL) or DMEM supplemented with 10% heat-inactivated FBS (Gibco/BRL) and 25 mM Hepes. Cell cultures were maintained at 37 °C under a humidified atmosphere of 5% CO_2 in an incubator. Cells (5000 cells) were seeded into 96 well plates in RPMI 1640, or DMEM containing 10% FBS. After 20–24 h, cells were replenished with fresh complete medium containing either a test compound or 0.1% DMSO. After incubation for 48 h, cell proliferation reagent WST-1 (Roche, Germany) was added to each well. The amount of WST-1 formazan produced was measured at 450 nm using an ELISA Reader (Bio-Rad, CA).