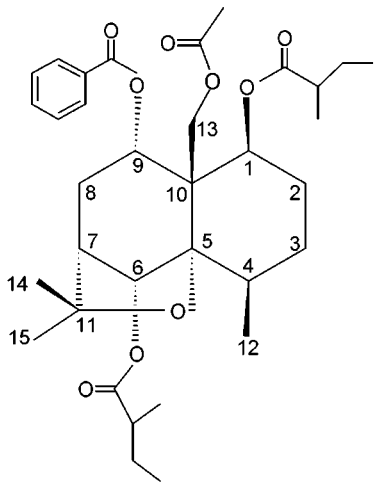
A New Antitumor β-Dihydroagarofuran Sesquiterpene Polyol Ester from the *Euonymus Nanoides*

Hong Wang, Xuan Tian,†,* and Yuan-Jiang Pan*

Department of Chemistry, Zhejiang University, Hanzhou 310027, P.R. China
†National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, P.R. China
Received February 4, 2003

Key Words : Antitumor, Sesquiterpene, *Euonymus nanoides*

The Celastraceae family is a rich source of β-dihydroagarofuran sesquiterpene skeleton with cytotoxic, antitumor-promoting, immunosuppressive, insecticidal and insect-antifeedant activities.[1] In a previous study of the chemical constituents of genus *Euonymus* (Celastraceae), we reported on the isolation of several β-dihydroagarofuran sesquiterpenes.[2,3] Recently, we examined sesquiterpene constituents of *Euonymus nanoides* Loes. (Celastraceae) and isolated a new (**1**) β-dihydroagarofuran sesquiterpene polyol ester. We report here the structure elucidation of new compound by a combination of 1D- and 2D- NMR techniques and antitumor activity of **1**.



Compound 1

Compound **1**, yellow oil, analyzed for $C_{34}H_{48}O_9$ by FABMS: *m/z* 601 $[M+1]^+$ and NMR spectra data (Table 1). IR spectrum revealed a characteristic ester absorption band at 1741 cm^{-1}. The NMR spectra suggested the presence of one acetate ester [δ_H 2.20 s (3H); δ_C 20.7, 170.5], one benzoate ester [δ_H 7.45 t (2H), 7.55 t (1H), 8.04 d (J = 7.2 Hz, 2H); δ_C 128.3 (2C), 129.4, 130.2 (2C), 133.3, 165.4] and two α-methyl-butanoate esters [δ_H 0.55 t (6H), 0.80 d (J = 6.8 Hz, 3H), 0.86 d (J = 6.8 Hz, 3H), 0.90 m (1H), 0.92 m (1H), 1.18 m (2H), 2.01 m (1H), 2.02 m (1H); δ_C 11.5, 11.8, 16.8, 17.0, 25.1, 25.4, 40.6, 40.7, 172.8, 173.2].

The ^{1}H NMR of **1** showed the presence of two tertiary methyl groups at δ 1.34 s (H-15), 1.31 s (H-14) and one secondary methyl groups at δ 1.22 d (J = 7.7 Hz, H-12). The ^{1}H-^{1}H COSY spectrum signals at δ 5.27 t (H-1), 5.70 s (H-6) and 5.33 t (H-9) were assigned to three protons attached to carbon atoms bearing secondary ester groups, while signals at δ 4.85 d (J = 12.8 Hz, H-13a) and δ 4.51 d (J = 12.8 Hz, H-13b) were assigned to the two protons attached to carbon atoms bearing primary ester groups. The ^{13}C NMR (DEPT) spectrum of the parent skeleton of **1** showed three methyls at δ 16.8, 24.8 and 29.1, three methylene at δ 31.0, 31.8 and 33.5, one methylene attached to an oxygen function at δ 66.3, two methine at δ 32.2 and 43.4, three methines attached to an oxygen function at δ 68.3, 68.8 and 69.4, one quaternary carbon at δ 51.2, and two quaternary carbons attached to an oxygen function at δ 83.8 and 89.8, whose chemical shifts were very similar to those of reported β-dihydroagarofurans.[4] It was determined that compound **1**

Table 1. The NMR data of **1** (400 MHz, $CDCl_3$)

No.	δ_C (DEPT)	δ_H (J, Hz)	HMBC (carbon)[a]
1	68.8 (CH)	5.27 t	(2), 9, (10), 13, MeBuO (172.8 ppm)
2	31.0 (CH_2)	2.29 m	(1), (3), 4
		2.08 m	(1), (3), 4
3	31.8 (CH_2)	2.04 m	(4), 5
		1.61 m	(4), 5
4	32.2 (CH)	2.33 m	(5), 6, 10
5	89.8 (C)		
6	69.4 (CH)	5.70 s	(5), (7), 8, 10, MeBuO (173.2 ppm)
7	43.4 (CH)	2.31 m	(8), 9, 11
8	33.5 (CH_2)	2.37 m	(7), (9), 10
		2.03 m	(7), (9), 10
9	68.3 (CH)	5.33 t	5, (8), (10), 13, BzO (165.4 ppm)
10	51.2 (C)		
11	83.8 (C)		
12	16.8 (CH_3)	1.22 d (7.7)	3, (4), 5
13	66.3 (CH_2)	4.85 d (12.8)	1, 5, 9, (10), AcO (170.5 ppm)
		4.51 d (12.8)	1, 5, 9, (10), AcO (170.5 ppm)
14	29.1 (CH_3)	1.31 s	(11), 15
15	24.8 (CH_3)	1.34 s	(11), 14

[a]Two-bond correlations are indicated in parentheses.

*To whom correspondence should be addressed. Tel and Fax: +86-571-87951264; E-mail: cheyjpan@zju.edu.cn (Y. J. Pan); xuant@lzu.edu.cn (X. Tian)

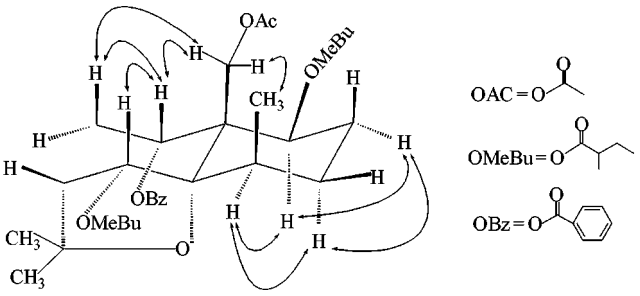


Figure 1. Major NOESY correlations in **1**.

was a β-dihydroagarofuran sesquiterpene substituted with one acetate, one benzoate and two α-methyl-butanoate esters.

The ester group distributions were determined from the HMBC spectrum, which showed cross-peaks between H-9 and the carbonyl at δ 165.4 of the benzoate ester, H-13 and the carbonyl at δ 170.5 of the acetate ester, H-1, H-6 and the carbonyl at δ 172.8, 173.2 of two α-methyl-butanoate ester, respectively. In skeleton of β-dihydroagarofuran sesquiterpene, H-1 and H-6 have axial stereochemistry.[5,6] From the results of the NOESY spectrum of **1**, the correlation between H-6 and H-9 indicated the presence of H-9eq (Fig. 1). Therefore, compound **1** was elucidated as 1β, 6α-di (α-methyl)-butanoyl-9α-benzoyloxy-13-acetoxy-β-dihydroagarofuran.

The compound **1** was tested for in *vitro* antitumor against HL 60 (leukemia neoplasm) and BEL 7402 (liver carcinoma).[7] IC_{50} values were determined for compound **1** (HL 60: 41.70 μg/mL; BEL 7402: 43.95 μg/mL). These results show that compounds **1** was able to inhibit activity with IC_{50} values below 100 μg/mL.

Experimental Section

General Methods. IR spectra were measured on a Nicolet 170-5X-FT-IR instrument KBr. UV spectra were measured on a Shimadzu UV-260 spectrometer. 1D and 2D NMR spectra were measured on a Bruker AM-400FT-NMR spectrometer with TMS as internal standard. MS spectra were measured on the EI. 70 eV and HP-5988MS spectrometer. Optical rotation was measured by Perkin Elmer Model 341. Silica gel (200-300 mesh) was used for CC, silica GF_{254} for TLC of compound isolated by pre. TLC.

Plant Material. The seed of *Euonymus nanoides* Loes. were collected in Luqu country, Gansu province of China in October 1997, and identified by Prof. J. Zh. Sun of Department of Biology, Lanzhou University. A voucher specimen (No. 971001) is deposited in Department of Biology, Lanzhou University.

Extraction and Isolation. Dried, powdered seed (1.2 kg) of *E. nanoides* were extracted with acetone by percolation at room temperature to give a residue (102.8 g) after evaporation. This residue was separated on CC over 800 g silica gel with a gradient of petroleum ether (60-90 °C) acetone as eluent. Compound **1** was isolated during elution with petroleum ether (60-90 °C)-acetone (5 : 1). TLC using solvent systems for **1** and obtained 12.3 mg.

Compound 1: $C_{34}H_{48}O_9$, yellow oil, $[\alpha]_D^{20}$: +16.0° ($CHCl_3$, c 1.20); IR *v*: 2926, 1741, 1632, 1380, 1232, 1060, 891, 712 cm^{-1}; UV λ_{max}^{MeOH} : 203, 231, 274 nm; EIMS: *m/z* (%) 600 $[M]^+$ (9.8), 478 $[M\text{-}BzOH]^+$ (3.5), 388 $[M\text{-}2MeBuO\text{-}AcOH]^+$ (18.2), 262 (21.0), 50 (100); FABMS: *m/z* 601 $[M+H]^+$; ^1H and ^{13}C NMR ($CDCl_3$, 400 MHz) see Table 1.

Acknowledgment. This work was supported by Lanzhou Medicine institute, Lanzhou of P. R. China.

References

1. Tincusi, Z. B. M.; Jimenez, I. A.; Ravelo, A. G.; Missico, R. *J. Nat. Prod.* **1998**, *61*, 1520.
2. Wang, H.; Tian, X.; Chen, Y. Z. *Chin. Chem. Lett.* **2002**, *13*, 1063.
3. Wang, H.; Yang, L.; Tian, X.; Chen, Y. Z. *Pharmazie* **2001**, *56*, 889.
4. Kim, S. E.; Kim, H. S.; Hong, Y. S.; Kim, Y. C.; Lee, J. J. *J. Nat. Prod.* **1999**, *62*, 697.
5. Huang, H. C.; Shen, C. C.; Chen, C. F.; Wu, Y. C.; Kuo, Y. H. *Chem. Pharm. Bull.* **2000**, *48*, 1079.
6. Wu, W.; Wang, M.; Zhu, J.; Zhou, W.; Hu, Z.; Ji, Z. *J. Nat. Prod.* **2001**, *64*, 364.
7. Bergeron, R. J.; Cavanaugh, P. F.; Kline, S. J.; Hughes, R. G.; Elliot, G. T.; Porter, C. W. *Biochem. Bioph. Res. Comm.* **1984**, *121*, 848.