Antitumor Sesquiterpenes from *Euonymus nanoides*

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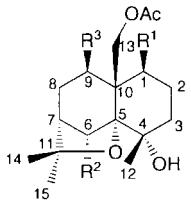
The isolation, structure elucidation, and antitumor activity of four new sesquiterpene polyol esters, *i.e.*, of 6α,13-bis(acetyloxy)-9β-(cinnamoyloxy)-1β-(furan-3-ylcarbonyl)oxy]-4α-hydroxy-β-dihydroagarofuran (**1**), 13-(acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β,6α-bis[(2-methylbutanoyl)oxy]-β-dihydroagarofuran (**2**), 1β,6α,13-tri(acetyloxy)-9β-(cinnamoyloxy)-4α-hydroxy-β-dihydroagarofuran (**3**), and 6α,13-bis(acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β-[(2-methylbutanoyl)oxy]-β-dihydroagarofuran (**4**), and of five known sesquiterpene polyol esters **5**–**9** from the seed oil of *Euonymus nanoides* LOES. are reported (β-dihydroagarofuran = octahydro-2,2,5a,9-tetramethyl-2*H*-3,9a-methano-1-benzoxepin).

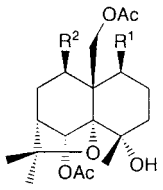
Introduction. – Plants of the Celastraceae family comprise *ca.* 60 genera and 850 species worldwide [1]. Many of them have been used as traditional medicinal plants [2]. β-Dihydroagarofuran (= octahydro-2,2,5a,9-tetramethyl-2*H*-3,9a-methano-1-benzoxepin) sesquiterpene polyol esters and alkaloids are biologically active natural products found almost exclusively in species of the *Euonymus* (Celastraceae) genus [3–5]. The common structural features of this class of compounds are a terpene C_{15} skeleton and a β-dihyroagarofuran moiety. Other structural elements can vary considerably. Pharmacological activities described for β-dihydroagarofuran sesquiterpenes include their cytotoxic [6], antitumor-promoting [7], immunosuppressive [8], insecticidal [9], and inset-antifeedant activities [10] and reversing multi-drug resistance in cancer cells [11]. Recently, several β-dihydroagarofuran sesquiterpenes were shown to have anti-HIV activity [12]. As part of our interest in β-dihydroagarofuran sesquiterpene polyol esters with antitumor activity, we investigated the chemical constituents of the seed oil of *Euonymus nanoides* LOES., which showed antiproliferative activity in the A-549, HL-60, BEL-7402, and P-388 cell-line antitumor screens. We now wish to report the isolation, structure determination, and biological activity of the four new compounds **1**–**4** and of the five known compounds **5**–**9** from the seed oil of *E. nanoides.*

Results and Discussion. – Column chromatography (CC), vacuum liquid chromatography (VLC), thin layer chromatography (TLC), and reversed-phase column chromatography (*RP-18*) of the acetone extract of the seed of *E. nanoides* yielded compounds **1**–**9**. The structures of compounds **5**–**9** were determined by spectral data as 1β,2β,6α-tris(acetyloxy)-9α-(benzoyloxy)-4α-hydroxy-13-[(2-methylbutanoyl)oxy]-β-dihydroagarofuran[1]) (**5**) [13], 2β,6α-bis(acetyloxy)-1β-[(furan-3-ylcarbonyl)oxy]-4α-

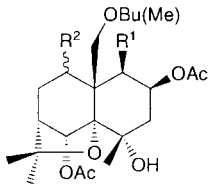
[1]) Arbitrary numbering (see *Formula* **1**); for systematic names of **1**–**4**, see *Exper. Part*



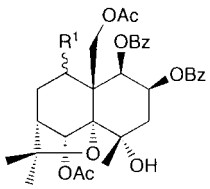
1[1]) R^1 = FuO, R^2 = AcO, R^3 = CinO
2 R^1 = R^2 = Bu(Me)O, R^3 = BzO



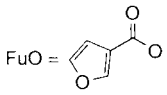
3 R^1 = AcO, R^2 = CinO
4 R^1 = Bu(Me)O, R^2 = BzO

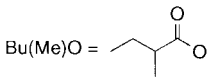


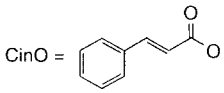
5 R^1 = AcO, R^2 = α-BzO
6 R^1 = FuO, R^2 = β-Bu(Me)O
9 R^1 = BzO, R^2 = α-Bu(Me)O



7 R^1 = α-AcO
8 R^1 = β-BzO







hydroxy-9β,13-bis-[(2-methylbutanoyl)oxy]-β-dihydroagarofuran[1]) (**6**) [14], 6α,9α,13-tris(acetyloxy)-1β,2β-bis(benzoyloxy)-4α-hydroxy-β-dihydroagarofuran[1]) (**7**) [14], 6α,13-bis(acetyloxy)-1β,2β,9β-tris(benzoyloxy)-4α-hydroxy-β-dihydroagarofuran[1]) (**8**) [15], 2β,6α-bis(acetyloxy)-1β-(benzoyloxy)-4α-hydroxy-9α,13-bis[(2-methylbutanoyl)-oxy]-β-dihydroagarofuran[1]) (**9**) [14]; similarly, the structures of the new compounds **1**–**4** were established.

Compound **1** was isolated as an optically active yellow oil. The HR-MS of **1** exhibited a quasi-molecular-ion peak at m/z 611.2494 ($[M+H]^+$), corresponding to the molecular formula $C_{33}H_{38}O_{11}$. The IR spectrum revealed a characteristic ester absorption at 1732 cm^{-1} and a free OH absorption at 3432 cm^{-1}. The complete assignments of the ^{1}H- and ^{13}C-NMR data of **1** were successfully carried out by ^{1}H,^{1}H COSY, HMQC, and HMBC experiments (*Tables 1* and *2*). Thus, compound **1** was identified as 6α,13-bis(acetyloxy)-9β-(cinnamoyloxy)-1β-[(furan-3-ylcarbonyl)oxy]-4α-hydroxy-β-dihydroagarofuran[1]).

The NMR spectra of **1** suggested the presence of two acetate moieties (δ(H) 1.79, 2.20 (2*s*, 2 Me); δ(C) 20.6 (*q*), 21.3 (*q*), 169.7 (*s*), 170.8 (*s*)), a cinnamate function (δ(H) 6.38 (*d*, J = 15.6 Hz, PhCH=C*H*), 7.40 (*m*, 2 H, Ph), 7.47 (*m*, 1 H, Ph), 7.55 (*m*, 2 H, Ph), 7.70 (*d*, J = 15.6 Hz, PhC*H*=CH); δ(C) 117.7 (*d*), 128.8 (2*d*), 129.7 (2*d*), 130.5 (*d*), 134.2 (*s*), 145.8 (*d*), 165.8 (*s*)), a furan-3-carboxylate moiety (δ(H) 6.87 (*d*, J = 1.2 Hz, 1 H, CH=CH), 7.41 (*d*, J = 1.2 Hz, 1 H, CH=CH), 8.27 (*s*, CH=C); δ(C) 109.7 (*d*), 118.9 (*s*), 144.0 (*d*), 148.4 (*d*), 161.9 (*s*)), and a free OH group (δ(H) 2.71 (*s*)). The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **1** showed the presence of three tertiary Me groups at δ 1.34 (*s*, Me(12)), 1.46 (*s*, Me(14)), and 1.49 (*s*, Me(15))[1]). The ^{1}H,^{1}H

Table 1. *^{1}H-NMR Data* (400 MHz) *of Compounds* **1**–**4** *in $CDCl_3$*. δ(H) in ppm, *J* in Hz.

	1	**2**	**3**	**4**
H–C(1)	5.66 (*d*, *J* = 4.0)	5.57 (*d*, *J* = 4.0)	5.48 (*d*, *J* = 3.2)	5.59 (*d*, *J* = 3.6)
CH_2(2)	2.45, 2.07 (2*m*)	2.48, 2.08 (2*m*)	2.40, 2.01 (2*m*)	2.46, 2.11 (2*m*)
CH_2(3)	1.15, 0.90 (2*m*)	1.50, 2.01 (2*m*)	1.20, 0.86 (2*m*)	1.50, 2.02 (2*m*)
H–C(6)	5.67 (*s*)	5.69 (*s*)	5.58 (*s*)	6.15 (*s*)
H–C(7)	2.31 (*m*)	2.33 (*m*)	2.21 (*m*)	2.30 (*m*)
CH_2(8)	2.31, 2.11 (2*m*)	2.31, 2.15 (2*m*)	2.25, 2.11 (2*m*)	2.32, 2.17 (2*m*)
H–C(9)	5.28 (*d*, *J* = 6.8)	5.34 (*d*, *J* = 6.8)	5.21 (*d*, *J* = 6.8)	5.34 (*d*, *J* = 6.4)
Me(12)	1.34 (*s*)	1.34 (*s*)	1.31 (*s*)	1.33 (*s*)
CH_2(13)	5.04, 4.42 (2*d*, each *J* = 12.8)	4.87, 4.52 (2*d*, each *J* = 12.8)	4.75, 4.44 (2*d*, each *J* = 12.8)	4.79, 4.56 (2*d*, each *J* = 12.8)
Me(14)	1.46 (*s*)	1.43 (*s*)	1.43 (*s*)	1.42 (*s*)
Me(15)	1.49 (*s*)	1.46 (*s*)	1.46 (*s*)	1.44 (*s*)

Table 2. *^{13}C-NMR Data* (100 MHz) *of Compounds* **1**–**4** *in $CDCl_3$*. δ(C) in ppm.

	1[a])	**2**[a])	**3**[a])	**4**[a])
CH(1)	70.1	70.0	70.1	70.0
CH_2(2)	31.0	31.1	30.9	31.0
CH_2(3)	40.5	41.7	40.5	40.5
C(4)	69.5	69.4	69.5	69.4
C(5)	89.7	89.6	89.7	89.8
CH(6)	69.1	70.2	69.0	68.5
CH(7)	43.3	43.4	43.2	43.3
CH_2(8)	33.6	33.5	33.4	33.3
CH(9)	69.0	68.3	68.6	68.1
C(10)	50.8	51.2	50.9	51.3
C(11)	83.7	83.8	83.6	83.8
Me(12)	30.0	29.9	29.9	29.9
CH_2(13)	66.3	66.2	65.6	65.6
Me(14)	25.3	26.5	25.0	25.1
Me(15)	24.2	24.4	24.2	24.3

[a]) Attached protons by DEPT.

COSY plot exhibited signals at δ 5.66 (*d*, *J* = 4.0 Hz, H–C(1)), 5.67 (*s*, H–C(6)), and 5.28 (*d*, *J* = 6.8 Hz, H–C(9)) assigned to CH groups bearing an ester group, while signals at δ 4.42 (*d*, *J* = 12.8 Hz, H_a–C(13)) and δ 5.04 (*d*, *J* = 12.8 Hz, H_b–C(13)) were assigned to the a CH_2 group bearing an ester group. The ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of **1** showed 3 Me (δ 24.2, 25.3, and 30.0), 3 CH_2 (δ 31.0, 33.6, and 40.5), 1 OCH_2 (δ 66.3), 1 CH (δ 43.3), 3 OCH (δ 69.0, 69.1, and 70.1), 1 quaternary C-atom (δ 50.8), and 3 quaternary C-atoms attached to an O-atom (δ 69.5, 83.7, and 89.7), whose chemical shifts were very similar to those of reported β-dihydroagarofuran sesquiterpene polyol esters [16][17]. The above data suggested that **1** was a β-dihydroagarofuran sesquiterpene substituted with two acetyloxy, one (furan-3-ylcarbonyl)oxy, one cinnamoyloxy, and one free OH groups. The ^{1}H,^{13}C long-range correlation spectrum (HMBC) confirmed the location of the ester groups. Thus, the C=O signals at δ(C) 161.9 and 165.8 were correlated with the signals at δ(H) 5.66 (H–C(1)) and 5.28 (H–C(9)), respectively, revealing that the (furan-3-ylcarbonyl)oxy group was located at C(1) and the cinnamoyloxy group at C(9). Similarly, the acetyloxy groups were positioned at C(6) and C(13) because the C=O signals at δ(C) 169.7 and 170.6 were correlated with the proton signals of H–C(6) and CH_2(13), respectively. As usually found in this class of compounds, H–C(1) and H–C(6) were axially positioned [9][18]. From the NOESY spectrum of **1**, the strong correlation between H–C(1) and H–C(9) indicated also an axial conformation for H–C(9)).

Compound **2** was isolated as an optically active yellow oil that analyzed for $C_{34}H_{48}O_{10}$ by HR-MS (*m/z* 639.3134 ($[M+Na]^+$)). The IR spectrum revealed a characteristic ester absorption at 1727 cm^{-1} and a free-OH absorption at 3432 cm^{-1}. In accord with the ^1H- and ^{13}C-NMR data (*Tables 1* and *2*), compound **2** was elucidated as 13-(acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β,6α-bis[(2-methylbutanoyl)oxy]-β-dihydroagarofuran[1]).

The NMR spectra of **2** suggested the presence of two 2-methylbutanoate moieties (δ(H) 0.55 (*t*, 2 *Me*CH_2), 0.80 (*d*, *J* = 6.8 Hz, 2 *Me*CH), 0.92 (*m*, MeCH_2), 1.20 (*m*, MeCH_2), 1.90 (*m*, MeCH_2C*H*), 2.00 (*m*, MeCH_2C*H*); δ(C) 11.2 (*q*), 11.6 (*q*), 15.4 (*q*), 16.5 (*q*), 25.1 (*t*), 25.4 (*t*), 40.6 (*d*), 40.8 (*d*), 174.4 (*s*), 175.2 (*s*)), an acetate moiety (δ(H) 2.21 (*s*, Me); δ(C) 21.4 (*q*), 170.5 (*s*)), a benzoate function (δ(H) 7.45 (*t*, 2 H, Ph), 7.57 (*t*, 1 H, Ph), 8.05 (*d*, *J* = 7.6 Hz, 2 H, Ph); δ(C) 128.3 (2*d*), 129.4 (*s*), 130.2 (2*d*), 133.3 (*d*), 165.4 (*s*)), and a free OH group (δ(H) 2.68 (*s*)). The NMR data for the parent ring system was very similar to those of **1**, suggesting that **2** also contains the 1,4,6,9,13-pentasubstituted β-dihydroagarofuran skeleton, and the locations of the protons were confirmed by the ^1H,^1H COSY plot. As for **1**, the free OH group at C(4) was in equatorial position, and the locations of the ester groups were apparent from the HMBC spectrum, which showed cross-peaks between H−C(9) (δ(H) 5.34, *d*, *J* = 6.8 Hz) and the C=O group at δ(C) 165.4 of the benzoate moiety between CH_2(13) (δ(H) 4.52, 4.87, 2*d*, each *J* = 12.8 Hz, 2 H) and the C=O group at δ(C) 170.5 of the acetate function, and between H−C(1) (δ(H) 5.57, *d*, *J* = 4.0 Hz) and H−C(6) (δ(H) 5.69, *s*) and the C=O groups at δ(C) 175.2 and 174.4, respectively, of the two 2-methylbutanoate moieties. In the NOESY of **2**, a strong correlation between H−C(1) and H−C(9) was observed, which further supported that H−C(9) was in axial position.

Compounds **3** and **4** were obtained as yellow oils. The conclusions drawn from the HR-MS, ^1H-NMR, NOESY difference measurement, and ^{13}C-NMR of **3** and **4** were confirmed by ^1H,^1H COSY, HMQC, and HMBC experiments (*Table 1* and *2*) and by comparison with data of **1** and **2**. Therefore, compound **3** was elucidated as 1β,6α,13-tris(acetyloxy)-9β-(cinnamoyloxy)-4α-hydroxy-β-dihydroagarofuran[1]) and compound **4** as 6α,13-bis(acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β-[(2-methylbutanoyl)oxy]-β-dihydroagarofuran[1]).

Compound **3** analyzed for $C_{30}H_{38}O_{10}$ by HR-MS (*m/z* 559.2534 ($[M+H]^+$). The NMR spectra suggested the presence of three acetate functions (δ(H) 1.79, 2.12, 2.16 (3*s*, 3 Me); δ(C) 20.6 (*q*), 21.1 (*q*), 21.3 (*q*), 169.7 (*s*), 170.2 (*s*), 170.4 (*s*)), a cinnamate moiety (δ(H) 6.35 (*d*, *J* = 16.0 Hz, PhCH=C*H*), 7.38 (*m*, 2 H, Ph), 7.52 (*m*, 1 H, Ph), 7.55 (*m*, 2 H, Ph), 7.67 (*d*, *J* = 16.0 Hz, PhC*H*=CH); δ(C) 117.7 (*d*), 128.2 (2*d*), 128.8 (2*d*), 130.6 (*d*), 134.2 (*s*), 145.7 (*d*), 165.8 (*s*)), and a free OH group (δ(H) 2.66 (*s*)). The NMR data for the parent ring system were very similar to those of **1** and **2**, suggesting that **3** also has the 1,4,6,9,13-pentasubstituted-β-dihydroagarofuran skeleton, and the locations of the protons were confirmed by the ^1H,^1H COSY plot. As for **1**, the positions of the ester groups were determined from the HMBC spectrum, which showed cross-peaks between H−C(9) (δ(H) 5.21, *d*, *J* = 6.8 Hz) and the C=O group at δ(C) 165.8 of the cinnamoate moiety, between CH_2(13) (δ(H) 4.44, 4.75 2*d*, each *J* = 12.8 Hz, 2 H), H−C(1) (δ(H) 5.48, *d*, *J* = 3.2 Hz) and H−C(6) (δ(H) 5.58, *s*) and the C=O groups at δ(C) 170.4, 169.7, and 170.2, respectively, of the acetate functions. In the NOESY of **3**, the correlation between H−C(1) and H−C(9) indicated the axial position of H−C(9).

Compound **4** analyzed for $C_{31}H_{42}O_{10}$ by HR-MS (*m/z* 575.2860 ($[M+H]^+$). The NMR spectra suggested the presence of a 2-methylbutanoate group (δ(H) 0.54 (*t*, *Me*CH_2), 0.79 (*d*, *J* = 6.8, *Me*(CH), 0.91 (*m*, 1 H, MeCH_2), 1.18 (*m*, 1 H, MeCH_2), 2.00 (*m*, MeCH_2C*H*); δ(C) 11.1 (*q*), 15.7 (*q*), 25.3 (*t*), 40.6 (*d*), 174.3 (*s*)), two acetate moieties (δ(H) 2.08, 2.19 (2*s*, 2 Me); δ(C) 21.0 (*q*), 21.2 (*q*), 169.4 (*s*), 170.4 (*s*)), a benzoate group (δ(H) 7.44 (*t*, 2 H, Ph), 7.56 (*t*, 1 H, Ph), 8.03 (*d*, *J* = 7.6 Hz, 2 H, Ph); δ(C) 128.2 (2*d*), 129.3 (*s*), 130.1 (2*d*), 133.5 (*d*), 165.3 (*s*)), and a free OH group (δ(H) 2.68 (*s*)). The assignments of the other ^1H- and ^{13}C-NMR signals of **4** were successfully carried out with ^1H,^1H COSY and HMQC experiments by comparison with data of **1**–**3**, and the positions of the ester groups were determined from the HMBC spectrum, which showed cross-peaks between H−C(9) (δ(H) 5.34, *d*, *J* = 6.4 Hz) and the C=O group at δ(C) 165.3 of the benzoate moiety, between H−C(1) (δ(H) 5.69, *d*, *J* = 3.6 Hz), and the C=O group at δ(C) 174.3 of the 2-methylbutanoate function, and between CH_2(13) (δ 4.56, 4.79, 2*d*, each *J* = 12.8 Hz, 2 H), and H−C(6) (δ(H) 6.15, *s*) and the C=O group at

δ(C) 170.4 and 169.4, respectively, of the two acetate functions. The NOESY of **4** indicated the axial position of H–C(9).

All new and known compounds were tested for *in vitro* antitumor activity against three human-tumor cell lines, *i.e.*, A-549 (lung carcinoma), HL-60 (leukemia neoplasm), BEL-7402 (liver carcinoma), and one mouse-tumor cell line, *i.e.*, P-388 (leukemia neoplasm). IC_{50} Values were determined for compounds **1**–**9** (*Table 3*). These results show that the compounds are able to inhibit tumoral activity, with IC_{50} values below 100 μM.

Table 3. in vitro *Antitumor Activities* (IC_{50} [μM]) *of Compounds* **1**–**9** *and VP-16*.

	P-388	HL-60	A-549	BEL-7402
1	64.41	39.72	83.05	46.45
2	72.24	29.30	> 100	52.72
3	> 100	91.12	> 100	–
4	68.06	45.59	> 100	62.02
5	> 100	98.24	–	> 100
6	13.26	8.68	> 100	12.08
7	91.53	54.46	> 100	74.13
8	80.86	17.48	–	41.13
9	71.83	12.05	> 100	6.23
VP-16	5.89	5.08	8.89	13.17

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Quingdao of China*); reversed-phase, *RP-18* short column, 25 × 250 mm, *Merck*. Vacuum liquid chromatography (VLC): silica gel *60* (35–70 mesh; *Merck*). TLC: precoated silica gel *60*, *F 254* plates (*Merck*); detection by UV light or by heating after spraying with 5% H_2SO_4 in EtOH. Optical rotation: *Perkin-Elmer-341* polarimeter. IR: *Nicolet-AVATAR-360-FT-IR* spectrometer; on KBr disks; in cm^{-1}. UV: *Shimadzu-UV-260* spectrometer; in nm. ^{1}H- and ^{13}C-NMR: at 400 and 100 MHz, resp.; *Bruker-Avance-400* spectrometer; chemical shifts δ in ppm, coupling constants *J* in Hz; $SiMe_4$ as internal standard; 2D NMR experiments included ^{1}H,^{1}H COSY, HMQC, NOESY, DEPT, and HMBC. MS: *HP-5988-MS* and *APEX*^{TM}*II-Bruker-4.7TAS* spectrometer.

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

Extraction and Isolation. Dried, powdered seeds (1.2 kg) of *E. nanoides* were extracted 3× for 7 d with acetone by percolation at r.t. to give a residue (102.8 g) after evaporation. This residue was separated by VLC and CC with a petroleum ether (60–90°)/acetone gradient. Compounds **1**, **3**, and **4** were eluted with petroleum ether/acetone 3:1, and **2** with petroleum ether/acetone 5:1. TLC purification of the crude compounds gave **1** (petroleum ether/acetone 3:2; 15.0 mg), **2** (petroleum ether/acetone 5:2; 7.0 mg), **3** (petroleum ether/acetone 2:1; 59.1 mg), and **4** (petroleum ether/acetone; 2:1 33.2 mg). Compounds **5**–**9** were eluted with petroleum ether/acetone 1:1 and purified by CC (*RP-18* MeOH/H_2O 6:1 → 2:1): **5** (5.0 mg), **6** (5.0 mg), **7** (6.7 mg), **8** (4.8 mg), and **9** (6.3 mg).

6α,13-Bis(acetyloxy)-9β-(cinnamoyloxy)-1β-[(furan-3-ylcarbonyl)oxy]-4α-hydroxy-β-dihydroagarofuran (= *Furan-3-carboxylic Acid (3*R,*5*R,*5a*S,*6*S,*9*S,*9a*S,*10*R*)-10-(Acetyloxy)-5a-[(acetyloxy)methyl]-octahydro-9-hydroxy-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-6-yl Ester*; **1**): Yellow oil. $[\alpha]_D^{20} = +8.5$ ($c = 0.75$, $CHCl_3$). UV (MeOH): 205, 217, 251, 280. IR (KBr): 3432, 2931, 1732, 1638, 1230, 1159, 1023, 877, 721. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Table 1* and *2*. EI-MS: 550 (0.7, $[M - AcOH]^+$), 462 (15.0, $[M - CinO]^+$), 380 (64.0, $[M + H - 2AcO - FuO]^+$), 230 (100). HR-MS: 611.2494 ($[M + H]^+$, $C_{33}H_{39}O_{11}^+$; calc. 611.2487).

13-(Acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β,6α-bis[(2-methylbutanoyl)oxy]-β-dihydroagarofuran (=*2-Methylbutanoic Acid (3*R,*5*R,*5a*S,*6*S,*9*S,*9a*S,*10*R)*-5a-[(Acetyloxy)methyl]-5-(benzoyloxy)-octahydro-9-hydroxy-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-7,10-diyl Ester*; **2**): Yellow oil. $[\alpha]_D^{20} = +29.0$ ($c = 0.70$, $CHCl_3$). UV (MeOH): 204, 231, 274. IR (KBr): 3432, 2928, 1727, 1642, 1460, 1238, 1105, 1028, 880, 725. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. EI-MS: 616 (13.0, M^+), 493 (8.0, $[M - H - BzOH]^+$), 394 (32.0, $[M - BzO - MeBuO]^+$), 312 (100), 264 (54), 154 (73). HR-MS: 639.3134 ($[M + Na]^+$, $C_{34}H_{48}O_{10}Na^+$; calc. 639.3140).

1β,6α,13-Tris(acetyloxy)-9β-(cinnamoyloxy)-4α-hydroxy-β-dihydroagarofuran (=*(2*E*)-3-Phenylprop-2-enoic Acid (3*R,*5*R,*5a*S,*6*S,*9*S,*9a*S,*10*R)*-6,10-Bis(acetyloxy)-5a-[(acetyloxy)methyl]-octahydro-9-hydroxy-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-5-yl Ester;* **3**): Yellow oil. $[\alpha]_D^{20} = +57.0$ ($c = 5.90$, $CHCl_3$). UV (MeOH): 218, 223, 281. IR (KBr): 3451, 2933, 1742, 1635, 1370, 1233, 1151, 1032, 880, 712. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. EI-MS: 410 (8.0, $[M - CinO]^+$), 290 (15.0, $[410 - 2AcOH]^+$), 230 (64.0, $[290 - AcOH]^+$), 50 (100). FAB-MS: 559 ($[M + H]^+$). HR-MS: 559.2534 ($[M + H]^+$, $C_{30}H_{39}O_{10}^+$; calc. 559.2538).

6α,13-Bis(acetyloxy)-9β-(benzoyloxy)-4α-hydroxy-1β-[(2-methylbutanoyl)oxy]-β-dihydroagarofuran (=*2-Methylbutanoic Acid (3*R,*5*R,*5a*S,*6*S,*9*S,*9a*S,*10*R)*-10-(Acetyloxy)-5a-[(acetyloxy)methyl]-5-(benzoyloxy)-octahydro-9-hydroxy-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-6-yl Ester*; **4**): Yellow oil. $[\alpha]_D^{20} = +45.3$ ($c = 3.30$, $CHCl_3$). UV (MeOH): 203, 231, 274. IR (KBr): 3427, 2928, 2357, 1740, 1632, 1380, 1236, 1145, 1033, 888, 710. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. EI-MS: 470 (14.0, $[M + 2H - MeBuO]^+$), 410 (15.0, $[470 - AcOH]^+$), 394 (25.0, $[M + 2H - AcO - BzO]^+$), 230 (80), 50 (100). FAB-MS: 575 ($[M + H]^+$). HR-MS: 575.2860 ($[M + H]^+$, $C_{31}H_{43}O_{10}^+$; calc. 575.2851).

Antitumor Assays. Compounds **1**–**9** were tested for antitumor activity against the following cell lines: P-388, suspension culture of a leukemia neoplasm from a mouse; A-549, monolayer culture of a human-lung carcinoma; HL-60, suspension culture of a leukemia neoplasm from a human; BEL-7402, monolayer culture of a human-liver carcinoma. Cells were maintained in DMEM medium, supplemented with 5% fetal calf serum (FCS), 10-2M sodium hydrogen carbonate and 0.1 g/l penicillin G + 0.1 g/l streptomycin sulfate. The compounds assayed were dissolved in DMSO and tested following the method described by *Bergeron et al.* [19].

H. W. gratefully acknowledges the postdoctoral fellowship awarded by the Zhejiang University, Hangzhou, China, and support by the Lanzhou Medicine Institute, Lanzhou, China.

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Received May 20, 2003