Two New Sesquiterpene Polyesters from the Seeds of *Euonymus nanoides* Loes.

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Two new β-dihydroagarofuran sesquiterpene polyesters, 1*S*,13-diacetyloxy-4*S*-hydroxy-6*R*-(3-)furancarbonyloxy-9*S*-cinnamoyloxy-β-dihydroagarofuran (1) and 1*S*,6*R*-di(2-)methylbutanoyloxy-4*S*-hydroxy-8*S*-benzoyloxy-9*R*-(3-)furancarbonyloxy-13-acetyloxy-β-dihydroagarofuran (2), together with one known compound (3) were isolated from the seeds of *Euonymus nanoides* Loes. Their structures were elucidated by spectroscopic data interpretation. Compound 2 showed moderate antitumor activity against BEL-7402, P-388, HL-60 and A-549.

Key words *Euonymus nanoides*; antitumor activity; β-dihydroagarofuran

Plants belonging to the family Celastraceae have used worldwide as medicines and have been the subject of many investigations for their biologically active components.[1)] In a previous study on the chemical constituents of Celastraceae, we have recently reported on the isolation and structural elucidation of several new sesquiterpene polyesters with β-dihydroagarofuran skeletons[2,3)] and triterpenes.[4,5)] Some of these novel isolates exhibited antitumor activities. Continuing studies on the seeds of *E. nanoides* have provided two additional new: 1*S*,13-diacetyloxy-4*S*-hydroxy-6*R*-(3-)furancarbonyloxy-9*S*-cinnamoyloxy-β-dihydroagarofuran (**1**), 1*S*,6*R*-di(2-)methylbutanoyloxy-4*S*-hydroxy-8*S*-benzoyloxy-9*R*-(3-)furancarbonyloxy-13-acetyloxy-β-dihydroagarofuran (**2**) and one known 1*S*-diacetyloxy-4*S*,6*R*-dihydroxy-9*S*-benzoyloxy-13-cinnamoyloxy-β-dihydroagarofuran (**3**). The pure compounds were tested on a panel of three human and one mouse cancer cell lines. Details of the isolation and structure determination of **1** and **2** are presented here.

Results and Discussion

Silica gel column chromatography and vacuum liquid chromatography (VLC) of the acetone extract of the seeds of *E. nanoides* afforded 20 fractions. Further separation of these fractions using thin layer chromatography (TLC) and reversed phase column chromatography (RP-18) yielded compounds **1**—**3**. The known compound **3** was identified by comparison of their physical and spectral data with those reported in the literature.[6)]

The molecular formula of **1** was established as $C_{33}H_{38}O_{11}$ by analysis of NMR and HR-ESI-MS data. IR spectrum revealed a characteristic ester absorption band at 1735 cm^{-1} and a free OH absorption band at 3427 cm^{-1}. The NMR spectra suggested the presence of two acetate esters [δ_H 1.78 (3H, s, Me), 2.20 (3H, s, Me); δ_C 21.0 (q), 21.3 (q), 169.7 (s), 170.5 (s)], one cinnamate ester [δ_H 6.38 (1H, d), 7.41 (2H, m), 7.47 (1H, m), 7.55 (2H, m), 7.70 (1H, d); δ_C 117.7 (d), 128.3 (2×d), 129.7 (2×d), 130.5 (d), 134.2 (s), 145.7 (d), 165.8 (s)], and one (3-)furancarboxylate ester [δ_H 6.73 (1H, d), 7.50 (1H, d), 8.27 (1H, s); δ_C 109.8 (d), 118.9 (s), 144.1 (d), 148.7 (d), 161.8 (s)]. The ^{1}H-NMR spectrum of **1** showed the presence of three tertiary methyl groups at δ 1.35 (s, H-12), 1.42 (s, H-14), 1.50 (s, H-15). The ^{1}H signals observed at δ 5.66 (1H, dd, H-1), 5.72 (1H, s, H-6) and 5.28 (1H, d, H-9) were assigned to those of the protons of three methine groups bearing an acyloxy group by the ^{1}H–^{1}H COSY spectrum, while signals at δ 4.50 (d, H-13a) and δ 5.09 (d, H-13b) were assigned to the two protons of one methylene group bearing an acyloxy group. In addition, the ^{13}C-NMR and DEPT spectra indicated that the parent skeleton consists of fifteen carbons: three methyl carbons (δ_C 29.1, 25.3, 24.2), four methylene carbons (δ_C 31.0, 40.7, 33.3, 66.3), four methine carbons (δ_C 70.2, 69.5, 43.3, 69.3) and four quaternary carbons (δ_C 69.4, 89.7, 50.9, 83.7). These data were in good agreement with the spectral values assigned to the 5-substituted β-dihydroagarofuran parent skeleton.[7,8)] Moreover, the molecular composition suggested the presence of one free hydroxyl group. One free hydroxyl group and the four esters mentioned above locates at C-1, C-4, C-6, C-9 and C-13 of β-dihydroagarofuran.

The carbonyl signal at δ 161.8 and 165.8 were correlated with the proton signals at H-6 and H-9 respectively, revealing that the (3-)furancarboxylate ester was located at C-6 and cinnamate ester was located at C-9. Also, two acetate esters were positioned at C-1 and C-13 respectively, because the carbonyl signals at δ 169.7 and 170.5 were correlated with the proton signals H-1 and H-13, respectively.

The relative stereochemistry of **1** was determined on the

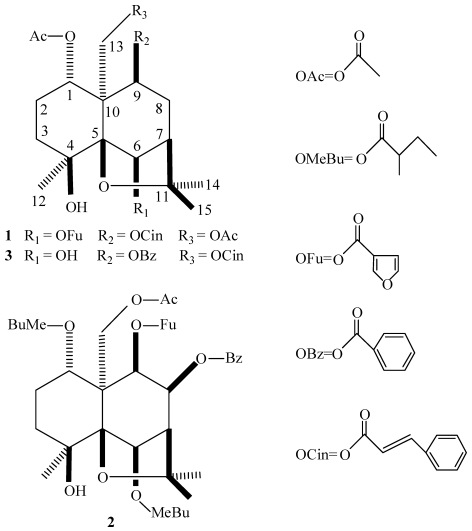

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Table 1. NMR Data for Compounds **1**—**3** in $CDCl_3$ (δ ppm)

No.	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	70.2	5.66 dd, 3.6, 11.4	68.0	5.59 dd, 3.5, 11.6	72.5	5.50 dd, 4.4, 11.2
2	31.0	2.45 m	31.7	2.40 m	23.6	2.04 m
		2.10 m		2.08 m		2.07 m
3	40.7	2.04 m	41.6	1.50 m	37.1	1.78 m
		1.45 m		2.05 m		1.98 m
4	69.4		69.6		73.0	
5	89.7		89.7		91.2	
6	69.5	5.72 s	68.2	6.71 s	79.2	4.81 d, 5.4
7	43.3	2.45 m	43.3	2.31 d, 3.2	49.9	2.31 m
8	33.3	2.66 m	69.7	5.31 dd, 6.0, 3.2	34.2	2.25 m
		2.07 m				2.47 m
9	69.3	5.28 d, 6.9	69.9	5.19 d, 6.0	68.4	5.68 d, 7.3
10	50.9		50.9		53.4	
11	83.7		83.6		84.7	
12	29.1	1.35 s	29.0	1.30 s	23.3	1.45 s
13	66.3	5.09 d, 12.8	66.1	4.84 d, 12.8	65.0	4.95 d, 12.2
		4.50 d, 12.8		4.46 d, 12.8		4.73 d, 12.2
14	25.3	1.42 s	26.4	1.42 s	30.0	1.61 s
15	24.2	1.50 s	24.1	1.40 s	26.6	1.58 s
Ac–O	21.0	1.78 3H, s	21.3	2.17 3H, s	20.6	1.45 3H, s
	169.7		170.4		169.7	
Ac–O	21.3	2.20 3H, s				
	170.5					
Bz–O			128.2	7.39 2H, t, 7.3	128.5	7.40 2H, t, 7.5
			128.7	7.43 1H, t, 7.3	129.0	7.45 1H, t, 7.5
			130.1	8.02 2H, d, 8.0	130.9	8.05 2H, d, 8.2
			133.3		134.0	
			165.3		165.2	
Fu–O	109.8	6.73 1H, d, 1.2	109.8	6.73 1H, d, 1.2		
	118.9	7.50 1H, d, 1.2	118.8	7.53 1H, d, 1.2		
	144.1	8.27 1H, s	143.6	7.99 1H, s		
	148.7		148.7			
	161.8		161.9			
MeBu–O			11.2	0.55 t, 3H),		
			15.9	0.86 3H, d, 6.8		
			25.0	0.90 1H, m		
			40.6	1.18 2H, m		
			174.5			
MeBu–O			11.5	0.66 3H, t		
			16.4	0.88 3H, d, 6.8		
			25.5	0.92 1H, m		
			40.7	2.00 2H, m		
			175.1			
Cin–O	117.7	6.38 1H, d, 15.6			117.2	6.50 1H, d, 16.6
	128.3	7.41 2H, m			128.3	7.43 2H, m
	129.7	7.47 1H, m			129.5	7.48 1H, m
	130.5	7.55 2H, m			130.5	7.57 2H, m
	134.2	7.70 1H, d, 15.6			134.8	7.78 1H, d, 16.6
	145.7				146.2	
	165.8				166.4	
H–O						5.18 d, 5.4

Figures after δ_H values are coupling constants in Hz. All signals were assigned using 1D and 2D NMR.

basis of NOESY and ^{1}H-NMR data. As usually found in this class of skeleton, H-1, H-6 and Me-4 have axial stereochemistry, the free hydroxyl group was assumed to be at C-4 and the H-6 was assigned to single peak because the dihedral angles between H-6 and H-7 was *ca.* 90°.[8—10] From the NOESY spectrum of **1**, the strong correlation between H-7/H-9 and H-9/H-13 indicated the presence of *cis*-orientation of H-9. Thus, compound **1** was identified as 1*S*,13-diacetyloxy-4*S*-hydroxy-6*R*-(3-)furancarbonyloxy-9*S*-cinnamoyloxy-β-dihydroagarofuran.

Compound **2** was obtained as yellow oil and assigned the molecular formula $C_{39}H_{50}O_{13}$ on the basis of its ^{13}C-NMR, DEPT and HR-ESI-MS. IR spectrum revealed a characteristic ester absorption band at 1727 cm^{-1} and a free OH absorption band at 3432 cm^{-1}. The NMR spectra suggested the presence of two 2-methylbutanoate esters, one acetate ester, one benzoate ester and one (3-)furancarboxylate ester. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, except that it was apparent that **2** has one more substituted moiety at C-8, suggesting that **2** contain the 1,6,8,9,13-pentasubstituted-β-dihydroagarofuran skeleton and that **2** has the same stereochemistry for H-1, H-6 and OH-4 as **1**. The cou-

pling constant, $J_{8,9}$=6.0 Hz suggested that H-8 and H-9 have a different orientation.[9,10] The stereochemistry for H-8 and H-9 was determined from the NOESY spectrum, which showed cross-peaks between H-7/H-8, H-7/H-9, H-8/H-13 and H-9/H-13, suggesting that H-8 is axial and H-9 is equatorial, respectively. The locations of the ester groups were also identified by the HMBC cross-peaks between H-8 and the benzoate carbonyl signal at δ 165.3, H-9 and the (3-)furancarboxylate carbonyl at δ 161.9, the H-13 and the acetate carbonyl at δ 170.4, and H-1 and H-6 between 2-methylbutanoate carbonyl groups at δ 174.5 and 175.1, respectively. Therefore, the structure of compound **2** was assigned as 1*S*,6*R*-di(2-)methylbutanoyloxy-4*S*-hydroxy-8*S*-benzoyloxy-9*R*-(3-)furancarbonyloxy-13-acetyloxy-δ-dihydroagarofuran.

Using a previously published protocol,[2] the compounds **1**—**3** were tested for *in vitro* antitumor activity against three human tumor cell lines: A-549, HL-60, BEL-7402 and one mouse tumor cell line of P-388. These results showed that compounds were able to inhibit activity with IC_{50} values below 100 μM. Compounds **1** and **3** were inactive with all IC_{50} >100 μM; compound **2** showed moderate activity against BEL-7402, HL-60, P-388 and A-549 with IC_{50} values of 6.9, 51.4, 51.2 and 81.7 μM, respectively.

Experimental

General experimental procedures have been described elsewhere.[2] ^{1}H-NMR data were obtained at 400 MHz (Bruker AM-400), and ^{13}C-NMR data were obtained at 100 MHz (Bruker AM-400). NMR data were recorded in $CDCl_3$, and the chemical shifts were referred to the residual solvent signals (δ_H 7.24/δ_C 77.0). Mass spectra (EI-MS, FAB-MS and HR-ESI-MS) were recorded on a HP-5988 MS and APEX ^{TM}II Bruker 4.7TAS spectrometer. Optical rotation (OR): Perkin Elmer Model 341.

Plant Material The collection and identification of the seeds of *E. nanoides* (No. 971001) were described previously.[2]

Extraction and Isolation Dried, powdered seed (1.2 kg) of *E. nanoides* were extracted with acetone (3000 ml×7 d×3) by percolation at room temperature to give a residue (102.8 g) after evaporation. This residue was separated on VLC and CC with a gradient of petroleum ether (60—90 °C)–acetone as eluent to give 20 fractions. Fraction 10 (3.0 g) was subjected to CC and eluted with petroleum ether–acetone (3 : 1) obtaining compound **1**, was purified by TLC yielding pure **1** (42.1 mg, petroleum ether–acetone (3 : 2)). Compounds **2** and **3** were yielded by CC with petroleum ether–acetone (1 : 1) from fraction 16 and purified by RP-18 short column (20×250 mm, MeOH–H_2O 4 : 1): **2** (63.1 mg) and **3** (1.4 mg).

Compound **1**: Yellow oil. $[\alpha]_D$ +87.5° (c=4.20, $CHCl_3$). UV λ_{max} (MeOH): (log ε) 202 (4.00), 225 (3.72), 279 (3.48). IR ν_{max} (KBr): 3427, 2933, 1735, 1635, 1230 cm^{-1}. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS: *m*/*z* 550 (M^+−AcOH, 4.4), 438 (550−FuOH, 4.0) 290 (438−CinOH, 27.0), 230 (290−AcOH, 100). FAB-MS: *m*/*z* 611 $[M+H]^+$. HR-ESI-MS: *m*/*z* 611.2494 $[M+H]^+$ (Calcd for $C_{33}H_{39}O_{11}$, 611.2487).

Compound **2**: Yellow oil. $[\alpha]_D$ +39.4° (c=6.30, $CHCl_3$); UV λ_{max} (MeOH): (log ε) 205 (3.77), 225 (3.70), 272 (3.01). IR ν_{max} (KBr): 3432, 2928, 1727, 1642, 1460 cm^{-1}. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS: *m*/*z* 614 (M^+−FuOH, 14.0), 604 (M^+−BzOH, 12.0), 492 (614−BzOH, 12.0), 392 (492+H−MeBuO, 54.0), 230 (392−MeBuOH−AcOH, 92), 50 (100). FAB-MS: *m*/*z* 727 $[M+H]^+$. HR-ESI-MS: *m*/*z* 727.3338 $[M+H]^+$ (Calcd for $C_{39}H_{51}O_{13}$, 727.3330).

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