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Sesquiterpene polyol esters from *Euonymus phellomana* Loes

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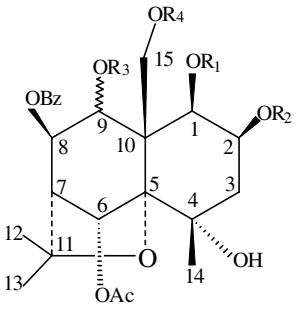
Three new (**1**–**3**) and six known (**4**–**9**) β-dihydroagarofuran sesquiterpene polyol esters were isolated from a methanol extract of the seed oil of *Euonymus phellomana* Loes. and their structures were established on the basis of spectral analysis, including 2D-NMR spectroscopy.

1. Introduction

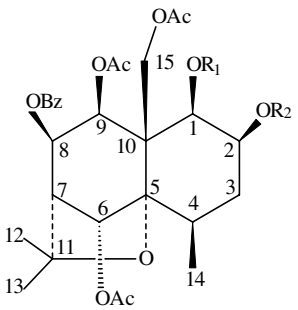
Plants of the Celastraceae family have been the subject of continued and growing interest, due to the range of biological activities shown by many members of this family. Some of them have been used in folk medicine from ancient times as a stimulant and to invigorate the circulation of blood. In the last 30 years, various β-dihydroagarofuran sesquiterpene polyol esters have been isolated from Celastraceae. Some of these sesquiterpene polyol ersters exhibit insect antifeedant and/or insecticidal activities as well as antitumor activity [1, 2]. Recently, we have investigated the chemical constituents of *Euonymus phellomana* Loes. (Celastraceae). *Euonymus phellomana* Loes., a defoliate shrub mainly distributed in the west of China, has been used for the treatment of rheumatism and fractures as a folk medicine. From the methanol extract of the seed oil, three new (**1**–**3**) and six known (**4**–**9**) β-dihydroagarofuran sesquiterpenoids have been isolated. The structure of compounds **1**–**3** was established by 2D-NMR spectra.

2. Investigations, results and discussion

Repeated column chromatography of the methanol extract of seed oil of *Euonymus phellomana* Loes. yielded compounds **1**–**9**. The strurctures of compounds **4**–**9** were determined by spectral data as 1β-hydroxy-2β,6α,9β,12-tetraacetoxy-8β-benzoyloxy-β-dihydroagarofuran [3], 1β-(β-)furancarboxy-2β,6α-diacetoxy-4α-hydroxy-9α,12-di(α-methyl) butanoyl-β-dihydroagarofuran [4], 1β-benzoyloxy-2β,6α-diacetoxy-4α-hydroxy-9α,12-di(α-methyl)butanoyl-β-dihydroagarofuran [4], 1β,8β-dibenzoyloxy-2β-hydroxy-6α,9β,12-triacetoxy-β-dihydroagarofuran [5], 1β, 8β-dibenzoyloxy-2β,6α,9β,12-tetraacetoxy-β-dihydroagarofuran [4], and 1β,2β-dibenzoyloxy-4α-hydroxy-6α,9α,12-triacetoxy-β-dihydroagarofuran [4].



	R_1	R_2	R_3	R_4
1	Ac	Ac	α-Fu	α-MeBu
2	Ac	Ac	α-Bz	α-MeBu
3	Bz	Bz	β-Bz	Ac
5	Fu	Ac	α-MeBu	α-MeBu
6	Bz	Ac	α-MeBu	α-MeBu
9	Bz	Bz	α-Ac	Ac



	R_1	R_2
4	H	Ac
7	Bz	H
8	Bz	Ac

Compound **1** was analyzed for $C_{31}H_{42}O_{13}$ by spectral data. It showed on ester carbonyl band at 1745 cm^{-1} and a hydroxyl at 3420 cm^{-1} in IR. UV showed the presence of an acetate and a furan moiety. FABMS: *m/z* 623 (M + 1). The ^1H and ^{13}C NMR spectrum revealed the presence of three acetoxy [δH: 2.09 (3 H, s), 2.11 (3 H, s), 2.27 (3 H, s); δC: 21.1, 21.2, 21.5, 169.4, 170.2, 170.5], one (β-)furancarboxy [δH: 6.72 (1 H, d), 7.45 (1 H, d), 8.01 (1 H, s); δC: 109.9, 118.7, 143.8, 148.9, 161.8] and one (α-methyl)butanoyl [δH: 0.68 (3 H, t), 0.88 (3 H, d), 1.26 (2 H, m), 1.98 (1 H, m); δC: 11.3, 15.6, 25.3, 40.7, 174.4] esters, and the MS showed peaks due to the loss of acetic acid, β-furoic acid and (α-methyl)butanoic acid. In addition, the ^{13}C NMR spectrum showed signals attributed to three methyls (δ 29.3, 25.5, 25.0), three methylenes (δ 65.4, 41.9, 34.5), five oxygenated methines (δ 78.1, 69.9, 69.1, 68.2, 49.1) and four quaternary carbons (δ 91.1, 84.5, 69.7, 55.0). The ^1H NMR spectrum contained signals assignable to protons on the carbon atoms carrying four secondary ester groups, i.e. δ 6.11 (1 H, s, H-6), 5.58 (1 H, d, J = 3.4 Hz, H-1), 5.47 (1 H, d, J = 3.4 Hz, H-2), 5.22 (1 H, d, J = 7.0 Hz, H-9) and a primary ester group, i.e. δ 4.99, 4.41 (2 H, ABq, J = 13.0 Hz, H-15a, b) on the basis of the multiplicity and the ^1H-^1H COSY spectrum. These data suggested that **1** is a β-dihydroagarofuran sesquiterpene substituted with five ester groups.

The positions of the ester groups were unambiguously established by HMBC experiments. The carbonyl ^{13}C signal at δ 161.8 showed long range correlation with the ^1H signals at δ 5.22 (H-9). Thus, the furoyl group must be located on C-9. The long range couplings of the carbonyl signals at 170.5, 170.2 and 169.4 with the proton signals at δ 5.47 (H-2), 5.58 (H-1) and 6.11 (H-6) and the acetyl methyl signals at δ 2.09, 2.11 and 2.27 indicated the presence of acetyl groups on C-2, C-1 and C-6. The last carbonyl ^{13}C signals at δ 174.4 were correlated with the ^1H signals at δ 4.99, 4.41 (H-15a, b), 0.88 (H-16), 1.26 (H-18) and 1.98 (H-19), respectively. Thus, the isobutyryl groups had to be situated on C-15. Therefore, a planar structure for **1** was established.

The relative stereochemistry of **1** was confirmed via the NOESY difference spectra. In NOESY there are correlation between H-1, H-2/H-3ax ($J_{1,2} = 3.4$), showing H-1ax,

Table 1: ^{1}H NMR data of compounds 1–4, 7 and 8 (δ in $CDCl_3$, J(Hz))*

H(J_{Hz})	1	2	3	4	7	8
1	5.58 d (3.4)	5.65 d (3.4)	5.95 s	4.34 dd (8.5, 3.5)	5.79 d (4.0)	5.83 d (4.0)
2	5.47 d (3.4)	5.51 d (3.4)	5.94 t	5.23 td (3.5, 3.5, 2.7)	4.29 m	5.51 dt (4.0, 4.0, 2.5)
3	2.02 m	2.20 m	2.27 m	2.31	2.43 m	2.52 ddd (15.2, 6.2, 4.0)
		1.99 m	2.10 m	1.29	1.89 m	
4				2.29	2.34 m	2.37 brp (7.7)
6	6.11 s	6.13 s	6.25 s	6.30 s	6.88 s	6.09 s
7	2.23 m	2.18 m	2.24 s	2.59 d (4.0)	2.58 d (3.5)	2.59 d (4.0)
8	2.57 dd	2.59 m	2.63 m	5.72 dd (4.0, 5.6)	5.66 dd (3.5, 5.5)	5.65 dd (4.0, 5.7)
	2.53 dd	2.55 m				
9	5.22 d (7.0)	5.33 d (8.0)	5.53 d (7.1)	5.63 d (5.6)	5.54 d(5.5)	5.54 d(5.7)
12	1.47 s	1.48 s	1.58 s	1.42 s	1.59 s	1.60 s
13	1.47 s	1.49 s	1.57 s	1.58 s	1.46 s	1.47 s
14	1.55 s	1.56 s	1.61 s	1.15 d (7.7)	1.22 d (7.0)	1.15 d (7.7)
15a	4.99 d (13.0)	5.00 d (13.0)	5.42 d (12.7)	5.11 d (12.5)	5.59 d (13.0)	5.47 d (13.5)
15b	4.41 d (13.0)	4.44 d (13.0)	4.69 d (12.7)	4.77 d (12.5)	4.68 d (13.0)	4.60 d (13.5)

* **2**: AcO × 3[2.09 (s, 3H), 2.12 (s, 3H), 2.28 (s, 3H)]; BzO[7.43~7.47 (t, 2H), 7.56~7.60 (t, 1H), 8.02~8.04 (d, 2H)]; α-MeBu[0.79 (d, J = 8.0, 3H), 0.54 (t, 3H), 1.15 (m, 1H), 0.91 (m, 1H), 1.87 (m, 1H)]. **3**: AcO × 2[2.14 (s, 3H), 2.34 (s, 3H)]; BzO × 3[7.12~7.16 (t, 2H), 7.30~7.36 (m, 5H), 7.45~7.57 (m, 4H), 7.74~7.76 (d, 2H)].

H-2eq; H-6: δ 6.11(s) indicated angle of 7β, 6 should be 90° [6, 7]. Cross signals of H-1ax and δ 8.01, 6.72(-OFu) determined α substituted ester group at C-9.

Considering the above data for **1** the structure was elucidated to be 1β,2β,6α-triacetoxy-4α-hydroxy-9α-(β-)furancarboxy-15-(α-methyl)butyroyloxy-β-dihydroagarofuran.

Compound **2** was found to be spectroscopically similar to **1**. FAB MS *m/z*: 633 (M + 1). Its spectral data (Tables 1 and 2) suggested the presence of three acetate esters, one benzyl ester and one isobutyrate ester and the 1β,2β,6α,9α,15-quinquesterified-β-dihydroagarofuran parent. Close comparison of the ^{1}H and ^{13}C NMR spectra of **1** and **2** revealed the benzoyl at C-9 of **2** replacing a furoylate of **1**. In the same way as in **1** (HMBC experiment), the positions of the three acetate esters were shown to be situated at C-1, C-2 and C-6, the benzoyl esters at C-9, and the one isobutyrate ester at C-11, respectively.

Thus, the structure of **2** is 1β,2β,6α-triacetoxy-4α-hydroxy-9α-benzoyloxy-15-(α-methyl)butyroyloxy-β-dihydroagarofuran.

Compound **3** was found to be spectroscopically very similar to **2**. Its spectral data (Table 1 and 2) suggested the presence of two acetate esters and three benzoyl esters and the 1β,2β,6α,9,15-quinquesterified-β-dihydroagarofuran parent. Close comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **3** clearly revealed the two benzoyl at C-1, C-2 of **3** replacing two acetate of **1** and an acetate at C-15 of **3** replacing an isobutyrate of **1**. In the same way as in **2** (HMBC experiment), the positions of two acetate esters were shown to be situated at C-6 and C-15, the three benzoyl esters at C-1, C-2 and C-9, respectively. In the NOESY spectrum of **3**, the presence of cross signals between H-6ax and δ 8.08 (at C-9) showed that the stereochemistry of the substituted ester group at C-9 was β in compound **3.**

Thus, the structure of **3** is 1β,2β,9β-tribenzoyloxy-4α-hydroxy-6α,15-diacetoxy-β-dihydroagarofuran.

Table 2: ^{13}C NMR shifts of compounds 1–3 (δ in $CDCl_3$)*

C	1	2	3
1	69.9	69.9	69.3
2	68.2	68.1	69.3
3	41.9	41.9	42.1
4	69.7	69.7	69.6
5	91.1	91.1	91.2
6	78.1	78.1	78.1
7	49.1	49.1	49.2
8	34.5	34.5	34.7
9	69.1	69.5	71.2
10	55.0	55.1	55.3
11	84.5	84.6	84.7
12	25.0	25.0	25.8
13	25.5	25.7	25.3
14	29.3	29.3	29.3
15	65.4	65.4	66.3

* **2**: AcO × 3[21.1, 21.2, 21.5, 169.4, 170.2, 170.5]; BzO [128.3, 129.1, 130.2, 133.5, 165.2]; α-MeBu [11.1, 15.6, 25.3, 40.6, 174.2]. **3**: AcO × 2[21.3, 21.5, 170.3, 70.8]; BzO× 3[127.9(2C), 128.0(2C), 128.7(2C), 128.8(2C), 129.1(2C), 129.4(1C), 129.8(2C), 129.9(2C), 132.8(1C), 133.1(1C), 133.4(1C), 165.5(1C), 165.0(2C)]

3. Experimental

3.1. Equipment

MP: uncorr. $[\alpha]_D$: JASCO-20C. MS: VGZAB-HS and VG-Auto Spec-3000. ^{1}H and ^{13}C NMR, NOESY, HMQC and HMBC spectra: 400 MHz spectrometer, (Bruker AM-400) solvent, $CDCl_3$, int. Standard, TMS. CC: silica gel (200–300 mesh). Eluent, petrol-EtoAc (10 : 1–1 : 1). TLC: precoated silica gel (Merck 60F-254) plates. Reverse phase CC: RP-18, Merck short column, eluent, H_2O-MeOH (1 : 4).

3.2. Plant material

Plant material was collected from Wen county, Gansu province P.R. China in August 1996 and identified by Prof. Jizhou Sun, Department of Biology, Lanzhou University where a voucher is deposited.

3.3. Extraction and isolation

Air-dried and pulverized seeds (2.5 kg) of *Euonymus phellomana* Loes., were extracted with petrol at room temperature for a week. Removal of the solvent under reduced pressure afforded an oil. The oil was extracted two times with H_2O-MeOH (1 : 10). A portion (50.0 g) of this extract was chromatographed on a silica gel column using Petrol-EtoAc (10 : 1, 8 : 1, . . . , 1 : 1, EtoAc), as eluent to give 65 fractions. 52 highly polar fractions were combined, on the basis of TLC, divided it into 3 groups, which were chromatographed on a RP-18 short column using H_2O-MeOH (1 : 4) as eluent to yield compounds **1** (6.4 mg), **2** (14.7 mg), **3** (14.2 mg), **4** (3.9 mg), **5** (300.0 mg), **6** (50.0 mg), **7** (10.5 mg), **8** (5.6 mg) and **9** (21.2 mg).

3.4. 1β,2β,6α-Triacetoxy-4α-hydroxy-9α-(β-)furancarboxy-15-(α-methyl) butyroyloxy-β-dihydroagarofuran (**1**)

White needles crystals (petroleum ether/Me_2CO); m.p.: 154–156, $C_{31}H_{42}O_{13}$; $[\alpha]_D^{20}$ +22.8° (C = 0.30, MeOH); IR, ν_{max}^{KBr} (cm^{-1}): 3420, 1746, 1621, 1571, 1241, 1155, 1095; UV, λ_{max}^{MeOH} (nm): 206, 232; EIMS, *m/z* (%): 607 (M^+-CH_3, 3), 562 (M^+-HOAc, 4) 502 ($M^+-2HOAc$, 30), 95 (Fu^+, 100), 85 ($C_4H_9CO^+$, 37), 57 ($C_4H_9^+$, 46), 43 (Ac^+, 2); FABMS *m/z*: 623 (M + 1); ^{1}H NMR and ^{13}C NMR see Table 1 and Table 2.

3.5. 1β,2β,6α-Triacetoxy-4α-hydroxy-9α-benzoyloxy-15-(α-methyl) butyroyloxy-β-dihydroagarofuran (**2**)

White needles crystals; m.p.: 170−172 °C, $C_{33}H_{44}O_{12}$; $[\alpha]_D^{20}$ +39.7° (C = 0.23, MeOH); IR, ν_{max}^{KBr} (cm^{-1}): 3530, 1745, 1709, 1606, 1584, 1459, 1388, 1369, 940, 867; UV, λ_{max}^{MeOH} (nm): 231, 275, 281; EIMS, *m/z* (%): 617 (M^+-CH_3, 1), 572 (M^+-HOAc, 2), 530 (572 + H−OAc, 2), 512 (M^+ −2 HOAc, 56), 452 (512–HOAc, 1), 105 ($C_6H_5CO^+$, 100), 85 ($C_4H_9CO^+$, 20), 77 ($C_6H_5^+$, 30), 57 ($C_4H_9^+$, 20); FABMS *m/z*: 633 (M + 1); ^1H and ^{13}C NMR see Table 1 and Table 2.

3.6. 1β,2β,9β-Tribenzoyloxy-4α-hydroxy-6α,15-diacetoxy-β-dihydroagarofuran (**3**)

Colorless oil; $C_{40}H_{42}O_{12}$; $[\alpha]_D^{20}$ +81.3° (C = 0.48, MeOH); IR, ν_{max}^{KBr} (cm^{-1}): 3556, 1729, 1604, 1454, 1388, 1368, 1276, 1240, 1152 ; UV, λ_{max}^{MeOh} (nm): 231, 275, 281; EIMS, *m/z* (%): 594 (M^+-2 HOAc, 2), 532 (46), 428 (2), 397 (4), 337 (5), 228 (4), 202 (22), 166 (12), 105 ($C_6H_5CO^+$, 100), 77 ($C_6H_5^+$, 48), 45 (3); FABMS *m/z*: 715 (M + 1); ^1H and ^{13}C NMR see Table 1 and Table 2.

Acknowledgements: We would like to thank Prof. Jizhou Sun, Department of Biology, Lanzhou University for the identification of the plant and the National Laboratory of Applied Organic Chemistry and Analysis Center Lanzhou University, P.R. China for measuring NMR and MS, respectively. This work was supported by National Laboratory of Applied Organic Chemistry of Lanzhou University of China.

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Received September 5, 2000
Accepted October 13, 2000

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